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LECTURES

PL-1

SPATIAL AND TEMPORAL ASPECTS OF CALCIUM SIGNALLING

Michael J. BERRIDGE

Calcium (Ca^{2+}) is a highly versatile intracellular signal capable of regulating many different processes. To achieve this versatility, the signalling system operates in many different modes thus enabling it to function over a wide dynamic range. At the synaptic junction, for example, Ca^{2+} triggers exocytosis within microseconds, whereas at the other end of the scale Ca^{2+} has to operate over minutes to hours to drive processes such as gene transcription and cell proliferation. At any moment in time, the level of intracellular Ca^{2+} is determined by a balance between the ON reactions that introduce Ca^{2+} into the cytoplasm and the OFF reactions during which this signal is removed through the combined action of buffers, pumps and exchangers.

Cells have access to a very extensive Ca^{2+} signalling toolkit from which each cell type expresses a unique set of components to create Ca^{2+} signalling systems with widely different spatial and temporal properties. Spatial properties are particularly relevant for the fast responses where components of the ON reactions and their downstream effectors are closely associated. This spatial contiguity is less apparent for the slower responses such as gene transcription, fertilization and cell proliferation where Ca^{2+} signals tend to operate more globally and where temporal properties of signalling become increasingly important in that signalling is usually presented in the form of repetitive Ca^{2+} transients and waves. The Ca^{2+}-sensitive processes have effector systems tuned to respond to particular Ca^{2+} transients. At the fast end of the scale, such as synaptic transmission or cardiac contraction, the effector systems respond to pulses within the micro- to millisecond range. As one moves up the time scale, the transients tend to last for longer (seconds to minutes) and the resulting signal spreads out as a Ca^{2+} wave to reach targets distributed throughout the cell. During prolonged stimulation, these transients are repeated to set up regular Ca^{2+} oscillations that have been implicated in the control of many different processes.

Many of these Ca^{2+} signalling systems are organized into macromolecular complexes enabling Ca^{2+} to carry out its signalling function within a highly localized environment. These complexes can function as autonomous units or modules that can be multiplied up or mixed and matched to create larger more diverse signalling systems. A typical example is the cardiac Ca^{2+} release unit that can be recruited independently of its neighbours to produce graded contractions. This highly organized cardiac Ca^{2+} signalling unit illustrates a number of the important dynamic aspects of the ON/OFF reactions of Ca^{2+} signalling such as amplification, homeostasis, tunnelling and modulation through cross talk with other signalling pathways.

Such Ca^{2+} signalling systems are not fixed in stone, but are constantly being remodelled to adapt to changing circumstances to ensure that each specific cell type continues to deliver the Ca^{2+} signals that characterizes its unique function. If the spatiotemporal properties of this output signal change due to a loss or defect of a key component, compensatory mechanisms come into play to restore the normal output signal. This remodelling process implies an element of quality assessment in that the output of the signalling system is under constant review. It seems that Ca^{2+} itself plays a critical role in this internal assessment mechanism by remodelling its own signalling pathway.

Evidence to support this hypothesis of Ca^{2+}-induced Ca^{2+} signalling remodelling is the fact that Ca^{2+} is a potent activator of gene transcription and that some of these genes are known to code for components of the Ca^{2+} signalling toolkit such as the expression level of Ca^{2+} signalling components such as pumps and channels. For example, expression of the InsP3 receptor is mediated through the calcineurin/NFAT transcriptional cascade.

A number of important disease states (hypertension, congestive heart failure, manic depressive illness, Alzheimer's disease) may result from abnormal remodelling of Ca^{2+} signalling systems. A good example is congestive heart failure, a major cause of human morbidity and mortality, which usually develops when the heart tries to adapt to stress usually in the form of an increased workload. The initial response is for the heart to grow and to begin to display an altered phenotype by expressing neonatal genes. This hypertrophy is a compensatory mechanism in that the heart will return to its original phenotype and size if the abnormal inputs are reduced. However, if the stresses persist, this compensated hypertrophy shifts to the more irreversible state of congestive heart failure. The phenotypic remodelling that occurs during both cardiac hypertrophy and congestive heart failure is controlled by a number of signalling pathways of which Ca^{2+} seems to play a prominent role.

A major problem with trying to understand how Ca^{2+} controls cardiac hypertrophy is the fact that the heart is not quiescent but is continuously subjected to large periodic Ca^{2+} signals that flood through the cytoplasm and nucleus every time the heart contracts. Why is it then that cardiac cells that are subjected to this constant barrage of Ca^{2+} avoid triggering a hypertrophic response? It has been suggested that the normal functioning heart may not be transcriptionally silent but may be under constant Ca^{2+}-dependent surveillance. If this is the case, then the increase in transcription during hypertrophy may result from subtle differences in the spatiotemporal properties of the individual Ca^{2+} spikes. Indeed, a broadening of the Ca^{2+} transient or an increase in its amplitude have been recorded in cases where hypertrophy is induced by modifying the levels of proteins such as triadin or FKBP12.6. It is a change in the kinetics of the Ca^{2+} transient that seems to carry the information responsible for inducing hypertrophy. Such subtle changes in Ca^{2+} signalling might be sufficient to activate a distinctive programme of gene transcription.

A different phenotypic remodelling process, which appears to be irreversible, occurs during the onset of congestive heart failure when the Ca^{2+} signalling system is severely down regulated. The most noticeable change is a dramatic decline in the activity of the SERCA pump caused by a decrease in its mRNA and protein expression level. This decline in SERCA2 activity coincides with an increase in the activity of the NCX1, which will reduce the access of the SERCA2 pump to Ca^{2+} thus contributing to the severe depletion of the SR store that characterizes congestive heart disease.

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PL-2

ION CHANNELS AND ASSOCIATED PATHOLOGIES

Michel LAZDUNSKI

Ion channels are the targets of numerous drugs with antihypertensive, antidiabetic, antiepileptic or analgesic effects. The presentation will deal with the analysis of the molecular properties, pharmacology and relation with disease states of two new families of ion channels.

The first one consists of the 2P-domain K^+ channels. This class of channels plays a central role in the regulation of resting potentials as well as in the shaping of action potentials. Two particular sub-families will be discussed. The first one is that of TREK and TRAAK channels. These channels are mechanosensitive, but they are also activated potently by polyunsaturated fatty acids and lysophospholipids as well as by intracellular acidification (TREK). The second one comprises TASK channels that are involved in sensing small extracellular pH variations. Both TREK and TASK channels are regulated by numerous neurotransmitters. Through their action on both TREK and TASK channels, metabotropic receptors have a potent ionotropic function. Both TREK and TASK channels are major targets in the action of volatile anaesthetics. TREK and TRAAK channels are essential targets in neuroprotection against brain and spinal cord ischemia as well as against epilepsy and spectacular effects can be obtained by activating them. TREK channels also have a sensory function in nociceptors and may be involved in psychiatric diseases.

The second new family of ion channels (ASICs) is permeable to Na^+. The ASIC channels are non-voltage activated, proton-activated ion channels. They are the most simple ligand gated channels and are involved in sensing extracellular acidifications including those that probably occur at post-synaptic levels. They are present everywhere in brain as well as in nociceptors and they seem to be particularly important in pain perception. Their molecular properties as well as their pharmacology and their involvement in nociception will be discussed.

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PL-3

NEW ASPECTS OF RENAL POTASSIUM TRANSPORT

Gerhard GIEBISCH, Steven HEBERT and Wenhui WANG

The kidney's major role in potassium (K) homeostasis depends on its ability to respond effectively to changes in external K balance and to stabilize the extracellular concentration of K. The correction of deviations from normal plasma K levels and the maintenance of external K balance depend on the intrinsic ability of distal nephron segments to either secrete or reabsorb K. Following extensive reabsorption of K along the proximal tubule and the thick ascending limb of Henle's loop, net K secretion occurs mainly in principal cells. K secretion is suppressed in K depletion and replaced by K reabsorption in intercalated cells. Studies on single tubules and principal and intercalated cells have defined the determinants of K secretion and reabsorption including the electrochemical driving forces, specific carriers, ATPases and K channels. Recent studies on the properties and molecular

identity of renal K channels have also contributed significantly to understanding the renal mechanisms that transport and regulate K excretion.

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PL-4

ATP-SENSITIVE K CHANNELS AND INSULIN SECRETIONIN HEALTH AND DISEASE

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(abstract not received)

PL-5

FUNCTIONAL ARCHITECTURE OF THE NICOTINIC RECEPTOR AT THE AMINO ACID LEVEL : A MEMBRANE ALLOSTERIC PROTEIN

Jean-Pierre CHANGEUX

The nicotinic receptor is a transmembrane hetero-pentamer of 300MW which carries the binding sites for acetylcholine, and the ion channel together with structural elements which account for its fast opening and slow desensitization by the neurotransmitter (Changeux & Edelstein, 1998 ; Karlin, 2002 ; Unwin, 1999). The amino acids which compose the ACh binding sites have been identified by photolabeling and by site-directed mutagenesis. They belong to 6-distinct loops within the large NH2-terminal hydrophilic domain of the receptorsubunits located at the interface between subunits and including an a-subunit (Corringer et al., 2000). Molecular modeling of the ACh binding pocket on the basis of the Xray structure of snail acetylcholine binding protein offers opportunities for docking and thus for design of nicotinic ligands at various neuronal subunit interfaces (Le Novère et al., 2002) Furthermore, in T. marmorata, the relative contribution of several different loops changes upon stabilization of the high-affinity desensitized state by the allosteric effector meproadifen (Galzi et al., 1991). The channel blocker chlorpromazine covalently labels, upon UV irradiation, all the subunits when bound to its unique high-affinity site located in the ion channel. The labeled amino acids belong to the hydrophobic segment MII and form three superimposed rings assuming MII α helical (Giraudat et al., 1986, 1987 ; Hucho et al., 1986 ; Revah et al., 1991). Mutations, within or in the neighborhood, of MII from α_7 neuronal nicotinic receptor selectively alter the ionic selectivity for Ca^{++} vs Na^+/K^+ (Bertrand et al., 1993) and for cations vs anions (Galzi et al., 1992). On the basis of systematic mutagenesis experiments, a model of the ion channel is proposed which locates the ion selectivity filter at the level of the cytoplasmic loop linking MII and MI (Corringer et al., 2000). Mutations of AA rings from MII cause "gain of function" pleiotropic phenotypes with, altogether, loss of desensitization, enhanced affinity for agonists and conversion of the competitive antagonists into agonists (Révah et al., 1991 ; Bertrand et al., 1992). Homologous genotypes and phenotypes have been recently identified in human patients with congenital myasthenia gravis and frontal lobe nocturnal epilepsy (rev. Ohno & Engel, 2002 ; Raggenbass & Bertrand, 2002). The data are interpreted in terms of a four-state "allosteric" model (Edelstein et al., 1997 ; Changeux & Edelstein, 1998 ; Corringer et al., 2000). Furthermore, obtention of functional chimaera between α_7 and 5HT3 receptors reveals a functional autonomy of the neurotransmitter binding and channel domains (Eiselé et al., 1993) supporting a common functional organization of these different receptors (Le Novère et al., 1999, 2002).

Implications of the allosteric model to the understanding of short-term memory and cognitive functions are discussed (Heidmann & Changeux, 1982 ; Dehaene & Changeux, 1997 ; Dehaene et al., 1998).

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PL-6

THE REGULATION OF EPITHELIAL Na^+ CHANNELS IN EXOCRINE EPITHELIA

David I. COOK

Epithelial Na^+ channels are expressed in the apical membranes of epithelia such as the renal collecting duct, the distal colon, the respiratory epithelium and the ducts of salivary and sweat glands. They are a key component in the mechanism by which these epithelia transport Na^+ ions across their apical membranes and have been shown to regulate blood pressure and extracellular fluid volume. They also play a critical role in controlling the thickness of the fluid layer covering the surface of the respiratory and gastrointestinal tracts. Abnormalities in the structure and regulation of these channels have been implicated in the pathogenesis of the autosomal dominant form of hypertension, Liddle's syndrome, salt-wasting conditions such as pseudohypoaldosteronism type I and cystic fibrosis. Consistent with the critical role of epithelial Na^+ channels in the normal regulation of blood pressure, of extracellular fluid volume and of the thickness of the fluid on the respiratory surfaces, transgenic mice in which the a-subunit of the channel has been deleted by homologous recombination die at birth due to failure to clear their lungs of fluid, and transgenic mice in which expression of the b- or g-subunits has been reduced, suffer from a salt-losing nephropathy characterised by hyperkalaemia and hypotension.

Given their importance for the regulation of the milieu intérieur, it is not surprising that epithelial Na^+ channels are regulated by a wide variety of hormones, including aldosterone, ADH and insulin-like growth factor I. They are also regulated by feedback systems that adjust the activity of the channels to ensure that the rate of Na^+ entry to the cytosol across the apical membrane does not exceed the capacity of the Na^+-K^+-ATPase to extrude it across the basolateral membrane. These feedback systems thus operate to ensure stability of the volume and ionic composition of the cytosol.

The nature of these feedback systems has been the subject of investigation since their existence was first postulated by Ussing and MacRobbie over 40 years ago. The mechanisms that have been proposed include: (i) inhibition of the channels by an extracellular modifier site that binds extracellular Na^+, (ii) direct inhibition of the channels by increased intracellular Na^+, (iii) inhibition of the channels by increased intracellular Cl^- serving as a surrogate for cell volume, (iv) inhibition of the channels by the increase in intracellular Ca^{2+} which results from the slowing of Na^+-Ca^{2+} exchange produced by increases in intracellular Na^+, and (v) inhibition of the channels by the decrease in cytosolic pH which results from the slowing of the Na^+-H^+ exchanger produced by increased intracellular Na^+. Of these, the Na^+ and the Cl^- feedback systems are of particular interest.

The Na^+ feedback system is mediated by an ubiquitin-protein ligase, either Nedd4 or Nedd4-2, which binds to proline rich domains, the so-called PY motifs, in the b- and g-subunits of the Na^+ channels. Once bound to the channels, the ubiquitin protein ligases ubiquitinate and inactivate them. In mouse mandibular duct cells, it has been possible to further show that the concentration of intracellular Na^+ is sensed by a cytosolic receptor that can be blocked by compounds such as benzimidazolylguanidinium (BIG) and dimethylamiloride (DMA) which have been reported to block Na^+ feedback regulation in intact tissues. This receptor in turn activates the G protein, Go, the a-subunit of which then activates the ubiquitin protein ligase. Many cases of Liddle's syndrome are due to the mutation or deletion of the PY motif in the b- or the g-subunit of the Na^+ channel, the increased channel activity seen in this condition being attributable to disruption of the Na^+ feedback system. There have also been recent reports that the hormonal regulators of epithelial Na^+ channel activity, aldosterone and IGF-I, activate epithelial Na^+ channels as a consequence of increasing the activity of the serum- and glucocorticoid-inducible kinase, Sgk, which in turn phosphorylates the ubiquitin-protein ligase, Nedd4-2, leading to interruption of the Na^+ feedback regulatory system. Given that epithelial Na^+ channels are known to be extensively phosphorylated in vivo, these reports raise the issue of the extent to which other kinases may be able to modulate the activity of the Na^+ feedback system.

The Cl^- feedback system, on the other hand, does not involve ubiquitin-protein ligases or ubiquitination of the channel. In mouse mandibular duct cells, it has been shown to be mediated by the G protein, Gi2. Like the Na^+ feedback system, the Cl^- feedback system is probably mediated by an intracellular receptor for Cl, although it has not yet proven possible to demonstrate this conclusively. The Cl^- feedback system is not sensitive to agents such as BIG, although it is blocked, as is the Na^+ feedback system, by extracellular exposure of the channels to sulfyhydryl reactive reagents such a r-chloromercuriphenylsulfonate. Recently, Cl^- feedback regulation has been proposed as the mediator of the inhibitory action of the Cl^- channel, CFTR, on epithelial Na^+ channels. If this is correct, then the aberrant regulation of cytsolic Cl^-which accompanies mutations of CFTR would be the cause of the increased epithelial Na^+ channel activity that is observed in cystic fibrosis.

As mentioned above, epithelial Na^+ channels play a critical role in regulating the thickness of the fluid layer that coats the surface of the respiratory epithelium. Increased activity of the channels leads to dehydration of the surfaces of the respiratory epithelium as is observed, for example, in cystic fibrosis. Conversely, decreased activity of the channels, as is observed in the hereditary condition, pseudohypoaldosteronism type I, leads to fluid accumulation in the lungs and respiratory passages. Recently, it has become evident that many acquired diseases associated with the accumulation of

fluid in the lungs or in other parts of the respiratory tract, such as high altitude pulmonary oedema, respiratory distress syndrome and otitis media, are associated with decreased activity of the Na^+ channels in the lining epithelium. In particular, many pathogens, including both gram-negative bacteria and viruses, inactivate epithelial Na^+ channels. In the case of influenza virus this inactivation is due to the hemagglutinin in the viral coat binding a glycoprotein in the apical membrane in the epithelial cells, which in turn activates protein kinase C leading to inhibition of channel activity. Other pathogens appear to act by triggering the release of ATP which then acts in an autocrine manner on purinergic receptors in the apical membrane to inhibit the Na^+ channels. Irrespective of the detailed mechanism, the reduction in the rate of Na^+ transport will shift the net rate of fluid transport by the epithelium towards secretion and promote such manifestations of respiratory infections as pulmonary oedema, sinusitis and rhinorhoea.

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PL-7

GENOMICS AND PHYSIOLOGY
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(abstract not received)

PL-8

EVOLUTION OF THE GENETIC MAP OF CARDIOVASCULAR FUNCTION
Allen W. COWLEY, Jr

The genetics of multifactorial disorders such as hypertension, arthritis, and diabetes in human populations has proven to be very challenging due to the modest nature of gene effects and the heterogeneity of patient populations. With the genetic sequencing of the human, mouse, and rat genomes now nearly completed, there is a need to define and place gene function in the context of complex systems biology. This lecture will focus upon two experimental approaches that have begun to provide an understanding of the relationships among genes, environmental stressors, and blood pressure. The first utilizes linkage studies with total genome scans. This approach has led to the first genomic-systems biology map of cardiovascular function. In studies performed in the F2 offspring of an intercross between the Dahl salt-sensitive rat (SS./Mcw) and the Brown Norway rat (BN), more than 200 cardiovascular phenotypes were determined during normal and stressed conditions. Genomic regions accounting for a large degree of the variability of 81 of the traits in the male population and 126 in the female population were identified and mapped as quantitative trait loci (QTL) regions on the rat genome. In addition, a number of QTL of cardiovascular and renal traits were found to be determined by gender and mapped to discrete regions of the genome. The results of these linkage analyses have led to a richly annotated map of genome function. The second approach has been the development of consomic panels of inbred rats that are enabling a broad mapping of cardiovascular pathways and leading to more detailed identification of genes related to these pathways and providing controls for genetic background effects. BN chromosomes are introgressed onto the DS genomic background one chromosome at a time in each strain. Each inbred consomic rat strain enables the assessment of the contribution of genes specific to that chromosome and provides strains with uniform genetic backgrounds for various genetic and physiological studies. Environmental stressors such as hypoxia, exercise, and high salt intake are being used to unmask deficiencies in normal homeostatic mechanisms and idiopathic mechanisms that contribute to disease as determined by more than 300 measured phenotypes to characterize heart, lung, kidney, vasculature, and blood function. Comparative mapping strategies are used to link these traits to the genomes of the mouse and human. As major phenotypic differences are identified within the consomic stains, these strains are being made commercially available (Charles Rivers, Inc) and provide highly useful model systems to test both physiological and genetic hypotheses. When combined with the rapid derivation of even more discrete chromosomal substitutions of very narrow regions within chromosomes (congenic strains) together with DNA microtechnology and proteomics, these functionally and genomically annotated rat strains provide powerful new tools to link complex systems biology to related genetic pathways.

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S1 : CALCIUM SIGNALLING

ORAL SESSION

S1-1

LOCALIZATION OF Ca^{2+} TRANSPORTERS IN EXOCRINE ACINAR CELLS

Petersen O.

More than 30 years ago, I showed that neurotransmitters acting on exocrine gland cells release Ca^{2+} from a store in the endoplasmic reticulum (ER). 20 years ago, work on pancreatic acinar cells by Michael Berridge and his collaborators provided the original evidence for the Ca^{2+} releasing action of IP3. Ironically, further work on the pancreatic acinar cells presented some problems for the view that IP3 acts on the ER, since the primary Ca^{2+} release site was in the apical pole, which contains the secretory granules, but little ER. All cytosolic Ca^{2+} signal responses to stimulation with neurotransmitters, hormones and intracellular messengers (including IP3, cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate) are initiated in the secretory granule area and are mostly confined to this region. These local Ca^{2+} signals control not only exocytosis, but also fluid secretion via regulation of Ca^{2+}-activated Cl^-channels in the apical plasma membrane. We have mapped the Ca^{2+}-sensitive Ca^{2+} release sites, using local uncaging of caged Ca^{2+}, and shown that Ca^{2+}-induced Ca^{2+} release (which does not involve IP3 formation) can only be triggered in the apical pole and is dependent on both functional IP3 and ryanodine receptors. Ryanodine itself triggers Ca^{2+} waves, which always start in the apical pole. The distribution of ER in living acinar cells, visualized by ER-specific fluorescent probes with confocal and two-photon microscopy, shows that although the bulk of the ER is located in the basolateral area, there is significant invasion of ER into the granular pole and each secretory granule is surrounded by ER strands. This provides the framework for a coherent and internally consistent theory for cytosolic Ca^{2+} signal generation in the secretory pole, where the primary Ca^{2+} release occurs from ER terminals supplied with Ca^{2+} from the main store at the base via the tunnel function of the ER.

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S1-2

THE ROLE OF MITOCHONDRIA IN THE GENESIS OF THE CALCIUM SIGNAL IN EXCITABLE CELLS

Garcia-Sancho J., Alonso M.T., Villalobos C.

Ca^{2+} transport by organella contributes to shaping Ca^{2+} signals and exocytosis. Therefore, accurate measurements of $[Ca^{2+}]$ inside organella are essential for a comprehensive analysis of the Ca^{2+} redistribution that follows cell stimulation. On the other hand, $[Ca^{2+}]$ inside organella regulates by itself important physiological functions. Here we have combined virus-based expression of targeted aequorins with photon counting imaging to resolve dynamics of the cytosolic and mitochondrial Ca^{2+} signals at the single-cell level. Adrenal chromaffin and anterior pituitary cells were used as models for excitable cells.
On activation of plasma membrane voltage-gated Ca^{2+} channels, mitochondria took up large amounts of calcium through the mitochondrial Ca^{2+} uniporter. Results are consistent with the generation of cytosolic high-Ca^{2+} subplasmalemmal domains adequate for triggering exocytosis. At the cell core, a smaller increase of cytosolic Ca^{2+}, adequate for recruitment of the reserve pool of secretory vesicles to the plasma membrane, is produced. Most of the entering Ca^{2+} load is taken up by a mitochondrial pool, M1, closer to the plasma membrane. The increase of mitochondrial$[Ca^{2+}]$ stimulates respiration in these mitochondria, thus providing local support for the exocytotic process.
Anterior pituitary cells exhibit spontaneous electric activity and cytosolic Ca^{2+} oscillations that are responsible for basal secretion of pituitary hormones and are modulated by hypophysiotrophic factors. Aequorin reported spontaneous $[Ca^{2+}]$ oscillations in bulk cytosol, nucleus and mitochondria. Interestingly, a fraction of mitochondria underwent much larger Ca^{2+} oscillations, which were driven by local cytosolic high-$[Ca^{2+}]$ domains generated by the spontaneous electric activity. These oscillations were large enough to stimulate respiration, providing the basis for local tune-up of mitochondrial function by the Ca^{2+} signal.

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OC01-1

THE CALCIUM SIGNALLING ACTIVATED BY NORADRENALINE IN RAT ARTERIES IS REGULATED BY RHO-KINASE

Morel N., Ghisdal P., Vandenberg G.

In vascular smooth muscle cells, contractile agonists activate a complex chain of events to increase cytosolic Ca concentration and contract the arteries. The present study was aimed at investigating the potential role of Rho-kinase in the Ca signal activated by noradrenaline in rat aorta and mesenteric artery.
In fura-2 loaded arteries, the Rho-kinase inhibitor Y-27632 (10 μM) completely relaxed the contraction evoked by noradrenaline (1 μM) and simultaneously inhibited the Ca signal by 54 ± 1 % (mesenteric artery) and 71 ± 15 % (aorta), while in KCl-contracted arteries, Y-27632 decreased tension without changing cytosolic Ca. Similar effects were obtained with another inhibitor of Rho-kinase (HA 1077), but not with an inhibitor of protein kinase C (Ro-31-8220). In aorta bathed in Ca-free solution, noradrenaline response consisted of a rapid but transient increase in cytosolic Ca. Re-addition of Ca into the Ca-free solution evoked a slow, sustained increase in Ca signal, which was partly inhibited by 10 μM Y-27632 (=65 %) and completely blocked by 1 μM Gd. In the presence of nimodipine, 10 μM Y-27632 or 1 μM Gd^{3+} completely blocked the entry of Ba activated by noradrenaline. However, Y-27632 did not affect the production of inositol phosphates activated by noradrenaline, and the release of Ca from the sarcoplasmic reticulum evoked by IP3, measured by the activation of Ca-dependent K current using the patch-clamp technique. Finally, Y-27632 did not inhibit the Ca signals evoked by thapsigargin or by caffeine and the capacitative Ca entry activated by the depletion of intracellular Ca stores by thapsigargin.
These results indicate that Rho-kinase does not alter the capacity of intracellular Ca storage and release, but that it is involved in the activation of a phospholipase C-dependent Ca entry pathway in rat arteries.

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OC01-2

AN INTERACTIVE COMPUTER PROCEDURE FOR AUTOMATIC DETECTION AND MEASUREMENT OF MUSCLE CALCIUM SPARKS

Sebille S., Cantereau A., Vandebrouck C., Balghi H., Constantin B., Raymond G., Cognard C.

In muscle cells, contraction is controlled by Ca^{2+} ions, which are rapidly released from the sarcoplasmic reticulum during sarcolemmal depolarization. In addition to this synchronised spatially homogeneous calcium signal, discrete calcium release events, termed sparks, have been discovered with the use of confocal microscopy in diverse tissues as skeletal, cardiac, and smooth muscle. Determination of the calcium spark morphology parameters is of critical importance to understand the nature of elementary calcium release events in muscle. Because of multiple behaviours of release, it appeared necessary to evaluate parameters from several hundreds of sparks in a same cell population in order to obtain reliable statistics. Automatic detection algorithms without user intervention have been previously developed on single images to automatically detect and analyse sparks in confocal line scan (space-time: 512 * 512 pixels) images. Nevertheless, most of previous studies have been performed on isolated sparks without taking into account that events could originate from the same locus of release. Our first studies on myotubes clearly showed that many events were originating from the same space location. Thus, we have addressed the problem of recognizing polymorphic events on series of images in order to follow sparks morphology from one site during several seconds. Here, we describe an interactive procedure coded in the image-processing language IDL 5.3. , that can be applied on series of n images (512 x 512 x n) derived from the same scanning line. Computing simultaneously entire series of images permits to measure, with the conventional morphological parameters, location and frequency of release from each release site. The use of this procedure provides quickly much information about the properties of release sites in muscle cells and can be applied on any elementary calcium events whatever the cell type.

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OC01-3

ACTIVATION OF ICRAC BY STORE DEPLETION AND RECEPTOR STIMULATION IN FRESHLY ISOLATED RAT HEPATOCYTES

Rychkov G., Litjens T., Roberts M., Barritt G.

Activation of Ca^{2+}-conducting cation channels, or so-called store operated channels, in the plasma membrane in response to depletion of intracellular Ca^{2+} stores is a universal feature of the Ca^{2+}signalling mechanism in most non-excitable cells. One of the best-known store operated channels, Ca^{2+}release activated Ca^{2+} (CRAC) channel has been extensively characterised in a number of immortalised cell lines. There is little evidence, however, that ICRAC is activated under physiological conditions in cells in primary culture.

In the current series of experiments we have shown that depletion of the intracellular Ca^{2+} stores in freshly isolated rat hepatocytes by IP3, thapsigargin or 10 mM EGTA activated an inward current highly selective for Ca^{2+}, which could be blocked by sub-micromolar concentrations of trivalent cations and by 50 micromolar of 2-APB. Changes of the current amplitude with Ba^{2+} substitution for Ca^{2+} and the kinetics of the current inactivation at negative potentials were similar to that of ICRAC described in immortalised cell lines. The amplitude of ICRAC in rat hepatocytes varied between -20 and -120 pA at -100 mV, with an average density of about -1 pA/pF. The same current was activated by the application of 20 nM vasopressin or 5-50 micromolar ATP. Activation of ICRAC by 20 nM of vasopressin or 5 microM ATP occurred with a considerable delay (2-4 minutes). While the delay could be shortened using higher concentrations of the agonist, the rate and extent of ICRAC development were largely unaffected.

It is concluded that in rat hepatocytes ICRAC is the major pathway of Ca^{2+}entry in rat hepatocytes, regulated by phospholipase C coupled receptors.

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OC01-4

NAADP MAY INTERACT WITH THE RYANODINE RECEPTORS IN THE NUCLEAR ENVELOPE OF PANCREATIC ACINAR CELLS

Gerasimenko J.V., Maruyama Y., Tepikin A.V., Petersen O.H., Gerasimenko O.V.

We have investigated possible functional interactions of Ca^{2+} release pathways mediated by nicotinic acid adenine dinucleotide phosphate (NAADP), inositol trisphosphate (IP3) and cyclic ADP-ribose (cADPR) in the envelope of isolated pancreatic acinar nuclei. After isolation, the nuclei were loaded with the calcium sensitive dye Mag Fura Red and changes of fluorescence intensity of the dye in the nuclear envelope were monitored using a Leica laser scanning confocal system.

Recently the existence of possible functional interactions between Ca^{2+} releasing pathways regulated by NAADP, IP3 and cADPR was shown for intact isolated pancreatic acinar cells. We used caffeine to inhibit IP3 receptors (IP3R) in isolated nuclei. We have found that caffeine itself can induce calcium release from the envelope of isolated nuclei loaded with Mag-Fura Red. Subsequent addition of NAADP in the presence of caffeine induced further calcium release.

We have also used ryanodine (100 μM), which is known as an inhibitor of ryanodine receptors (RyR) when used at a high concentration. Ryanodine did not affect IP3 induced responses, but completely prevented caffeine-induced calcium release. Ryanodine also completely inhibited NAADP-induced and cADPR-induced calcium responses.

These data indicate that NAADP is functionally interacting with the RyR without involving the IP3R. We conclude that NAADP-induced Ca^{2+} release from the nuclear envelope could be explained by direct or indirect functional interaction of NAADP with the RyR

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S1-3

MITOCHONDRIAL REGULATION OF THE STORE-OPERATED CALCIUM CURRENT ICRAC

Bakowski D., Parekh A.

Mitochondria are important regulators of store-operated calcium entry under physiological conditions. By taking up some of the calcium that has been released from the stores by InsP3, mitochondria enable the stores to deplete sufficiently for the store-operated calcium current ICRAC to activate. Furthermore, by buffering incoming calcium, mitochondria reduce calcium-dependent slow inactivation of CRAC channels. Recent work suggests mitochondria might have an addition role in regulating ICRAC. The ability of thapsigargin, which depletes stores independently of InsP3 receptors by inhibiting SERCA pumps, to activate ICRAC is compromised by mitochondrial depolarisation. The involvement of mitochondria here is distal to store depletion and kinetic considerations argue against a role for calcium feedback inactivation of ICRAC. To test the latter more directly, we have carried out experiments using barium to carry ICRAC. Barium is not able to trigger calcium-dependent inactivation of ICRAC in RBL cells. We find that barium permeates CRAC channels, with a macroscopic conductance around 70% that of calcium. In weak buffer, no store-operated barium current is seen unless mitochondria are energised. In experiments monitoring divalent cation entry with fura 2, mitochondrial depolarisation suppressed barium influx. Hence calcium-feedback mechanisms do not account for the regulation of ICRAC by mitochondria under these conditions. Surprisingly, barium influx following store depletion in fura 2-loaded cells was smaller than expected from the electrophysiological recordings of ICRAC. We find barium permeates ICRAC in a voltage-dependent manner and depolarises the membrane potential by blocking potassium channels. This combination accounts for the low barium influx seen in fluorescence experiments. Our results suggest that caution is needed in interpreting data that uses barium to monitor calcium influx in non-excitable cells.

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S1-4

VDAC AND APOPTOSIS: REGULATION AND STRUCTURE-FUNCTION RELATIONSHIP

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(abstract not received)

S1-5

THE MODE OF ACTION OF POTASSIUM ON Ca^{2+} SIGNALLING AND ALDOSTERONE PRODUCTION IN GLOMERULOSA CELLS

Spät A., Koncz P., Makara J.K., Pitter J.G.

Potassium balance is controlled by aldosterone, secreted by adrenal glomerulosa cells. These cells are uniquely sensitive to extracellular K^+ concentration. Hyperkalaemia depolarizes the glomerulosa cell, thus activating voltage-dependent Ca^{2+} channels. Rat glomerulosa cells display Ca^{2+} signal in response to raising $[K^+]$ from the control 3.6 mM by as little as 0.5 mM and at 4.6 mM aldosterone production rate is already doubled. Resting membrane potential is ~ -80 mV and the activation threshold of T-type Ca^{2+} channels is between –70 and –80 mV. Activation of these channels by K^+ in rat glomerulosa cells is attributed exclusively to depolarization (Lotshaw: Mol. cell. Endocrin. 2001) and increased hormone secretion is attributed to Ca^{2+}-induced transport of cholesterol into mitochondria. We studied both the generation and action of Ca^{2+} signal. Microfluorimetry, digital imaging and patch-clamp techniques were applied on primary cultures of rat glomerulosa cells.

Swelling induced by hyposmosis (measured on calcein-loaded cells) shifts the activation threshold of T-type channels to more negative potentials, enhances K^+-induced Ca^{2+} signal as well as aldosterone production. Swelling can be induced also by raising $[K^+]$ (from 3.6 to 5 mM) and this swelling coincides with a second phase of elevation of cytoplasmic $[Ca^{2+}]$. Prevention of K^+-induced swelling with appropriate hyperosmosis attenuates the Ca^{2+} signal. Therefore, swelling seems to amplify the action of K^+-induced depolarization on Ca^{2+} channels.

Physiological increase in $[K^+]$ evokes elevated mitochondrial NAD(P)H level in a Ca^{2+}-dependent manner. The reoxidation of NAD(P)H depends on the rate of steroid synthesis. Low submicromolar elevation of cytoplasmic $[Ca^{2+}]$ raises mitochondrial $[Ca^{2+}]$ and the ensuing reduction of pyridine nucleotides may support increased steroid production.

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POSTER SESSION

P01-01

INHIBITION OF RETICULAR CALCIUM UPTAKE ALTERS THE EFFECT OF PASSIVE TENSION ON RAT AORTA CONTRACTION

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The length-tension relationship, as a determinant of muscle contraction, is highly variable among smooth muscles and the mechanisms remain elusive. We investigated the effect of passive tension on the contractile response of the de-endothelised isolated rat aorta and the influence of reticular calcium pump inhibitors therein. De-endothelised aorta rings (2 mm wide) from male adult Wistar rats were studied in isometric conditions; oxygenated saline solution (HCO_3^- buffer, pH 7.2-7.4), at 37 °C. Each ring equilibrated for 2 h under a passive tension of 2 g, then was contracted by 0.01 mM phenylephrine (PE); results expressed as % active tension of this reference value in each preparation (mean ± S.E.M.; n = 6 in all series). All rings were randomly subjected to 1 h re-equilibration under 0.5, 2, or 3 g, then a concentration-effect curve for either PE alone or in the presence of 100 nM thapsigargin (THAP) or 0.01 mM cyclopiazonic acid (CPA), to eliminate the reticular pump influence upon cytosolic calcium signals. The curve is shifted to the right at the lower passive tension, while effects are significantly increased by the higher resting tension only for low PE doses. In agreement with previous findings, elevation of cytosolic calcium by THAP or CPA enhances contractions induced by low and moderate PE concentrations. We found this effect to be prominent at low passive tension and weaker with higher stretch. Reticular pump inhibition enhances aortic contraction presumably by elimination of the buffering effect of reticular calcium uptake. Beside other mechanisms investigated so far, the potentiating effect of passive tension could involve inhibition of the reticular calcium pump.

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P01-02

PLASMA MEMBRANE AND NUCLEAR ENVELOPE NPY AND Y1 RECEPTOR IN HUMAN ENDOCARDIAL ENDOTHELIAL CELLS

Perreault C., Jacques D.

Using 3D confocal microscopy and immunofluorescence, we tested the hypothesis that endocardial endothelial cells do possess NPY and NPY receptors and that activation of these receptors may modulate cytosolic and nuclear free calcium. Our results showed that effectively, NPY is present in human endocardial endothelial cells at both the cytosolic and nuclear levels. This peptide was found to be secreted by these cells upon sustained increase of intracellular calcium. In addition, our results showed that Y1 receptors are present all through the cell including the nuclear membranes. Activation of NPY receptors induced a dose-dependent increase of both cytosolic and nuclear calcium. This effect of NPY was largely due to activation of Y1 receptors. In conclusion, our results demonstrate that human endocardial endothelial cells do secrete NPY via a calcium-dependent mechanism. In addition, NPY and its receptors modulate intracellular calcium, thus affecting excitation-secretion coupling of endocardial endothelial cells. This work was supported by the Canadian Institutes of Health Research.

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P01-03

MODULATION OF INTRACELLULAR CALCIUM BY SARCOLEMMA AND NUCLEAR MEMBRANES Ang II AT1 RECEPTORS.

Nader M., Bkaily G.

The objective of the study was to verify if human (h) AngII type-1 receptor (hAT1R) undergoes transcellular trafficking in human aortic vascular smooth muscle cells (hVSMCs)and if overexpression of this receptor modulates cytosolic and nuclear free calcium. Three-dimensional (3D) confocal microscopy was used to monitor hAT1R-GFP (green fluorescence protein fusion. Using 3-D imaging technique, hAT1Rs were localized at the sarcolemma, in the cytosol and in the nuclear compartments. Stimulation of sarcolemma membrane hAT1Rs by Ang II induced internalization and nuclear translocation of this type of receptor. The internalization of hAT1Rs was found to be mediated via clatherin-coated pits and vesicles pathway. The internalization and translocation of hAT1Rs was associated with a de novo-synthesis of this type of receptor. Overexpression of hAT1Rs induced a decrease of both cytosolic and nuclear free Ca^{2+}. In conclusion, our results susggest that hAT1Rs are the predominant type of Ang II receptors in aortic hVSMCs and are present in the sarcolemma, the cytosolic and nuclear compartments. Overexpression of these receptors modulate cytosolic and nuclear free calcium. This work is supported by a grant from the Canadian Institute of Health Research (CIHR).

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P01-04

REGULATION OF GABA RELEASE BY CALCIUM TRANSIENTS AT A SINGLE HIPPOCAMPAL TERMINAL

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We correlated dynamic changes in free Ca^{2+} concentration ($[Ca^{2+}]i$) within single presynaptic terminal of cultured hippocampal neurones with postsynaptic GABA-mediated currents. For this purpose local changes in $[Ca^{2+}]i$ and evoked inhibitory postsynaptic currents (eIPSCs) were recorded simultaneously using Fura-2 fluorescence and whole-cell patch-clamp. The Ca^{2+} signals and eIPSCs were evoked by direct extracellular electrical stimulation of a single presynaptic terminal by short depolarizing pulses. All experiments were performed in 0.25 *M TTX-containing solution to suppress action potential generation.

The presynaptic Ca^{2+} transient was changed by varying the amplitude of the extracellular stimulating pulses. The probability of release event, estimated for the each stimulation strength, changed from 0 to 1. The release probability reached P = 1 since the Ca^{2+} signals attained maximal value and remained at this level at higher stimulation strength despite the decrease in the amplitude of the Ca^{2+} transients. In the range of stimulating amplitudes, where release probability was $P < 1$, a Ca^{2+} signal of the same amplitude could result in either failure of the postsynaptic response or an IPSC of any random amplitude.

Linear gradual increase in stimulation amplitude (Vstim) resulted in a bell-shaped dependence of the averaged amplitudes of Ca^{2+} signals and corresponding averaged amplitudes of eIPSCs. Aanalysis of eIPSC demonstrated that decrease in mean eIPSC amplitude as well as reduction in quantal content of release resulted from a reduction in the probability of multivesicular release i.e. in the disappearance of failures and decrease in individual eIPSC amplitude. Ca^{2+} signals of similar amplitude resulted in both random and non-random release characteristics. We concluded that depolarization-induced $[Ca^{2+}]i$ elevation within the terminal is necessary but not sufficient for activation of vesicular release.

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P01-05

CILIARY NEUROTROPHIC FACTOR RAPIDLY INHIBITS VOLTAGE-GATED SODIUM CHANNELS IN SKELETAL MUSCLE

Talon S., Metges-Giroux M.-A., Pennec J.-P., Gioux M., Léoty C.

The ciliary neurotrophic factor (CNTF) is known to exert long-term myotrophic effects, but it is not yet evidenced if this cytokine could also induce a rapid biological response in skeletal muscles. The present in vitro study brings up the possibility that CNTF could affect the nerve-muscle coupling implied in the rapid triggering of muscle fibre contraction, particularly by influencing sodium channel activity. Therefore, we investigated the effects of an external 10-min application of 2ng.ml-1 CNTF on macroscopic sodium current (INa) of rat native fast-twitch skeletal muscle (flexor digitorum brevis, FDB) by using a cell-attached macro-patch technique. The fibres were isolated by enzymatic dissociation then cultured in 35mm Petri dishes for the experiments duration. Compared to control conditions, CNTF rapidly reduced the peak value of INa by 30.4 ± 6.3% ($n=10$, $p<0.005$) with a voltage step depolarising the path membrane from –100 to –10mV. No significant change was observed in activation and inactivation kinetics. Normalized current-voltage (I/V) curves in the presence and in absence of CNTF were superimposed, indicating the lack of CNTF effect on the voltage-dependence of sodium channel activation in FDB muscles. In the opposite, the relative INa blockade induced by CNTF was accompanied by a shift of inactivation curves to more negative potentials, the shift in half-maximal potential being DVh1/2 = -5.7 ± 1.3 mV ($n=10$, $p<0.005$) and DVs1/2 = -8.8 ± 2.1 mV ($n=5$, $p<0.005$) for the steady-state fast and slow inactivation respectively. These results suggest that CNTF can rapidly induce a decrease in skeletal muscle sodium currents, probably through an intracellular mechanism different from the well-characterized

JAK/STAT pathway. The present study would then contribute to better understand the physiological role of endogenous CNTF.

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P01-06

IMPROVEMENT OF CELL SURVIVAL AND CALCIUM SIGNALING PROPERTIES IN MUSCLE CELLS BY BMD MINIDYSTROPHIN

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Defective expression of dystrophin in muscle cells is the primary feature of Duchenne Muscular Dystrophy (DMD). Absence of 427kDa dystrophin is accompanied with a chronic elevation in intracellular calcium concentration, leading to fiber necrosis. However, direct evidence that dystrophin can control calcium handling in muscle cells has not yet been proved. Mutations of the dystrophin gene lead to DMD or the milder Becker Muscular Dystrophy (BMD) which is associated with the expression of a truncated 229kDa protein. A 6.3 kb minidystrophin cDNA has been cloned from an asymptomatic BMD patient. Its size is sufficiently small to be accommodated by current retroviral vector systems and it has been successfully expressed in the myogenic Sol8 dystrophin-deficient cell line with accumulation of the minidystrophin at the sarcolemma. Forced expression of the minidystrophin reactivates appropriate sarcolemmal expression of dystrophin-associated proteins, and leads to a decrease in cell death. We have measured the calcium influx with a cytophotometer and the fluorescent calcium probe Indo-1 as well as the intramitochondrial calcium by transfection with Aequorin, a luminescent calcium probe targeted to mitochondria. Minidystrophin forced expression decreases the amplitude of cytosolic calcium transients induced by membrane depolarisation. We have observed that depletion of sarcoplasmic reticulum leads to a store-operated calcium influx, which is less sustained in myotubes expressing minidystrophin. These store-operated calcium influx also lead to calcium entry into mitochondria, a major calcium buffer of muscle cells. These intramitochondrial entries are also shorten in myotubes expressing minidystrophin. We propose that dystrophin could regulate sarcolemmal calcium channels likely through linkage with Alpha-syntrophin, but also on intracellular calcium channels behaviour.

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P01-07

CHANGES IN SR CA-ATPASE EXPRESSION AND CYCLOPIAZONIC ACID SENSITIVITY IN EDL MUSCLE FROM MDX MICE

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Duchenne muscular dystrophy (DMD) results from the lack of dystrophin, a cytoskeletal protein associated with the inner surface membrane in skeletal muscle. Although increased sarcolemmal Ca^{2+} influx in dystrophic muscle is proposed as an early event in DMD pathogenesis, Ca^{2+} handling mechanisms are not clearly understood. In this study, we investigated the sarcoplasmic reticulum (SR) properties in fast- (edl) and slow- (soleus) twitch muscles from 4-week-old control and mdx (C57BL/10mdx) mice, an animal model for DMD. The results show that in saponin skinned fibres, where the SR was functional, the Ca^{2+} uptake was slower in mdx muscles while the maximal Ca^{2+} loading capacity was maintained. In both types of mdx muscles, the time to load the SR was significantly increased but this was more pronounced in soleus fibres. Cyclopiazonic acid (CPA), an inhibitor of the SR Ca^{2+}-ATPase, induces a decrease in the Ca^{2+} uptake and the CPA sensitivity was decreased by 50% in mdx edl skinned fibres (control: IC50=10.1±1.7 µM CPA, mdx: IC50=20.2±1.7 µM CPA; n=8). In SR vesicles, the Ca^{2+}-ATPase activity and CPA sensitivity was not affected by the dystrophic process in both types of muscles. The expression of the slow Ca^{2+}-ATPase isoform (SERCA2a) at the mRNA and protein level was significantly increased in mdx edl muscle (SERCA1/SERCA2a mRNA: control=163.7±13.4, mdx=74.0±16.2; n=3). The expression of SERCA1 and SERCA2a was not modified in mdx soleus. The results show that the SR is involved in the abnormal Ca^{2+} homeostasis in both types of skeletal muscles. In mdx soleus muscle, the increase in SR Ca^{2+} loading time was not related to the Ca^{2+}-ATPase function and expression, and then could be explained by an increase of the SR Ca^{2+} leakage. In mdx edl muscle, the decrease in CPA sensitivity in skinned fibres could be explained by the presence of the slow Ca^{2+}-ATPase isoform. Then some SR properties of fast mdx muscles are similar to those observed in slow-twitch muscles.

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P01-08

EXTRACELLULAR ATP-EVOKED CALCIUM FLUXES ON CULTURED MOUSE SKELETAL MUSCLE CELLS

Gönczi M., Szappanos H., Cseri J., Kovács L., Csernoch L.

Changes in intracellular calcium concentration ($[Ca^{2+}]i$) were measured on cultured skeletal muscle cells from mice following the application of extracellular ATP. Established methods were used to determine the calcium flux, entering the myoplasmic space, from the measured changes in $[Ca^{2+}]i$ in order to assess the contribution of different sources of calcium in the formation of the ATP-evoked calcium transient. The resting $[Ca^{2+}]i$ decreased, from 99±4 (n=64) to 51±2 nM (n=104), while the transport capacity of the Ca-ATPase increased, from 107±10 to 596±36 µM/s, with differentiation. The calcium flux, evoked by 30-40 s long application of ATP, displayed an early peak (74±9 µM/s, n=35; in cells with less than 5 nuclei) and then declined to a quasi-maintained steady level (Sl; 17±3 µM/s). The peak was strongly reduced on depolarised cells and was completely missing on large myotubes. The removal of external calcium on the other hand, suppressed the quasi-steady level as well. Suramin, in a concentration of 10 µM, reduced Sl by 40±7% (n=7), while 300 µM suramin produced an almost complete block. Immunofluorescent labeling revealed the presence of both P2X and P2Y receptors in the surface membrane of these cells. The results demonstrate that extracellular ATP elevates $[Ca^{2+}]i$ through four, interconnected mechanisms: an influx through P2X receptors and voltage gated calcium channels, the release of calcium from intracellular stores through calcium-induced calcium release and IP3-sensitive channels.

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P01-09

FLUORESCENT IMAGING STUDIES OF NO PRODUCTION IN PANCREATIC ACINAR CELLS

Chvanov M., Gerasimenko O., Petersen O.H., Tepikin A.

We have analysed the synthesis of nitric oxide (NO) in acutely isolated pancreatic acinar cells using the fluorescent probes – membrane-permeable 4,5-diaminoflurescein diacetate (DAF-2 DA) and impermeable 3-amino-4-(N-methylamino)-2',7'-difluorofluorescein (DAF-FM). Application of 10 µM acetylcholine (ACh) caused a rise in fluorescence in only 21 out of 107 cells loaded with DAF-2 DA. Much higher proportion of cells (14 of 20) displayed fluorescence changes to ACh application when the membrane-impermeable form has been used. In these experiments the indicator was delivered into the cytosol via patch pipette. The recordings of calcium-dependent chloride currents allowed us to correlate Ca^{2+} signals with changes of DAF-FM fluorescence. The DAF-FM responses were also seen when cells were stimulated with physiological (5 pM) and supramaximal (10 nM) doses of cholecystokinin (CCK), as well as at low doses (50 nM) of ACh. The DAF-FM responses to secretagogues were abolished by 300 µM melatonin and 300 µM carboxy-PTIO but remained intact in the presence of 300 µM uric acid, indicating specific detection of NO by DAF-FM. Addition 10 mM of calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) to patch pipette completely eliminated DAF-FM responses triggered by the secretagogues. These results suggest that pancreatic acinar cells produce NO during physiological activity and when stimulated with pathological doses of secretagogues.

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P01-10

CHARACTERIZATION OF CALCIUM RELEASE EVENTS IN DYSTROPHIN DEFICIENT CELL LINES FROM SKELETAL MUSCLE

Balghi H., Sebille S. Cantereau A., Monory A., Tanguy S., Constantin B., Raymond G., Cognard C.

Skeletal muscle depolarization induces a massive release of stored calcium from the sarcoplasmic reticulum (SR) through the ryanodine receptors (RyR). Previous data suggest that an elevation in myoplasmic IP3 may be a secondary triggering signal for SR Ca^{2+} release during muscle activation. At rest, localized quantal Ca^{2+} release events (sparks) have been shown using laser scanning confocal fluorescence microscopy. Alterations of Ca^{2+} homeostasis are involved in Duchenne muscular dystrophy characterized by a lack of the dystrophin protein. The link between the absence of dystrophin and the Ca^{2+} mishandling remains unclear. Furthermore, there are only few data concerning a possible role of Ca^{2+} stored in the SR. The present study aims to characterize various events of Ca^{2+} release in a dystrophin deficient cell line (SolC1) and in SolD7, a stable dystrophin forced-expression derivative clone. Both spontaneous sparks and global Ca^{2+} release induced by perfusion of hyperpotassium (47 mM) depolarizing solutions were recorded. Using confocal microscopy, measurements of Ca^{2+} signals have been performed in myotubes loaded with the Ca^{2+} probe Fluo-4. SolC1 myotubes showed an intense sparks activity though SolD7 exhibited either low activity or not. During KCl perfusion, SolC1 myotubes show a slow kinetic both for Ca^{2+} increase and recovery. Although SolD7 myotubes displayed a recovery kinetic similar to SolC1 myotubes one, they showed a faster kinetic of Ca^{2+} release. Myotubes incubated with 2-APB (an inhibitor of IP3 receptors) showed a faster recovery kinetic than the control myotubes. These data suggest that IP3 could play a substantial role in Ca^{2+} release from SR in dystrophic cells. The characterization of both the expression and the organization of the calcium release channels (IP3 receptors) involved in this pathway has to be investigate more precisely in dystrophin-deficient models.

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P01-11

NAADP ACTIVATES A Ca^{2+} CURRENT WHICH IS DEPENDENT ON F-ACTIN CYTOSKELETON

Moccia F., Lim D., Nusco G.A., Ercolano E., Santella L.

NAADP is involved in the Ca^{2+} response observed at fertilization in the oocytes of several species, including starfish. In this study, we have employed Ca^{2+} imaging and the single-electrode voltage-clamp technique to investigate whether the NAADP-mediated Ca^{2+} entry discovered in our laboratory in starfish oocytes was mediated by a membrane current and whether the response to NAADP required an intact cytoskeleton. Uncaging of pre-injected NAADP evoked a cortical Ca^{2+} flash which was followed by the spreading of the wave to the remainder of the cell. No Ca^{2+} increase was detected in Ca^{2+}-free sea water. Under voltage-clamp conditions, the photoliberation of NAADP activated an inward rectifying membrane current, which reversed at potentials more positive than +50 mV and was abolished by removal of Ca^{2+}, but not of Na^{+}. The current was affected by pre-incubation with verapamil, SK&F 96356 and thapsigargin, but not by pre-injection of heparin, 8-NH2-cADPr, or both antagonists. The membrane current and the Ca^{2+} wave were inhibited by latrunculin A and jasplakinolide, which depolymerize and stabilize actin cytoskeleton, respectively. These data offer the first demonstration that NAADP initiates a Ca^{2+} sweep by activating a Ca^{2+}-permeable membrane current, which requires an intact F-actin cytoskeleton as other Ca^{2+}-permeable currents, such as ICRAC and IARC.

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P01-12

LIPOXINE A4 STIMULATES A CALCIUM MOBILIZATION IN HUMAN AIRWAY EPITHELIAL CELL

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Lipoxins (LX) are biologically active eicosanoids possessing anti-inflammatory properties. Using a calcium imaging system we investigated the effect of LXA4 on intracellular [Ca^{2+}] of human bronchial epithelial cell. LXA4 produced a dose-dependent increase in [Ca^{2+}]i followed by a recovery to basal values in primary culture of bronchial epithelial cell and in 16HBE14o- cells. The LXA4-induced [Ca^{2+}]i increase was completely abolished by pertussis toxin (G protein inhibitor). The [Ca^{2+}]i response was not affected by the removal of external [Ca^{2+}] but inhibited by thapsigargin (Ca^{2+}-ATPase inhibitor) treatment. Pre-treatment of the bronchial epithelial cells with either MDL hydrochloride (adenylate cyclase inhibitor) or Rp-cAMP (cAMP dependent protein kinase inhibitor) inhibited the Ca^{2+} response to LXA4. However the response was not affected by chelerytrine chloride (protein kinase C inhbitor) or montelukast (cysteinyl leukotriene receptor antagonist). The lipoxin A4 receptor mRNA was detected, by RT-PCR, in human bronchial epithelium. The functional consequence of the LXA4 effect on [Ca^{2+}]i have been investigated on Cl^{-} secretion, measured using the short-circuit techniques on 16HBE14o- monolayers grown on permeable filters. LXA4 produced a sustained stimulation of the Cl^{-} secretion through 16HBE14o- monolayers, which was inhibited by BAPTA-AM, a chelator of Ca^{2+}i. Taken together our results provided evidence for the stimulation of a [Ca^{2+}]i increase by LXA4 through a mechanism involving its specific receptor and protein kinase A activation and resulting in a subsequent Ca^{2+}-dependent Cl^{-} secretion by human airway epithelial cells.

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P01-13

THE ROLE OF DEFECTIVE MITOCHONDRIA IN REGULATION OF Ca^{2+} INFLUX INTO OSTEOSARCOMA CELLS.

Szczepanowska J., Zablocki K., Duszynski J.

In non-excitable cells, the depletion of intracellular calcium stores localized in the lumen of endoplasmic reticulum leads to the opening of plasma membrane calcium channels termed SOC and finally, to an activation of Ca^{2+} influx into the cells. This regulatory phenomenon is also known as a capacitative Ca^{2+} entry. To explain the molecular mechanism of capacitative Ca^{2+} entry several hypotheses have been employed. One of them postulates that mitochondria can play an important role in the regulation of SOCs. In our study we examined a few osteosarcoma cell lines with mutated mitochondrial DNA (mtDNA). MtDNA contains 13 genes coding polypeptides required for the mitochondrial respiration and oxidative phosphorylation. In our investigations we used cells with different levels of heteroplasmy (mtDNA point mutation ATP6 gene, encoding subunit 6 of mitochondrial ATPase) and cells lacking mtDNA (without complete respiratory chain and ATPase). It has been shown that SOC activity was not reduced despite the impairment of mitochondrial energy status resulted from the mutations in mtDNA.
Moreover, we have examined the effect of thapsigargin-induced depletion of calcium stores localized in the ER and the CCCP – discharge of the mitochondrial electrochemical proton gradient on the mitochondrial and cytoskeletal organization and mitochondrial membrane potential in all the osteosarcoma cell lines. Confocal microscopy was used to visualize intracellular structures. Filamentous mitochondria were distributed along the cell body in the control cell line and in cells with only slight mitochondrial disorders. In the cells with low mitochondrial membrane potential due to high level of heteroplasmy as well as lack of mitochondrial mtDNA, mitochondria were small, and had round and cylindrical shape.
In conclusion, the results show that the regulation of capacitative calcium entry into osteosarcoma cells does not depend on the mitochondrial energy status.

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P01-14

EFFECTS OF CHOLINERGIC BLOCKADE AND APAMIN ON RABBIT JEJUNUM MOTILITY AND ADRENERGIC INHIBITION

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Our aim was to investigate the influence of the cholinergic system and apamin-sensitive Ca^{2+}-activated K^{+} channels on spontaneous contractions in rabbit jejunum and on the a1- and b-adrenoceptor-mediated inhibition. Jejunum segments were placed in baths containing Tyrode solution at 37°C, gassed with O_2/CO_2 and connected to an isotonic force transducer. Atropine (ATR) and tetrodotoxin (TDX) inhibited almost totally the spontaneous activity amplitude. Despite the presence of ATR or TDX, tissue contraction gradually recovered (within 5-10 min) to about 50% of baseline value; a second addition of ATR or TDX left the amplitude of the recovered contractions unchanged. Yet, after washout and a 45-min rest the contraction

amplitude returned to baseline values and a further exposure to ATR or TDX markedly reduced it. In preparations pre-stimulated for 10 min with ACh, ATR abolished the TDX-resistant recovered spontaneous activity. Adrenaline and phenylephrine caused inhibition of tissue motility both in naïve and ATR- (or TDX)-exposed tissues; washout caused a rebound increase in contraction amplitude. Isoproterenol (up to 2.8 x 10-7 M) produced no inhibitory response in naïve tissues, but it caused (at 7.0 x 10-8 M) inhibition of the recovered spontaneous activity in tissues exposed to ATR or TDX, which was not affected by apamin. In naïve tissues, apamin caused a rapid and persistent increase in the contraction amplitude and blocked the inhibition by adrenaline and phenylephrine. The apamin-induced amplitude increase correlated with the rebound responses to adrenaline or phenylephrine. These results indicate that spontaneous motility in rabbit jejunum is predominantly mediated by neuronal release of ACh and by some other unidentified neuronal activity. Released ACh inhibits myogenic activity and strongly antagonizes b-adrenoceptor-induced apamin-insensitive inhibition but leaves a1agonist-induced apamin-sensitive inhibition unchanged.

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P01-15

BRADYKININ INDUCES CHANGES IN $[Ca^{2+}]i$ IN EPITHELIAL NORMAL BREAST CELLS IN PRIMARY CULTURE

Greco S., Muscella A., Elia M.G., Cimaglia F., Storelli C., Marsigliante S.

The effects of bradykinin (BK) on intracellular calcium concentration ($[Ca^{2+}]i$) in epithelial normal breast cells in primary culture were evaluated by using Fura 2-loaded cells. BK induced an increase of $[Ca^{2+}]i$ in a dose-dependent manner, showing maximal effect at 1 mM. 1 mM BK induced $[Ca^{2+}]i$ increase, after a 10-15 sec delay, to a peak of 678±45 nM above resting level (96±11 nM), and a subsequent decay to 165±37 nM. Both preincubation with B2 BK receptor and phospholipase C inhibitors blunted the BK effect, while pre-treatment with B1 BK receptor inhibitor did not, showing that B2 receptor and phospholipid hydrolysis are involved in BK signalling. The source of $[Ca^{2+}]i$ increase evoked by BK may be due to two mechanisms: release of Ca^{2+} by the intracellular Ca^{2+} pools and/or entry of Ca^{2+} through membrane channels. In this view, we incubated breast cells in Ca^{2+}-free Krebs Ringer Hepes medium (KRH), without $CaCl_2$ before BK stimulation; we found that the $[Ca^{2+}]i$ increase was reduced by 42% respect to the control, cells incubated in KRH with Ca^{2+}, indicating that Ca^{2+} could entry through membrane channels. In addition, when 1.0 mM thapsigargin (TG), the inhibitor of the endoplasmic reticulum Ca^{2+} pumps, was used to discharge Ca^{2+} before BK treatment, BK increased the $[Ca^{2+}]i$ of only 50% with respect to the control, i.e. cells stimulated with BK only. This result indicates that the release of Ca^{2+} from TG-sensitive intracellular stores is involved in the BK-induced $[Ca^{2+}]i$ increase. The addition of 2 mM CaCl2 to cells previously treated with BK for 3.5 min in Ca^{2+}-free KRH medium, induced a Ca^{2+} entry with a net peak height of 567±28 nM, indicating that stores operated membrane Ca^{2+} channels (SOCs) are involved in $[Ca^{2+}]i$ increase. In conclusion, in this study we demonstrated that in normal breast, BK through a functional B2 receptor evokes changes in $[Ca^{2+}]i$ by opening Ca^{2+} membrane channels and emptying intracellular stores.

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P01-16

MITOCHONDRIAL CONTROL OF CALCIUM SIGNALLING IN MOUSE EGGS AND ZYGOTES.

Dumollard RPL., Duchen M., Carroll J.

Similar to numerous somatic cells that elicit calcium oscillations upon stimulation, mammalian eggs respond to sperm entry by long lasting calcium oscillations. These calcium signals up-regulate mitochondrial ATP production by stimulating oxygen consumption and increasing the mitochondrial NADH concentration. However, in eggs, the effect of calcium signals on mitochondrial physiology remains unknown.
We imaged NADH and flavoprotein autofluorescence of mouse eggs, the mitochondrial electrical potential, simultaneously with the cytosolic Ca^{2+} concentration ($[Ca^{2+}]c$). We manipulated mitochondrial oxidative phosphorylation by adding different substrates (Me-succinate, lactate, pyruvate, glucose) as well as perfusing inhibitors of the mitochondrial respiratory chain (Complexes I to V) onto mouse oocytes. Intracellular Ca^{2+} was manipulated by the addition of sperm or uncaging IP3. This experimental model is aimed at assessing potential roles for mitochondria in the regulation of the Ca^{2+} homeostasis in the oocyte and early embryo.
Perfusion of substrates and mitochondrial inhibitors perfusions revealed that mitochondria in eggs use the complex I of the respiratory chain (NADH-Ubiquinone oxidoreductase) to build up the electrical potential and synthesise ATP. Such synthesis of ATP by the mitochondria is necessary to maintain a low resting $[Ca^{2+}]c$ and to allow sperm-triggered Ca^{2+} oscillations.
Confocal imaging of live oocytes showed that the mature mouse egg possesses numerous aggregates of phosphorylating mitochondria often embedded in sheets of endoplasmic reticulum. Finally we observed oscillations of the redox state (albeit without any mitochondrial potential changes) that are dependant on sperm-triggered Ca^{2+} oscillations or an IP3-mediated Ca^{2+} signal.
Together our observations provide evidence that, functional interactions exist between ER and mitochondria to regulate the pattern of calcium oscillation seen at the onset of development of the mouse embryo.

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P01-17

THE N-TERMINAL DOMAIN OF THE INOSITOL TRISPHOSPHATE RECEPTOR IS A TARGET FOR THIMEROSAL

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The N-terminal domain (aa 1-225) of the type 1 inositol 1,4,5-trisphosphate (IP3) receptor (IP3R1) is considered as a suppressor of IP3 binding. In order to study the function of this domain we constructed a deletion mutant of mouse IP3R1 lacking those first 225 amino acids (D1-225) and expressed it in IP3R-knockout R23-11 B-lymphocytes. Although D1-225 was still able to bind IP3, it did not exhibit any measurable Ca^{2+}-release activity. The thiol-reactive agent thimerosal potentiated the IP3-induced Ca^{2+} release and IP3-binding activity of the wild type mouse IP3R1 expressed in the R23-11 cells, but the stimulation of the binding could not be detected in cells expressing D1-225, suggesting that critical cysteine residues are lacking. By a $45Ca^{2+}$ flux technique a bell-shape dependence of the IP3 induced Ca^{2+}-release on thimerosal is found, which was shifted to higher sensitivity in the presence of Ca^{2+}. Using GST-IP3 binding core (aa 226-604) affinity chromatography, we identified an interaction between aa 1-225 and aa 226-604 of the mouse IP3R1. This interaction was regulated by Ca^{2+}, strengthened by the addition of thimerosal and in the presence of this agent weakened by calmodulin and calcium-binding protein, whose binding sites are localized in the 1-225 region. The stimulatory effect of thimerosal for this interaction was mimicked by site-directed mutation of two well-conserved cysteine residues (C56A and C61A). Furthermore, GST-pull down experiments demonstrated a Ca^{2+}-dependent interaction between aa 1-225, aa 226-604 or aa 1-604 and the C-terminus. These data provide evidence that amino acids residues 1-225 play an important role in the transduction of the activating stimulus from the IP3-binding domain to the gate of the channel. The target sites of thimerosal are localized presumably in this domain with cysteine residues C56 and C61 as possible candidates.

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P01-18

REGULATION OF INOSITOL TRISPHOSPHATE RECEPTORS BY PROTEIN-PROTEIN INTERACTIONS AND PHOSPHORYLATION

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Most cell types express more than one type of inositol trisphosphate receptors (IP3Rs). Interestingly, these various IP3R isoforms can have different intracellular localizations. Moreover, their exact localization was recently shown to be dependent on the physiological state of the cell. The aim of this study was therefore to compare IP3R distribution in different cell types and to ascertain the mechanisms physiologically relevant for determining their localization. For this purpose, immunolocalizations experiments were supplemented by immunoprecipitation and phosphorylation analysis. In A7r5 smooth muscle cells, IP3R1 redistributed in a protein kinase C (PKC)-dependent and microtubule-dependent way by a mechanism most likely involving vesicle trafficking. IP3R3 however did not seem to redistribute. We therefore investigated IP3R localization in a number of cell lines that have IP3R3 as predominant isoform. In HeLa cells and K41 fibroblasts, both IP3R1 and IP3R3 display a homogenous distribution. In calreticulin-deficient K42 fibroblasts, no difference in subcellular

localization of IP3R1 and IP3R3 was observed compared to the wild type. In bronchial epithelial cells (16HBE14o-), IP3R3 clusters were observed in the perinuclear region. Immunoprecipitation experiments in A7r5 cells, which contain both IP3R1 and IP3R3, demonstrated no interaction between either IP3R isoform with cytoskeletal proteins such as zyxin, vinculin or tubulin, while talin immunoprecipitated with both IP3R1 and IP3R3. To further investigate the role of PKC in the redistribution process, we investigated in which conditions and to what degree IP3R1 and IP3R3 could be phosphorylated by PKC. Purified IP3R1 and IP3R3 were both phosphorylated in vitro by PKC. These results indicate that in various cell types, IP3R isoforms are regulated by protein-protein interactions and by phosphorylation, which may be determinants of their intracellular localizations and function.

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P01-19

STIMULATION OF P2Y2 RECEPTOR INDUCES PROLONGED ACTIVATION OF ERK BY PKC-EPSILON.
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Extracellular purine nucleotides elicit a diverse range of biological responses through binding to specific cell surface receptors. Recently, we showed that in PC-Cl3 cells, a rat thyroid cell line that retains most of the features of differentiated follicular thyroid cells, ATP and UTP elevated the $[Ca^{2+}]i$ through the Gaq-coupled P2Y2 receptor.
To further elucidate the intracellular signalling mechanisms, we examined the effects of UTP on mitogen-activated protein kinase MAPK and proliferation. By Western blot analysis with an anti-phospho-p42/p44 MAPK antibody, we demonstrated that UTP activates ERK1/ERK2 in a time- and dose-dependent manner. The phosphorylation reached maximal levels after 3 min and returned to baseline in 6 h. ATP-induced activation of ERK1/ERK2 is dependent on the dual-specificity kinase mitogen-activated protein kinase/ERK kinase (MEK). In addition, UTP-stimulated MAPK activation was blocked by the protein kinase C (PKC) inhibitors staurosporine but not by Gö 6976, a preferential inhibitor of calcium-dependent PKC isoforms. The involvement of PKC in the signal transduction pathways was further supported by the ability of UTP to induce translocation of PKC-epsilon. PKC- epsilon isoform was translocated by a 0.5 min UTP stimulation and returned to the cytosol after 15 min. In many cells, the extracellular signal-regulated kinase (ERK) cascade plays an important role in cellular proliferation. We evaluated the effects of UTP on PC-Cl3 cell proliferation by a) a spectrophotometric 3-(4,5-dimethylthazol-2-yl)–2,5-diphenyl-2H tetrasodium bromide (MTT) assay; b) direct cell count and c) total protein assay. PC-Cl3 cells were incubated with different concentrations of UTP (0.1, 1 and 10) for 24 and 48 hours. UTP had no effects on cellular proliferation and total cellular protein. In conclusion, UTP induced prolonged activation of ERK1/2 through PKC- epsilon without affecting cell proliferation.

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P01-20

ENDOPLASMIC RETICULUM MORPHOLOGY AND POLARITY OF Ca^{2+} SIGNALLING IN PANCREATIC ACINAR CELLS
Gerasimenko O.V., Gerasimenko J.V., Rizzuto R. R., Treiman M., Tepikin A. V., Petersen O. H.

Pancreatic acinar cells are highly polarised cells with a distinct secretory granule area; the mitochondria are positioned outside the secretory granule region. The basal part of the cell contains the nucleus and the highly developed endoplasmic reticulum. It has been shown that IP3-induced calcium release initiates in the secretory granule area, which contains very little ER. Using confocal and two-photon microscopy, we investigated the morphology of the ER in the secretory granule area of living pancreatic acinar cells. The positioning of the ER was compared with the distribution of other cellular organelles. We found, that although the main part of the ER is located in the basal part of the cells, there are strands of the ER in the secretory granule area. The strands of ER projecting into the granular region are connected with the main ER structures in the basal area of the cells. This is the first visualization of the ER strands in the secretory granule area in living pancreatic acinar cells. The density of the ER decreases abruptly at the apical/basal border. These data confirm our recent findings demonstrating the tunnel function of the ER, which allows high Ca^{2+} mobility in the ER lumen. Ca^{2+} is released from the ER terminals in the granular area and this Ca^{2+} releasable store is re-supplied from the main calcium store at the basal area of the cell by the ER tunnel function.

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P01-21

MEMBRANE IP3 RECEPTOR IS MEDIATOR OF THE SYNERGISM BETWEEN ATP AND ADENOSINE ON THE CILIARY BEAT
Barrera N., Torres S., Morales B., Villalon M.

ATP and adenosine induce a synergistic increase of the ciliary beat frequency (CBF) in cultured ciliated cells from hamster oviduct. To elucidate the mechanism involved in this interaction, we quantified the intermediaries of the ATP transduction pathways in the presence of adenosine. ATP activates the phospholipase C followed by IP3 receptor activation. Using the immunogold and electronic microscopy, the subcellular distribution of IP3 receptors types 1 and 3 in oviductal ciliated cells determined the presence of both receptors types in nucleus and reticulum endoplasmic, however only the type 3 was localized in plasma membrane. Using fluorescence spectroscopy, it was demonstrated that ATP or caged IP3 increased the intracellular Ca^{2+} free concentration ($[Ca^{2+}]i$), initially from intracellular reservoirs followed by a Ca^{2+} influx. Addition of adenosine or intermediaries of adenosine transduction pathways, such as 8 Br-cAMP (a cAMP permeable analogue) or protein kinase A (PKA) in the presence of ATP, induced a higher Ca^{2+} influx. Furthermore Ca^{2+} influx induced by caged IP3 was increased by the release of caged cAMP. Using the radioimmumoreceptor technique, it was observed a high correlation between the time course of the IP3 production and both sources of the $[Ca^{2+}]i$ increase. Using the patch clamp technique in whole cell recording, ATP triggered an entry of Ca^{2+} which is blocked by Xestospongin C, a IP3 receptor inhibitor. In the presence of adenosine, we observed a higher ATP dependent-Ca^{2+} current, which is diminished by H-89, a PKA blocker. Furthermore, in the inside-out configuration, IP3 and the catalytic PKA subunit triggered a higher Ca^{2+} current compare to IP3 alone. These results suggest that the synergism of CBF increase between ATP and adenosine depend on IP3 receptor membrane activation by ATP and the modulation of these receptors by PKA dependent adenosine activation. Supported by CONICYT and FONDECYT 2010120.

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P01-22

FUNCTION, BUT NOT LOCATION OF MITOCHONDRIA, IS CRITICAL TO SUSTAIN STORE-OPERATED Ca^{2+} INFLUX
Frieden M., Castelbou C., James D., Martinou J.C., Demaurex N.

Mitochondria modulate, propagate, and synchronize Ca^{2+} signals by taking up and releasing Ca^{2+} at key locations near Ca^{2+} release or influx channels. Functional mitochondria are required to sustain the activity of store-operated Ca^{2+} channels (SOC) at the plasma membrane, but it is not clear whether mitochondria act as local Ca^{2+} buffers to remove Ca^{2+}-dependent channel inhibition or release a diffusible messenger. The location of mitochondria relative to SOC channels is difficult to ascertain, as mitochondria are dynamic structures that form a tubular network constantly remodeled by fusion and fission reactions. To distinguish between local and global effects of mitochondria on SOC channels, we transiently transfected HeLa cells with hFis1, a protein that promotes mitochondria fission. hFis1 expression induced mitochondrial fragmentation within 4h, all mitochondria appearing as punctuate organelles clustered around the nucleus. Despite the dramatic morphological change, the mitochondrial membrane potential and pH as well as the amplitude of mitochondrial Ca^{2+} transients, measured with targeted ratiometric pericam, were not altered by hFis1 expression. However, upon Ca^{2+} readdition to histamine-stimulated cells hFis1-fragmented mitochondria took up Ca^{2+} with a significant delay, consistent with their increased distance from the cell membrane. The delayed transfer of Ca^{2+} was not due to reduced Ca^{2+} entry, as hFis1 did not affect the amplitude and kinetics of cytosolic Ca^{2+} changes upon Ca^{2+} readdition. Regardless of hFis1 expression and mitochondria location, disruption of mitochondrial potential with oligomycin/rotenone or CCCP reduced Ca^{2+} entry by ~40%. These observations indicate that mitochondria remain functional despite drastic alteration in their morphology. Sustained Ca^{2+} entry requires functional mitochondria but not the presence of mitochondria near membrane channels, indicating that mitochondria exert a global, rather than a local effect on SOC channels.

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P01-23

RECOMBINANT NEURAL AGRIN AFFECTS THE EXCITATION-CONTRACTION MACHINERY IN HUMAN MYOTUBES IN VITRO
Lorenzon P., Bandi E., Formaggio E., Jevsek M., Mars T., Fumagalli G., Grubic Z., Ruzzier F.

It is generally accepted that the neural isoform of agrin is a critical molecule for the acetylcholine receptor clustering and/or stabilisation at the endplate. More recently, it has been shown that recombinant neural agrin mimics the synaptogenic effect of motor neurons inducing microprocess formation in uninnervated myotubes. Taking into account other possible unexplored mechanisms of action, we tested if neural agrin could also be involved in the maturation of the excitation-contraction coupling mechanism. Videoimaging experiments were performed on human myotubes which were either: i) cocultured with foetal rat spinal cord explants; ii) aneurally cultured in medium containing purified recombinant chick neural agrin or iii) aneurally cultured in control medium (without agrin). The maturation of the excitation-contraction coupling mechanism was followed by measuring the percentage of cells exhibiting an intracellular calcium transient when depolarised in the absence of extracellular calcium. The percentage of cells characterised by a mature excitation-contraction coupling mechanism was similar in myotubes cocultured (63.59 ± 7.44%; n = 66) or treated with agrin (70.44 ± 5.52%; n = 40). However, this percentage was significantly lower (28.58 ± 10.09%; n = 68) in myotubes cultured in control medium. Our results suggest that, besides other effects, agrin might also be responsible for the motor neuron-controlled maturation of the excitation-contraction coupling machinery. The molecular details by which agrin induces such process remains to be identified.

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P01-24

IDENTIFICATION OF TRANSMEMBRANE Ca^{2+} INFLUX IN CHOLINERGIC Ca^{2+}-SIGNALLING IN SALIVARY ACINAR CELLS
Kruglikov I., Fedirko N., Voitenko N.

The functioning of exocrine cells is under strict parasympathetic control, but the information about Ach-induced intracellular Ca^{2+} release and transmembrane Ca^{2+} influx remains obscure. Thus, in the present study we investigated the pathways of acetylcholine (Ach) induced Ca^{2+} signalling in isolated rat salivary acini. Fluorescent calcium measurements were done using fura-2/AM. Application of 5mkM Ach evoked $[Ca^{2+}]i$ transients with the amplitude of 215+/-22 nM (n=59). Second Ach application produced $[Ca^{2+}]i$ transient with the amplitude of 74+/-5% (n=10) from initial Ach response with no subsequent desensitization. Due to Ach ability to be endogenously hydrolyzed by acetylholinesterases (AchE) we did additional control adding the AchE inhibitor neostigmine (1mM). Application of neostigmine together with Ach did not significantly change the amplitude of Ach-induced $[Ca^{2+}]i$ transients (95+/-3%, n=7), thus showing the absence of active AchEs in our preparation. To study the subsets of Ach receptors responsible for generation of $[Ca^{2+}]i$ transients we used potent muscarinic receptor antagonist atropine. Application of Ach in the presence of atropine (10 mM), gave rise to $[Ca^{2+}]i$ transients with the amplitude of 21+/-5% (n=9) from initial Ach response. Ach-induced $[Ca^{2+}]i$ transients after acini preincubation with thapsigargin (500 nM, 20 min) were reduced by 79+/-6% (n=8). Application of 1 mM benzohexonium and 50 mkM tubocurarin (inhibitors of n type AchRs) decreased the amplitude of Ach-response by 26+/-4% (n=7) and 32+/-6% (n=7) respectively. Application of nAchRs agonist cytisine (100 mkM) induced $[Ca^{2+}]i$ transients with the amplitude of 21+/-2% from initial Ach response, these responses were completely blocked by either benzohexonium or tubocurarin. Thus we conclude that transmembrane Ca^{2+} influx induced by nicotinic receptors activation is present in salivary acinar cells, though activation of mAchRs is the main source for Ca^{2+} elevation in the cytoplasm.

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P01-25

ROLE OF CALCINEURIN IN CHRONIC HYPOXIA-INDUCED HYPERTROPHIC RESPONSE OF RIGHT VENTRICLE
Koulmann N., Sanchez H., Letout A., Ventura-Clapier R., Bigard A.X.

Chronic hypoxia leads to pulmonary hypertension, then to right ventricle (RV) hypertrophy. This process is associated with an increased proportion of beta isoform of myosin heavy chains (bMHC). Calcineurin seems to be involved as an hypertrophic transducing factor in cardiac myocytes. This experiment was designed to study the effects of treatment with cyclosporin A (CsA), an inhibitor of calcineurin, on: 1) the RV hypertrophy related to prolonged exposure to hypoxia; 2) the expression of bMHC in RV and left ventricle (LV).Male Wistar rats were exposed to hypobaric hypoxia (500 hPa) for 3 weeks and treated either by CsA (H-CsA) or by placebo (H-P). Their morphological and contractile properties were compared to those of normoxic rats treated either by CsA at the same dose (N-CsA), or by placebo (N-P). The body weight of hypoxic rats was less than that of normoxic rats (-10%, $P<0.001$). CsA also induced a depressed growth rate ($P<0.001$ compared with P groups). The hypoxia-induced RV hypertrophy (+139%, $P<0.001$)) was prevented by CsA treatment, whereas the overexpression of bMHC (+33%, $P<0.001$) was similar in H-CsA group (non hypertrophic RV) and H-P group (hypertrophic RV). Hypoxia also induced a slight increase in the LV weight normalized to body weight (+16%, $P<0.001$). CsA treatment did not prevent this response but in contrast induced a subtle hypertrophic process in LV (+16%, +21% for N-CsA and H-CsA, respectively, $P<0.001$), likely because of its well-known systemic hypertension effect. An increased expression of bMHC was observed in hypertrophic LV (+18%, +28% and +38% in N-CsA, H-P and H-CsA group, respectively).Then calcineurin seems to be involved in the RV hypertrophy in response to hypoxia. An overexpression of bMHC occured in response to the increased workload, independently of the activation of calcineurin. Whether the hypertrophy observed in LV in response to CsA treatment was minimized by calcineurin-induced inhibition has not been examined in this study.

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P01-26

INTERACTIONS BETWEEN Ca AND MITOCHONDRIA IN NEURONAL AGEING
Xiong J., Toescu EC.

Normal brain ageing is associated with a degree of functional impairment of neuronal activity that might result in a decrease in memory and cognitive functions. The relationship between mitochondrial function and Ca^{2+} homeostasis was studied in the cerebellum by use of both brain slices and primary neuronal cultures. The main parameters of Ca^{2+} homeostasis were not different between young and old neurones with the notable exception of a prolonged rate of Ca^{2+} recovery following neuronal stimulation (either depolarization or glutamatergic). In addition, in aged preparations, significantly more neurones showed an early Ca^{2+} dysregulation, resulting in neuronal death. The use of simultaneous loading with Ca^{2+} and mitochondrial membrane potential-sensitive dyes showed that increases in cytosolic $[Ca^{2+}]i$ over a threshold value (400 nM) evoked a mitochondrial depolarization response. In the aged neurones the mitochondria had a significantly longer repolarization response and quantitative analysis showed a direct correlation between the delays in mitochondrial repolarization and $[Ca^{2+}]i$ recovery, indicating the causal relationship between the two parameters. Inhibition of the mitochondrial permeability transition pore had several protective effects: it enhanced the rate of mitochondrial repolarization and Ca^{2+} recovery and decreased the percentage of neurones showing early Ca^{2+} dysregulation. Western blot analysis of the expression of several members of the Bcl-2 family showed no difference between young and old cerebella. The present results show that the changes in Ca^{2+} homeostasis associated with ageing are mainly due to a metabolic dysfunction in which the mitochondrial impairment play an important role.

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P01-27

ACTIVATION OF L-TYPE CALCIUM CHANNELS BY VIP INDUCES PROLACTIN GENE EXPRESSION IN AVIAN PITUITARY

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Our previous work demonstrated that Ca^{2+} influx through voltage-gated L-type calcium channels mediated the stimulatory effects of Vasoactive Intestinal Peptide)VIP(on prolactin (PRL) gene expression and release in cultured turkey anterior pituitary cells. The objective of this study was to examine the possible involvement of protein kinase C (PKC) in mediating VIP-induced Ca^{2+} influx and the subsequent stimulation of PRL gene expression and release. The level of PRL gene expression was determined by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) of PRL-mRNA. The homologous radioimmunoassay (RIA) was used to measure the level of PRL secretion from cultured turkey anterior pituitary cells. The PKC inhibitor bisindolylmaleimide I (BI) significantly ($P<0.05$) reduced VIP-stimulated PRL mRNA levels. In contrast, incubating the cells with the PKC activator, phorbol-12-mysterate-13-acetate (PMA), resulted in a significant ($P<0.01$) increase in PRL mRNA levels and PRL release. The stimulatory effects of VIP and PMA were not additive when combined together. Finally, PKC involvement in Ca^{2+} influx-stimulated PRL expression and release induced by the L-type Ca^{2+} channel agonist Bay K-8644 was examined. The PKC inhibitors staurosporine (ST) and bisindolylmaleimide I (BI) did not reduce PRL mRNA levels stimulated by Bay K8644. However, PRL secretion stimulated by VIP or Bay K-8644 was significantly ($P<0.05$) reduced by PKC inhibitors. The results of this study show clearly that: 1) PKC plays a major role in mediating VIP induction of Ca^{2+} influx through the voltage-gated L-type Ca^{2+} channels in cultured turkey primary anterior pituitary cells, and 2) PKC-dependent signal transduction pathway contributes to VIP effects on PRL gene expression and PRL release in avian species. Supported by USDA grant # 00-02127.

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P01-28

VASORELAXANT EFFECT OF TOTAL FLAVONES FROM DENDRANTHEMA MORIFOLIUM ON RAT THORACIC AORTA

Jin HF., Shan QX., Jiang HD., Tu J., Xia Q.

Objective: To investigate the vasorelaxant effect of total flavones from the dendranthema morifolium (Ramat.) Tzvel. cv. Hangju (FDM) in rat aortic rings. Methods: The isolated thoracic aortic rings were mounted on the organ bath and the tension of the vessel was recorded. Results: FDM completely relaxed, in a concentration-dependent manner, the contractions induced by either phenylephrine or a high concentration of KCl (60 mmol/L) in endothelium-intact rat aorta. Mechanical removal of endothelium did not significantly modify the vasorelaxant effects of this FDM. In endothelium-denuded aortic rings depolarized by 60 mmol/L KCl, FDM inhibited Ca^{2+}-induced contraction. It also reduced the transient contraction elicited by phenylephrine in Ca^{2+}-free medium, but had no effect on active phorbol ester-induced contraction. Pretreatment of endothelium-denuded aorta with propranolol, a beta-adrenoceptor antagonist, significantly attenuated the relaxant effect of FDM. Conclusion: These results indicate that FDM induces an endothelium-independent relaxation in rat aortic rings. The mechanisms may include the activation of beta-adrenergic receptor, reduction in Ca^{2+} influx through the voltage-dependent and receptor-operated channels, and inhibition of intracellular Ca^{2+} release in the vascular smooth muscle cells.

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P01-29

EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON SALIVARY SECRETORY CELL Ca^{2+}-ATPASES

Fedirko N., Vats J., Voitenko N.

Ca^{2+} pumps regulate $[Ca^{2+}]i$ thus playing an important role in exocytosis of secretory cells. Diabetes is associated with changes in cellular Ca^{2+} homeostasis and functional disorders in effector's organs. Particularly, the patients with diabetes mellitus suffered with hypo-salivation but its cellular mechanism is unknown. We assume calcium-dependence of this disorder. Because of that in the present research we studied the influence of diabetes on the Ca^{2+}-ATPases of salivary cells. The study was done on isolated cells and microsomes. Inorganic phosphor (Pi) content was measured using Fiske-Subbarow method. Fluorescent calcium measurements were performed using fura 2/AM. Diabetes was induced by intraperitoneal injection of streptozotocin (80 mg/kg proportion). Animals were taken into experiments 6 weeks after. The glucose concentration was 5-9 and 12-28 mM for normal and diabetic animals respectively. We showed that under diabetic neuropathy the resting $[Ca^{2+}]i$ increases by 66%. This increase could be due to modified functioning of Ca^{2+} extruding systems. Next we demonstrated that under the diabetes kinetic properties of total Ca^{2+}-ATPase activity are changed: Pmax decreased by 70%, $v0$ – by 56% and t – by 67%. Diabetes decreased specific PMCA and SERCA activities by 16±7% and 40±9% respectively. The substrate affinity of PMCA and SERCA of salivary cell under diabetes was also modified: Pmax decreased on 37% and 67%; Km for ATP decreased by 85% and 41% for PMCA and SERCA respectively, Hill's coefficient for PMCA did not changed while for SERCA it increased by 26%. We suppose that under diabetes lowered activities of PMCA and SERCA are associated with decreased amount of active molecules and/or enzyme rotation. Decreased Km and Hill's coefficient under diabetes testify about the enhanced affinity of Ca^{2+},ATP-ases to the lowered cellular ATP concentration that could be a physiological protection from their suppressed activity. Supported by CRDF grant to NV.

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S2 NEW ASPECTS OF IONIC TRANSPORT (I) THE PFLUGERS ARCHIV SYMPOSIUM

ORAL SESSION

S2-1

EPITHELIAL SODIUM CHANNELS: LESSONS FORM HUMAN DISEASES AND MOUSE MODELS

Rossier B.C.

According to the hypothesis put forward by Guyton, over 20 years ago , control of blood pressure at steady state and on a long-term basis is critically dependent on renal mechanisms. A number of genes expressed in various parts of the nephron have been shown to be directly involved in the control of blood pressure. The identification of mutations in monogenic diseases such as the Bartter's or the Gitelman's syndromes clearly indicate that defects in ion transporters expressed in the thick ascending limb (TAL) or in the distal convoluted tubule (DCT) may lead to a severe salt-loosing syndrome with a hypotensive phenotype. In the Aldosterone-Sensitive Distal Nephron (ASDN) i.e late distal convoluted tubule (late DCT), the connecting tubule (CNT), the cortical collecting duct (CCD) and, to some extent, the outer medullary collecting duct (OMCD) and inner medullary collecting duct (IMCD), the final control of sodium reabsorption is achieved through an amiloride-sensitive electrogenic sodium reabsorption which is under tight hormonal control, aldosterone playing the key role. The main limiting factor in sodium reabsorption in this part of the nephron is the apically located amiloride-sensitive epithelial sodium channel (ENaC). Two monogenic diseases have been linked to ENaC subunit genes; first, pseudohypoaldosteronism Type 1, a severe autosomal recessive form of a salt-loosing syndrome is due to loss (or partial loss) of function mutations in the a, b or g subunit genes of ENaC. Gain of function mutations in the b or g subunit of ENaC lead to a hypertensive phenotype (Liddle syndrome), a paradigm for salt-sensitive hypertension.
In this presentation, I will discuss conditional gene targeting experiments that offer new opportunities to study in vivo ENaC function in the collecting duct.

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S2-2

THE FAMILY OF EPITHELIAL CALCIUM CHANNELS

Bindels R.J.

The recent expression cloning of the epithelial calcium channels, TRPV5 and TRPV6 has provided a molecular basis to explore the characteristics of the rate-limiting entry step in transcellular calcium (re)absorption. These channels are primarily expressed in the distal part of the nephron, proximal small intestine and placenta, organs that play a key role in calcium homeostasis of the body. These channels of about 730 amino acids contain 6 putative membrane-spanning domains with an additional hydrophobic stretch predicted to be the pore region. TRPV5/6 resemble the recently cloned capsaicin receptor and the transient receptor potential-related ion channels with respect to its predicted topology. In kidney, TRPV5/6 are abundantly present in the apical membrane of calcium transporting cells and colocalize with 1,25-dihydroxyvitamin D3-dependent calbindin-D28K, sodium-calcium exchanger and plasma calcium ATPase. Several studies in animal models demonstrated that TRPV5/6 expression in kidney and intestine is positively controlled by the important calciotropic hormone, 1,25-dihydroxyvitamin D3. TRPV5/6 expression in eukaryotic cells confers calcium influx with properties identical to those observed in native distal renal cells including a high calcium selectivity and negative feedback regulation to prevent calcium overload during transepithelial transport. TRPV5/6 are co-expressed in several tissues forming homo- and heterotetrameric channel complexes with distinct channel properties. Consequently, regulation of the relative expression levels of TRPV5/6 may be a mechanism to fine-tune the calcium transport kinetics in TRPV5/6-expressing tissues. The S100A10 and annexin complex is an important regulator determining the plasma membrane localization of these channels. In conclusion, TRPV5 and TRPV6 constitute a new family of calcium channels with the expected properties for being the gatekeepers of 1,25-dihydroxyvitamin D3-dependent active calcium (re)absorption.

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OC02-1

INVOLVEMENT OF AN ANION EXCHANGER IN REGULATORY VOLUME DECREASE (RVD).

Borgese F., Gabillat N., Guizouarn H.

Trout erythrocytes possess multiple swelling-sensitive transport pathways: a KCl cotransport and an osmolyte channel permeable to diverse solutes (taurine, Na^+ and K^+Cl independent). This channel of broad specificity is activated by a decrease in intracellular ionic strength independently of the magnitude of cell swelling. The anion exchanger AE1 (also termed band 3) is a major constituent of erythrocyte plasma membrane. The particular sensitivity of the swelling-sensitive osmolyte channel to a wide range of drugs known as potent inhibitors of band 3 protein has prompted the suggestion that AE1 might be involved in volume regulation.
Indeed, when expressed in Xenopus oocytes, the trout red blood cell anion exchanger (tAE1) elicits, as expected, an anion exchange activity. But simultaneously tAE1 expression results in the appearance of both an anion conductance and a transport of taurine and cations. tAE1 forms an organic osmolyte channel of broad specificity, having a significant cation permeability. These permeabilities are expected if tAE1 serves as a route for volume regulatory efflux of osmolytes. In contrast, the homologous AE1 from mammalian erythrocytes are devoid of such volume-regulatory functions.
To define the structural domains involved in induction of the channel activity, chimeras have been done between trout and mouse AE1. Results have shown that only the spanning domain of tAE1 is linked to the channel activity and more precisely helices 6, 7, 12 and 13 are required for this function.
It remains to be shown whether other mammalian AE (e.g. AE2 and AE3) expressed in cell types regulating their volume, also possess a channel activity that may be involved in volume regulation.

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OC02-2

STRUCTURAL DOMAINS INVOLVED IN SUBSTRATE SELECTIVITY IN TWO NEUTRAL AMINO ACIDS TRANSPORTERS

Soragna A., Valli E., Castagna M., Mari S., Giovannardi S., Bossi E., Peres A.

Two high homologous Na^+/Cl^-dependent neutral amino acid transporters: KAAT1 and CAATCH1 cloned from the midgut epithelium of the larva Manduca sexta are useful tools to study protein domains involved in substrate selectivity. The ability of the two proteins to transport different amino acids depends on the cotransported ion, on pH and on the membrane voltage. Each organic substrate gives rise to transport-associated currents with its own characteristics, which are notably distinct between the two proteins. Differences in amplitude, kinetics and voltage-dependence of the transport-associated currents have been observed especially in the presence of the amino acids leucine, methionine, threonine and proline. These diversities were used to investigate the structural determinants involved in the substrate selectivity. To identify these protein regions, four chimera proteins between the two transporters were built.The high homology let us to exchange different fragments of the protein without introducing mutations. The chimera proteins obtained, heterologously expressed in Xenopus laevis oocytes were analysed by two-electrode voltage clamp and uptake measurements.The proteins where the first three domains were exchanged, C3K9 and K3C9 show electrophysiological characteristics and uptake of [H3]leucine and [H3]proline of KAAT1 and CAATCH1 respectively. These first results show that the transmembrane domains (TMs) 1-3 in KAAT and CAATCH are not involved in organic substrate selectivity. Consequently the substitution of the last four domains in C3K9 and K3C9 giving the proteins C3K5C4 and K3C5K4 shows again that these proteins have the same behaviour of KAAT1 and CAATCH1 in electrophysiological and transporter determination. We can conclude that in KAAT1 and CAATCH1 only the central TMs(from 4 to 8)of the protein is responsible of the substrate selectivity.

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OC02-3

DIACYLGLYCEROL DIRECTLY ACTIVATES A NON-SELECTIVE CATION CHANNEL IN DEDIFFERENTIATED CARDIOMYOCYTES
Guinamard R., Lenfant J., Bois P.

In the adult rat cardiomyocytes culture, proposed as an in vitro myocardial hypertrophy model, we have recently characterized a rise of the density of a calcium-activated non-selective cation channel (NSCCa) during dedifferentiation. The channel was selective for Na^+ and K^+ and impermeable for Ca^{2+} ions. It had a conductance of 20 pS in the inside-out configuration and was activated by rise in internal Ca^{2+}. A pre-stimulation by ATPgS or by a phorbol ester increased the channel detection, suggesting that PKC is involved in the regulation of NSCCa channels.
Here we reported the regulation of the channel by DAG analogues and PKC. In cell-attached configuration, it had a conductance of 20.2 pS and a reversal potential of +24 mV (n=7) (pipette and bath, 140 mM NaCl). Application of the permeable DAG analogue OAG (0.1 mM) or the PKC activator PMA (500 nM) increased the open probability (Po) from 0.06 to 0.55 (n=4) and from 0.05 to 0.46 (n=4) respectively. In the presence of the PKC inhibitor Calphostin C (0.001 mM), OAG still had an activating effect while PMA had no effect. In inside-out configuration, DAG analogues OAG (0.1 mM) or SAG (0.01 mM) applied to the inside of the membrane increased Po from 0.10 to 0.59 (n=6) and from 0.07 to 0.65 (n=9), respectively. We infer that the NSCCa channel is under the control of DAG via the PKC pathway but also via a direct interaction.
In models of hypertrophy it was shown that DAG contents and PKC activity increase during hypertrophy, that would increase channel activity. Thus the NSCCa channel is a candidate for the genesis of arrhythmias in ventricular cells. In addition, this new regulation of the channel by DAG and PKC could help to understand the physiological role of the NSCCa channels family.

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OC02-4

20-HETE INOTROPIC EFFECTS INVOLVE THE ACTIVATION OF NON-SELECTIVE CATIONIC CURRENT IN ASM
Rousseau E., Cloutier M., Campbell S., Basora N., Proteau S., Payet M.D.

Eicosanoids are important lipid mediators. 20-hydroxyeicosatetraenoic acid (20-HETE) controls several mechanisms such as vasoactivity, mitogenicity and ion transport in various tissues. Our goal was to quantify the effects of 20-HETE on the tone and electrophysiological properties of airway smooth muscle (ASM). Isometric tension measurements, performed on guinea pig ASM, showed that 20-HETE induced a dose-dependent inotropic effect, with an EC50 value of 1.5 µM and a Hill coefficient of 0.77. The sustained contraction, requiring Ca^{2+} entry, was partially blocked by 100 µM Gd^{3+} and 1 µM nifedipine, revealing the involvement of non-capacitative Ca^{2+} entry and L-type Ca^{2+} channels, respectively. Microelectrode measurements showed that 3 µM 20-HETE depolarized the membrane potential in guinea pig ASM by 13 ± 2 mV (n = 9). Depolarizing effects were observed in absence of epithelium as well as in the presence of OAG (a PKC and TRP channel activator). Patch clamp recordings demonstrated that 1 µM 20-HETE activated a non-selective cationic inward current which might be support by the activation of TRP channels. The presence of the TRPC mRNA was confirmed by RT-PCR in guinea pig ASM cell. Together our results suggest that an eicosanoid, such as 20 HETE, might activate a non-selective cationic current generated by a member of the TRP channel-receptor family. Supported by the CIHR

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S2-3

TRPV CHANNELS: STRUCTURE – FUNCTION RELATIONSHIP AND PROMISCUOUS GATING BEHAVIOUR
Nilius B.

Calcium signals control a plethora of short- and long-term cell functions. In most non-excitable cells, sustained entry of extracellular calcium upon various stimuli essentially contributes to those Ca^{2+} signals. Molecular candidates for this entry are cation channels of the “transient receptor potential” (TRP) superfamily. Activation of TRP channels, consisting of three subfamilies (TRPC, TRPV, TRPM), is still very little understood. Examples of activation of TRP channels from all three subfamilies will be discussed. Main focus is on the members of the TRPV subfamily, among which the TRPV4 channel shows a surprising gating promiscuity. It can be activated by cell swelling, heat, or phorbol esters. Endogenous activators of the channel have not yet been described. It will be shown that arachidonic acid (AA) is a robust activator of TRPV4 which may also explain activation of TRPV4 by the endocannaboids anandamide and 2- arachidonyl glycerol which likely requires metabolisation to AA. Lipid messengers downstream of arachidonic acid might act as endogenous TRPV4 activators. For TRPV5 and 6, the only highly Ca^{2+} - selective channels within the TRP super-family, a voltage dependent gating mechanism will be discussed, which includes an open pore block by Mg^{2+} and a highly Ca^{2+} - sensitive mechanism of inactivation. Regulation of channel availability by interaction with a protein bound to the C-terminus of both channels will be demonstrated. Functional consequences of these different mechanisms of gating will be discussed.

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S2-4

PHYSIOLOGY AND PATHOPHYSIOLOGY OF CHLORIDE TRANSPORT
Jentsch T.J.

Mouse models and human genetic diseases have recently shed considerable light on the function of CLC chloride channels and KCC K-Cl-cotransporters. This talk will focus on three pathologies, all affecting the kidney and two of them the inner ear.
ClC-5 is an endosomal chloride channel that is essential for the acidification of proximal tubular endosomes by providing an electrical shunt for the proton pump. Its disruption leads to a defect in endocytosis. This leads to secondary changes in calciotropic hormones (PTH and VitD) which eventually lead to kidney stones in Dent's disease.
ClC-K channels, by contrast, are plasma membrane channels involved in transepithelial transport. They need barttin, a small beta-subunit, for their transport to the surface. Mutations in ClC-Kb lead to Bartter syndrome type III and in barttin to Bartter syndrome IV that also includes deafness. The importance of these channels in ion transport in the kidney and the stria vascularis of the cochlea will be discussed.
KCC4 is an electroneutral K-Cl cotransporter that is e.g. expressed in renal proximal tubules and intercalated cells, and, in Deiter's cells that support outer hair cells in the inner ear. Its disruption in mice leads to deafness that is associated with renal tubular acidosis. This pathology will be compared to that of the Bartter syndrome type IV.

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S2-5

MODULATION OF ION CHANNELS BY ESTROGENS
Valverde M.A.

Estrogen and antiestrogens are capable of rapid modulation of Maxi Cl^- and Maxi K^+ channels in vascular smooth muscle cells (Valverde et al, 1999; Díaz et al. 1999). The mechanism of action leading to the modulation of these channels seems to be different. Modulation of Maxi K^+ involves a direct interaction between the hormone and the channel complex, as well the participation of second messengers. Rapid modulation of vascular smooth muscle ion channels by estrogens leads to endothelium-dependent and independent vasodilatation. A key player in the control of vascular smooth muscle tone is the Maxi-K channel. This channel consists of two subunits: a pore forming a subunit and a regulatory b subunit which confers the channel with a higher Ca^{2+} sensitivity. We have recently described the modulation by 17b-estradiol of both native and heterologously expressed Maxi-K channels and found that oestradiol activates the channels through its interaction with the b subunit (Valverde et al. 1999).
Maxi Cl^- channels have been recorded in many different cell types. We have described their modulation by estrogens and antiestrogens in vascular smooth muscle and neuroblastoma cells (Diaz et al. 1999, 2001), a process that requires the generation of intracellular signals, although its relevance to cell physiology remains unknown.
Our results suggest that estrogen and antiestrogens exert different rapid actions on the same cell type, an observation that fits the current view of multiple sites of action for estrogens (Nadal et al. 2001).
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POSTER SESSION

P02-02

EXHALED NITRIC OXIDE AS A MARKER OF ION TRANSPORT IMPAIRMENT IN CYSTIC FIBROSIS PATIENTS
Texereau J., Fajac I., Hubert D., Dusser D., Bienvenu T., Dall Ava-Santucci J., Dinh-Xuan A.

Because production of nitric oxide – a key molecule which regulates many important physiological functions of the airways – is reduced in cystic fibrosis, a condition characterized by defective ion transport, we aimed to test the hypothesis whether reduced nitric oxide production might affect airway ion transport in vivo in cystic fibrosis patients.
Pulmonary function, nasal potential difference and exhaled nitric oxide were measured in sixty adults with cystic fibrosis. A slope of lung function decline was determined retrospectively for each patient using simple linear regression with all available spirometric values obtained over a time period of five years that preceded patient's entry in the study.
The annual rates of decline in forced expiratory volume in one-second were directly correlated to abnormal nasal potential difference values ($P<0.05$). The latters were inversely related to exhaled nitric oxide concentrations ($P<0.01$). Cystic fibrosis patients with normal nasal potential difference had higher exhaled nitric oxide concentrations (20.1 ± 2.6 parts per billion) than healthy controls (12.1 ± 0.8 parts per billion, $P<0.01$) whose exhaled nitric oxide concentrations were significantly higher than those of cystic fibrosis patients with abnormal nasal potential difference (8.6 ± 0.5 parts per billion, $P<0.01$).
These data suggest that exhaled nitric oxide is related to nasal transepithelial potential difference and that nitric oxide could play a compensatory role on defective cystic fibrosis transmembrane conductance regulator protein activity.

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P02-03

ANION SELECTIVITY AND GATING OF TORPEDO CLC-0 CHLORIDE CHANNEL
Bennetts B., Roberts M., Bretag A., Rychkov G.

Members of the ClC family are ubiquitous, Cl specific channels that are expressed in both outer membranes and the membranes of intracellular organelles. The muscle-type ClC channels ClC-0 and ClC-1 are activated by membrane depolarisation, allowing Cl to enter the cell and repolarise the membrane. Activity of these channels is sensitive to Cl concentration in the external solution, and so they have been referred to as Cl activated Cl channels.
In the current experiments a series of anions was used to probe the permeation pathway and gating of ClC-0. Equilibrium selectivity for various anions, determined from reversal potential measurements corresponded to a moderately strong field site in the pore, and was similar to ClC-1. The selectivity of the site that regulates activity of the channel appeared to be different in the open and closed states, such that in the closed state the regulatory site specifically bound Cl in preference to larger anions such as ClO3 and ClO4, but in the open state these ions could block Cl conductance by binding to the regulatory site. Selectivity sequence and the relative ability of different anions to affect fast gating determined in the present study implied a smaller size of the pore and smaller dimensions of the regulatory Cl binding site in ClC-0 compared to ClC-1. Linear free energy relationships analysis suggested that the conformational changes at the regulatory site during open-closed transitions coincided with voltage sensation by the channel.

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P02-04

SODIUM AND CALCIUM CURRENTS IN CHICK EMBRYO DEVELOPING TYPE I AND TYPE II HAIR CELLS
Bosica M., Zucca G., Valli P., Masetto S.

By using the whole-cell patch-clamp technique in combination with the chick embryo crista slice preparation, we have recorded inward ionic currents from type I and type II hair cells in situ, at different stages of development. To block outward K^+ currents, KCl in the pipette solution was substituted by NMDG and CsCl. Voltage-clamp experiments showed that a large fraction of type I and type II hair cells express a sodium current (INa),

from embryonic day 14 (E14) up to hatching (E21). INa activated around –60 mV, peaked around –20 mV, displayed fast activation and inactivation, and was completely, and reversibly, blocked by tetrodotoxin (TTX; Kd = 3 nM). A peculiar property of INa concerned its steady-state inactivation, in that it was complete at –60 mV (V1/2 = -96 mV).
A very small sustained inward current was also present at voltages less negative than –60 mV, from the first developmental stage investigated (E10). This current was not blocked by TTX, whereas it was completely abolished by Cd^{++} 100 µm, which also blocked INa. The sustained inward current was increased by perfusing an extracellular solution containing Ba^{++} instead of Ca^{++}. INa conversely was reduced by Ba^{++} perfusion. IBa was present in all hair cells investigated. It activated around –60 mV and peaked around –20 mV. It showed rapid activation and little inactivation. Its time- and voltage-dependent properties appeared similar in type I and type II hair cells. This current has been identified as a Ba^{++} current flowing through voltage-dependent Ca channels.

Ca channels are involved in afferent synaptic release from the basal pole of the hair cells. Present results suggest a similar operational range for Ca channels in type I and type II hair cells. Na channels on the other side could reinforce, at least in a subpopulation of hair cells, membrane depolarization and thus boost synaptic output.

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P02-05

A NOVEL VOLTAGE-DEPENDENT CHLORIDE CURRENT ACTIVATED BY EXTRACELLULAR ACIDIC pH IN RAT SERTOLI CELLS

Auzanneau C., Thoreau V., Norez C., Becq F.

Sertoli cells from mammalian testis are involved in development and maintenance of spermatogenesis, support and nourishment of germ cells and synthesis and release of several proteins and a potassium-rich fluid into the lumen of seminiferous tubules. Sertoli cells express a variety of ionic channels among them voltage-dependent Ca^{2+} and calcium-dependent Cl^- channels. Using whole-cell patch clamp experiments and iodide efflux, a novel chloride current was identified. It is activated only in the presence of an extracellular acidic-pH with an estimated half maximal activation at pH 5.5. The current is strongly outwardly rectifying, activated with a fast time-dependent onset of activation but a slow time-dependent kinetic at depolarization pulses. The pH-activated chloride current was not detected at physiological or basic pH, and is not sensitive to intracellular nor extracellular Ca^{2+} variation. The pharmacology of this channel has been established by iodide efflux. Its anionic selectivity was $Cl^->Br^->I^->$gluconate. We have performed an RT-PCR analysis to search for voltage-dependent chloride rCLC channels in cultured rat Sertoli cells. Among the nine members of the family only rClC-2, rClC-3, rClC-6 and rClC-7 have been identified. The inwardly rectifying rClC-2 chloride current was activated by hyperpolarization but not by pH variation. A different depolarization-activated outwardly rectifying chloride current was activated only by hypotonic challenge and may corresponds either to rClC-3 or rClC-6. Immunolocalization experiments demonstrate that rClC-7 resides in intracellular compartment of Sertoli cells. This study provides the first functional identification of a native acid-activated chloride current. Based on our molecular analysis of rClC proteins, this new chloride current does not corresponds to rClC-2, rClC-3, rClC-6 nor rClC-7 channels.
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P02-06

NASAL POTENTIAL DIFFERENCE MEASUREMENT IN CYSTIC FIBROSIS AND ELECTRONIC DATA ACQUISITION

Ergonul Z., Yilmaz G., Balkanci ZD., Kiper N., Yalcin E., Dogru D., Ozcelik U., Gocmen A.

Objectives: Cystic fibrosis (CF) patients demonstrate more a negative potential difference on respiratory epithelia than normal controls. The CF gene product, cystic fibrosis transmembrane conductance regulator (CFTR), is a chloride channel that also acts as a regulator of heterologous ionic channels. Abnormalities of ion transport in respiratory epithelia of patients are associated with enhanced sodium absorption and defective cAMP mediated chloride secretion, which contributes to the dehydration of airway secretions. Transepithelial nasal potential difference (NPD) measurement has been used as a diagnostic test for CF. NPD measurement techniques commonly vary between centers. It has been shown that there are large differences in reproducibility of measurements between different study sites. In order to standardize measurement protocols, voltmeter input impedance standardizations and electronic data acquisition have been suggested.
Methods: In our study NPD was measured using an adaptation of the method described by Alton which involves an epicutaneous reference electrode and intranasal placement of an exploring electrode with a Foley catheter. The DA100B differential amplifier module of MP100 computer based data acquisition and analysis system replaced a high impedance voltmeter. Our study included 40 CF patients (18 females, mean age 9.3 yrs, range 2-20 yrs) and 37 controls (17 females, mean age, 17.08 yrs and range 2-34 yrs).
Results: The CF group NPD was significantly higher (mean ± SEM, -39.21 ± 1.74 mV) than that of controls (-18.24 ± 1.48 mV, $P< 0.00001$). No significant difference was noted between left and right sides in all groups. Neither the age nor sex of the subject influenced the measurements.
Conclusion: Our results are consistent with the published data. We suggest that MP-100 system provides suitable monitoring and recording during measurements, which could eliminate the bias due to different voltmeters and hand analysis.

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P02-07

SINGLE-CHANNEL Cl^- CURRENTS IN INSIDE-OUT PATCHES FROM BROWN FAT CELL MEMBRANES

Sabanov V., Cannon B., Nedergaard J.

The function of brown adipose tissue is sympathetically regulated heat generation. Norepinephrine induces in brown adipocytes a very fast and dramatic activation of metabolism, which is accompanied by complex electrical perturbations in the plasma membrane. The very first component of this electrical response has been shown to be due to Cl^- efflux. The present investigation was designed to explore the Cl^- permeability of the membrane at the single-channel level. Earlier we described Cl^- channel currents in the inside-out configuration that can be promoted by strong depolarisation of the excised patches, although could not be observed in the cell-attached mode (Sabanov & Nedergaard, 1995, BBRC, pp.639-647). They have relatively unstable amplitude and reveal multiple closed and open states and burst/gap behaviour. In an effort to characterize the channels functionally we examined their calcium dependence. According to their behaviour in Ca2-free solutions containing Ca-chelator (EGTA or BAPTA), the currents (channels?) can be divided into two groups. The average current amplitudes and, correspondingly, mean single-channel conductivities in these groups are different. The activity of the “large” (~59 pS) channels was completely blocked by Ca-free solutions, whereas in the “small” channels (~38 pS) a more specific rearrangement occurred. The possible explanation is that in either case both EGTA/BAPTA and Ca^{2+} can inhibit the channel activity. Therefore, in the Ca-free solutions these two factors – presence of chelator and absence of Ca, counteract. In the large channels the blocking effect of the chelator dominated over the stimulating effect of calcium absence; in the second group these two effects were much more equal and mask each other. On the bases of all-point amplitude histogram analysis a double–barrelled structure for both the “large” and “small” channels can be suggested.

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P02-08

PROPERTIES OF A CHLORIDE CHANNEL AT THE BASOLATERAL MEMBRANE OF THE MOUSE CONNECTING TUBULE

Nissant A., Teulon J.

We investigated the properties of basolateral Cl^- channels on microdissected connecting tubules (CNT) isolated from collagenase-treated mouse kidneys, using the cell-attached and excised configurations of the patch-clamp technique. The bath solution contained (in mM): 140 NaCl, 5 KCl, 1 MgCl2, 1 CaCl2, 10 glucose, 10 HEPES (pH 7.4). The pipette solution was similar except for NaCl (145 mM) and KCl (no KCl included).
In the cell-attached configuration, we recorded one channel, which had a linear i/v relationship with a unit conductance of 10.5 +/- 1.2 pS (n = 6, means +/- SEM) and a reversal potential (Er) close to zero (0.8 +/- 5.3 mV). Upon excision, it was possible to assess the anionic selectivity of the channel despite frequent channel rundown by changing the solution on the intracellular side. With a bath NaCl concentration of 14 mM, conductance

and Er were 9.8 +/- 0.3 pS and -41.3 +/- 2.6 mV (n = 6), respectively. The PNa/ PCl ratio was 0.08 +/- 0.02 (n = 6). We also investigated the relative permeabilities for halides and nitrate. We obtained relative permeabilities of 0.44 +/- 0.07 (n = 5) for Br-, 0.56 +/- 0.09 (n = 5) for NO_3^-, 0.77 +/- 0.08 (n = 3) for I- and 0.17 +/- 0.04 (n = 6) for F-. Thus the channel had the relative permeability sequence $Cl^- \sim I^- > Br^- \sim NO_3^- > F^-$.
The properties of the channel described here are similar to those of a Cl^- channel that we have previously described in the basolateral membrane of the mouse DCT.

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P02-09

NEUROHYPOPHYSIAL HORMONE REGULATION OF Cl^- SECRETION IN CULTURED GILL CELLS:PRESENCE OF V1 RECEPTORS.
Avella M. (1)., Guibbolini M.E. (2)

Neurohypophysial hormone receptors were studied in primary cultures of sea bass gill respiratory-like cells grown on permeable supports.
Under control conditions, the cultured monolayered epithelium had a short-circuit current (Isc) of 3.5±1.1µAxcm-2. This current had previously been identified as an active Cl^- secretion. Addition of increasing concentrations of the fish neurohypophysial hormones, arginine vasotocin (AVT) or isotocin (IT), elicited a concentration-dependent stimulation of the Isc. Maximal increases of 61±12% and 118±28% above the basal Isc value were obtained for 10-7M AVT and IT, respectively. Half-maximal effects were obtained for 3.1x10-9M AVT and for 1.4x10-9M IT. Mucosal application of 1mM DPC (a specific blocker of Cl^- channels) revealed a correlation with a hormone-dependent Cl^- transport.
Specific V1 or V2 analogues of vasopressin (mammalian hormone) were used to characterize pharmacologically the type of neurohypophysial hormone receptors. While the V1 agonist stimulated the basal Cl^- secretion with a similar profile to that of AVT or IT, the V2 agonist had no effect. The V1 antagonist used at a concentration of 5x10-7M totally reversed the 10-8M AVT-stimulated Cl^- secretion, whereas the V2 antagonist used at the same concentration had no significant effect. In contrast, similar experiments carried out in the presence of 10-8M IT showed that both antagonists significantly reduced the IT-stimulated Cl^- secretion, with an efficiency of the V1 antagonist significantly greater than that of the V2.
This study provides evidence for a neurohypophysial hormone control of Cl^- secretion in fish cultured gill respiratory cells. It suggests on physiological basis that the hormonal effect is shared by the two peptides present in fish neurohypophysis, acting by means of two distinct, although pharmacologically similar, V1-type receptors. These specific receptors are expected to play an important role in controlling ion homeostasis in seawater fish

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P02-10

VOLTAGE GATED ION CHANNELS IN TRANSDUCTION AND ADAPTATION IN CRAYFISH STRETCH RECEPTOR.
Rydqvist B., Swerup.C., Sand P.

The crayfish stretch receptor, an analogue to the human muscle spindle, is a classical model for the study of transduction in a mechanoreceptor. Analysis has shown that viscoelastic properties and mechano-gated channels are important determinants of transduction. However, voltage gated ion channels permeable to Na^+ and K^+ also contribute to the overall response of these sensory receptors and in particular to explain the difference in adaptive behaviour of the slowly and rapidly adapting neurone. To further investigate the transduction of these receptors we have studied the different ion channels present in these neurones and the possible spatial distribution of the voltage gated ion channels. Two electrode voltage clamp and patch clamp experiments in the stretch receptor neurones of the crayfish (Pacifastacus leniusculus) have demonstrated that the Na^+ channels are differently distributed in the two neurones. In the slowly adapting neurone the Na^+ channels seems to be present in both axon and soma whereas in the rapidly adapting neurone the Na^+ channels are present in the axon only. Three different types of K^+ channels are present in the neurones. One of them have been characterized as an outward rectifying channel of type Kv1.2. Two other potassium channels are of the transient type. Mathematical modelling also confirms that small changes in activation inactivation properties of the Na^+ and K^+ channels and spatial distribution result in considerable difference in adaptive properties.
It is concluded that the main voltage gated ion channels have a decisive importance for the adaptive properties of these neurones when stimulated both mechanically and electrically.

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P02-11

PROTEIN KINASE A AND PROTEINE KINASE C MODULATION OF NAV1.7 AND NAV1.8 NERVE SODIUM CHANNELS.
Vijayaragavan K., Chahine M.

Voltage-gated sodium channels (VGSC) are transmembrane proteins essential for initiation and propagation of action potentials in neuronal excitability.
Dorsal root ganglion specific VGSC Nav1.7 and Nav1.8, were expressed in Xenopus oocytes and the effects of protein kinase activation on
the Na^+ currents were studied using the two-electrode voltage clamp method. Our data show that PKC and PKA differentially regulate these channels.
A dose-dependent attenuation of Na^+ current is observed when phorbol esters are applied to both channels, Nav1.8 being more sensitive.
In addition, a 6mV shift of only the voltage-dependence of activation towards more depolarized potentials is observed for Nav1.7.
However, no shift of steady-state gating is observed for Nav1.8. The Nav1.8 decrease in peak current can be inhibited with epsilon PKC antagonist, while epsilon PKC and betaII PKC both seen to modulate the PMA induced effect on Nav1.7. PKA activation instead results in a dose-dependent increase in Nav1.8 Na^+ current
but decrease in Nav1.7 Na^+ current with no shifts in voltage-dependence of gating.
The PKA-mediated rise of Nav1.8 Na^+ current is inhibited by chloroquin that affects vesicular trafficking. This suggests that during nerve injury, increased PKA activity could enhance Nav1.8 trafficking to the synaptic membrane surface, which may cause C-fiber hyperexcitability.
However the functional consequence of these channels would depend on the fine balance between the activated PKA and PKC isozymes.

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P02-12

L 703,606 AS MODULATOR OF ELECTROGENIC IONIC TRANSPORT IN COLON
Mlodzik N., Lelinska A., Kaczorowski P., Tyrakowski T.

The C-fiber endings in the colon influence local physiological functions of the intestine by releasing of sensory peptides such as substance P, NKA and NKB.
In this study the effects of L 703,606 (the NK-1 antagonist) transepithelial potential difference were examined.
The experimental model was an isolated colon wall mounted in Ussing apparatus. The mechanical stimulation of C-fiber endings was by gentle rinsing of mucosal surface of the colon by jet-flux from peristaltic pump. The 35 specimens of isolated colonic walls from 11 rabbits were investigated. Every significant reaction was repeated at least ten times.
After mechanical stimulation the hyperpolarization of the tissue was noticed. In the presence of chloride transport inhibitor - bumetanide L 703,606 in the concentration of 10-7 M and 10-6 M were able to augment electrogenic ion current and in the concentration of 10–5 M were able to diminished the electrogenic ion current.
These electrophysiological data evidenced that colonic receptors for tachykinins influence electrogenic ion transport differently: augmenting it in smaller concentration and diminishing it in higher concentration. The hypothetical mechanism for opposite effects of the different concentrations of drug is the presence of the two different populations of tachykinin receptors in colon - autoreceptors on C-fiber ending (responsible for the augmentation of the reaction) and epithelial receptors (responsible for its diminution).

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P02-13

A NEW EXPRESSION SYSTEM FOR PANCREATIC Na^+/Ca^{2+} EXCHANGER NCX1.3 AND NCX1.7

Hansen MR., Amstrup J., Novak I.

Activity of the Na^+/Ca^{2+} exchanger (NCX) in rat pancreatic ducts is regulated by pancreatic secretagogues secretin, acetylcholine, insulin and ATP. The studies were carried out on intact rat pancreatic ducts, which express the NCX splice variants NCX1.3 and NCX1.7. Therefore regulation of Na^+/Ca^{2+} exchange can be due to any of these variants. In order to separately study NCX1.3 and NCX1.7, we require a model system with cell lines expressing those individually. The aim was therefore to generate plasmid constructs with rat pancreatic NCX1.3 and NCX1.7 cDNA.
Using an RT-PCR based approach on rat heart and pancreatic RNA, six different constructs with hybrid NCX1.3 and NCX1.7 cDNA coupled to Enhanced Green/Blue Fluorescent Protein (NCX1.3-EGFP, EGFP-NCX1.3, EBFP-NCX1.3, NCX1.7-EGFP, EGFP-NCX1.7 and EBFP-NCX1.7) were generated. The differential tagging allows us to assess the effect of tagging the N- or C-terminal part of the protein and to visualize co-localization of NCX1.3 and NCX1.7 chimeras in transfected cells. The constructs were successfully expressed in HEK293 cells. Expression of all six constructs and targeting of the NCX1.3-GFP chimera to the plasma membrane was verified with western blotting and confocal laser scanning microscopy, respectively. RT-PCR was used to check for expression of NCX isoforms, purinergic receptors and Ca^{2+} -binding proteins in model cell lines HEK293 and Capan-1 to compare the background of the cell lines with the known expression in native pancreas. Using the above mentioned constructs expressed in HEK293 and Capan-1 we are now carrying out experiments using fluorescent optical techniques to study the function and regulation of the pancreatic Na^+/Ca^{2+} exchangers. Thus, a new expression system useful for physiological studies on the function and regulation of pancreatic Na^+/Ca^{2+} exchange has been established.

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P02-14

P2X RECEPTOR MEDIATED INTRACELLULAR SIGNALLING

Amstrup J., Novak I.

Nucleotides are extracellular agonists of specific purinergic P2 receptor subtypes. The P2 receptors are well characterized by pharmacological and electrophysiological methods. However, relatively little is known about their intracellular signalling pathways, especially those via P2X type receptors that are widely expressed in exocrine glands. The coexistence of different types of P2 receptors, together with a general lack of selective agonists and antagonists, has made advances in P2 receptor characterization and P2 mediated signal transduction difficult using native tissues and physiological methods. The aim of the present study was to set up a model system using heterologous expression of the P2X4 receptor in HEK293 cells and study the signal transduction pathway(s).
Inserting cDNA coding for the P2X4 receptor into vectors containing a green fluorescent protein (GFP) at either the N- or C-termini of the receptor enabled us to detect the receptors by Western blot and by use of confocal laser scanning microscopy (CLSM).

CLSM studies showed that the GFP-P2X4 chimera was targeted to the plasma membranes in HEK293 cells. Stimulation of transfected HEK293 cells with 30 □M ATP mediated an activation of p44/42 MAP kinases. Further, we investigated which parts of the P2X4 receptor were involved in this activation by making constructs lacking part of either the intracellular C- or N-terminals. The constructs lacking a part of the intracellular N-terminal were still able to mediate an ATP activation of the p44/42 MAP kinases. In contrast, the construct missing the C-terminal part of the P2X4 receptor, were not able to mediate activation of p44/42 MAP kinases. Furthermore, an ATP-mediated tyrosine phosphorylation of the P2X4 receptor was detected. In conclusion, these experiments suggest that the C-terminal part of the P2X4 receptor is involved in ATP-induced activation of p44/42 MAP kinases, and that the receptor is tyrosine phosphorylated in response to ATP.

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P02-15

STIMULATION OF Na-K-2Cl CO-TRANSPORTER BY MOLECULAR INTERACTION WITH AE1 IN XENOPUS OOCYTE.

Guizouarn H., Gabillat N., Borgese F.

Regulation of membrane permeability could be achieved by direct modifications on transporters (as phosphorylations) or by interactions between transporters. These interactions could be functional (electric coupling, thermodynamic coupling or autocrine mechanism...) or they could also involve direct contacts between proteins.
An example of membrane transporters regulation by protein interactions that are not related to transport functions is given by the trout anion exchanger, tAE1, and the Na-K-2Cl co-transporter.
Xenopus oocyte expressing tAE1 for one day exhibits a strong Na and Cl-dependent Rb influx that is mediated by the endogenous Na-K-2Cl co-transporter. Other members of the AE1 family (skate or mouse AE1) were not able to stimulate the co-transporter. Our data previously showed that tAE1 but not other AE1, induced a Cl channel in oocyte. It was possible to specifically inhibit with glybenclamide the tAE1 anion channel without affecting stimulation of the Na-K-2Cl co-transporter. Measurements of intracellular Na^+, K^+ and Cl^- concentrations in control or tAE1 expressing oocytes could ruled out involvement of ion content modifications in co-transporter activation. Moreover, activation of the Na-K-2Cl co-transporter by tAE1 expression was abolished by alteration of the C-terminal end of tAE1. These alterations were obtained either by reaction with a specific antibody raised against the last amino-acids of tAE1 or by fusion of gyrase B to the carboxy terminal end of tAE1. By the use of chimeric AE1, it was possible to conclude that tAE1 stimulation of the co-transporter is not linked to tAE1 conductive properties but rather to interaction between the C-terminal end of tAE1 and the Na-K-2Cl co-transporter.
This interaction observed in tAE1 expressing oocytes could take place in physiological conditions to coordinate activity of different transporters and regulate trout erythrocyte membrane permeability.

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P02-16

INHIBITION OF THE MUCIN PRODUCTION BY ANION AND V-ATP-ASES BLOCKERS IN THE NCI-H292 CELL LINE

Chénafi O., Bogliolo S., Renard C., Bernard K., Ehrenfeld J.

Mucus overproduction is an important feature of airway diseases including cystic fibrosis, chronic obstructive pulmonary disease or asthma. Recently, a calcium-activated chloride channel, hCLCA1, was proposed to be responsible, in part, for the overproduction of mucus in asthmatic subjects. These preliminary findings suggest the inhibition of hCLCA1 may be an important new therapeutic approach to control mucus overproduction in chronic airway disorders. Up to now, the functional role of Cl^- channels in this process is unknown. We postulated that acidification of secretory mucin granules implicates the function of Cl^- channels associated with V-type H^+-ATPase and used NCI-H292, a cell line derived from a human pulmonary mucoepidermoid carcinoma, as a model of mucin secretion. An immunoassay of the MUC5AC protein was used to evaluate the effects of known anion channel and proton V-ATPase inhibitors on the mucin production in these cells. The involvement of K channels also present in secretory granules was also investigated.
Epidermal grow factor (EGF) application induced a three fold increase in the mucin synthesis and in the mucin secretion. Simultaneous application of EGF with one of classical anion channel inhibitors (NPPB, NFA, DIDS, DPC or glybenclamide) resulted in an inhibition of mucin synthesis without significant effect on mucin secretion. Charybdotoxin and clotrimazole, two KCa (SK4) channel inhibitors or chromanol 293B, a KcAMP channel blocker were ineffective on mucin production. The H^+ pump inhibitors, DCCD and oligomycin as the more specific V-ATPase blocker bafilomycin considerably reduced the EGF-stimulated mucin synthesis and totally abolished the mucin secretion. hCLCA2 as hCLCA4 mRNAs were detected by RT-PCR but not hCLCA1 transcripts. We conclude to a key role of central vacuolar V-ATPase in the process of mucin synthesis and secretion. Cl^- channels participate to the first event but their molecular identification remains to be precised.

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P02-17

PARACELLULAR REGULATION OF ION ABSORPTION IN NATURAL AIRWAY EPITHELIUM

Frederiksen O., Poulsen A.N., Willumsen N.J., Pedersen P.S.

Fast regulation of the depth of upper airway apical surface fluid layer (ASL) is accomplished by regulation of NaCl absorption in the airway surface epithelium. Natural airway epithelium is leaky and Na^+ absorption through ENaC channels is accompanied by passive Cl^- absorption through an anion selective paracellular pathway. In the present study we investigated the relative importance of regulation of cellular and paracellular pathways for the downregulation of NaCl absorption by luminal ATP/UTP.
Short circuit current (ISC), epithelial conductance (Gt), and tracer fluxes of Na^+, Cl^-, and mannitol were measured under short circuit conditions in native airway epithelium from the rabbit nasal septum mounted in Ussing chambers.
Mucosal nucleotides inhibited amiloride-sensitive Na^+ absorption but only slightly increased Cl^- secretion. From changes in Gt it was calculated that ATP/UTP caused a large decrease in paracellular conductance (GS) and in parallel passive (paracellular) Cl^- fluxes (but not passive Na^+ and mannitol fluxes) decreased. The effects of nucleotides were mimicked by ionomycin and pretreatment with ionomycin largely prevented the effects of ATP/UTP on ISC and Gt. Stimulation of cAMP by P1 (adenosine) receptor stimulation or by forskolin only slightly affected ISC but increased Gt to an extent that involved a substantial increase in GS.
The results suggest that ATP and UTP released to ASL exert an autocrine regulatory function on native airway epithelial ion transport, primarily by inhibiting net NaCl absorption, while stimulation of Cl^- secretion is of minor importance. This downregulation is caused by an activation of apical P2Y2 receptors leading to an increase in $[Ca^{2+}]i$ which inhibits apical ENaC channels and paracellular anion (Cl^-) permeability. The permeability of the anion-selective paracellular pathway is under dual and opposite control from $[Ca^{2+}]i$ and cAMP.

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P02-18

PANCREAS SECRETES PARTICULATE NUCLEOTIDASE CD39 – NEW ASPECTS OF PURINERGIC SIGNALLING

Novak I., Sørensen C.E., Amstrup J., Rasmussen H.N., Ankorina-Stark I., Møbjerg N.

Pancreatic acini release ATP and the excurrent ducts express several types of functional purinergic P2 receptors. Thereby, ATP might play a role as a paracrine regulator between acini and ducts. The aim of the present study was to elucidate whether this acinar-ductal signaling is regulated by nucleotidase/s, characterize and localize it within the rat pancreas.
In our studies we used physiological, biochemical and molecular biological methods to characterize nucleotidase activity in the rat pancreatic tissue and in pancreatic juice. RT-PCR and Western blotting revealed that the pancreas expresses the full length 78 kDa ecto-nucleoside triphosphate diphosphohydrolase, CD39. Immunofluorescence shows CD39 localization on basolateral membranes of acini, luminal membranes of small intercalated/interlobular ducts and basolateral membranes of larger ducts. Upon stimulation with CCK-8, acinar CD39 relocalized towards the luminal pole. Accordingly, pancreatic juice collected from intact pancreas stimulated with CCK-8 did not contain significant amounts of ATP, but nucleotidase activity, including that of CD39. Anti-CD39 antibodies detected a full length CD39 in pancreatic juice. This CD39 was only confined to the particulate and not the soluble fraction of the CCK-8 stimulated secretion. No CD39 activity was detected in the secretin-stimulated secretion. Electron microscopy shows that pancreas secretes microsomes that presumably contain the CD39 activity. The role of secreted CD39 would be to regulate intraluminal ATP concentrations within the ductal tree. The final product of ATP hydrolysis by CD39 and other nucleotidases is adenosine, and as we show by patch-clamp studies larger ducts possess adenosine receptors that regulate Cl channels. In conclusion, we show a novel inducible release of full length particulate CD39, and propose its role in physiological context of pancreatic secretion.

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P02-19

CHLORIDE EFFECTS ON THE FUNCTION OF THE GABA COTRANSPORTER RGAT1

Pisani R., Giovannardi S., Fesce R., Bossi E., Binda F., Peres A.

The effects of reducing external Cl^- on the electrophysiological properties of the Na^+/Cl^--dependent GABA transporter rGAT1 expressed in Xenopus oocytes were investigated. In agreement with a recently proposed kinetic scheme, the effects of Cl^- are complex but preserve the mutual relationship that links the transport-associated currents, Itr, measured in saturating GABA concentration, and the transient current Ipre, recorded in the absence of GABA following a voltage step from the holding potential Vh to V. In particular Itr(V)-Itr(Vh)= r[integral]Ipre(V)dt, where r is the relaxation rate of Ipre at the same membrane potential and Cl^- concentration. The model also predicts a relation between charge relaxation rate and apparent affinity for GABA, which is also verified in presence of lowered Na^+ or Cl^- concentrations. In these conditions the binding rate of GABA to the transporter is increased. All these effects are consistent with the hypothesis that interaction of the organic substrate with rGAT1 induces a conversion from a capacitive to a conductive mode of operation without strongly altering either the amount or the rate of charge movement.

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P02-20

NIFEDIPINE-ACTIVATED CATIONIC PERMEABILITY IN MDCK CELLS.

Melendez E. (*), Bidet M., Tauc M., Reyes J.L. (*), Poujeol P.

We have demonstrated that newborn rat distal cells express an apical Ca^{2+} channel that presents the characteristic to be activated by dihydropyridine drugs. With a similar approach (Fura2), we found that, in MDCK cells, nifedipine increases $Ca^{2+}i$ in a dose-dependent manner (IC50 = 4µM). The requirement of extracellular calcium was clearly established since this increase was abolished in EGTA containing solution. The Ca^{2+} channel antagonist isradipine as well as the agonist BayK8644 caused such an increase of $Ca^{2+}i$ indicating that this effect is related to the dihydropyridines as a substance class. Diltiazem (20µM) significantly inhibited the nifedipine effect (62% inhibition in calcium variation). Gadolinium (200µM) also had a significant inhibiting effect (43%). La^{3+} even at high concentrations (100 µM) was ineffective. Clamping membrane potential with valinomycin did not modify the nifedipine-induced $Ca^{2+}i$ increase, indicating that it was not related to potassium flux. Results obtained with fura2-loaded cells suggested that nifedipine activates an electrogenic mechanism. On that account, we performed whole cell clamp experiments. When MDCK cells were maintained at –50mV in a perfusion solution containing10 mM CaCl2, the addition of 20µM nifedipine induced an increase of the current (1.2±0.3nA) which, was inhibited by Gd^{3+}. No significative current was observed when nifedipine was added in the presence of 0.5 mM EGTA. To precise the effects of nifedipine on the membrane potential, we then performed oxonol fluorescence experiments. Addition of nifedipine or BayK8644 induced a depolarization, which was highly dependent of the presence of sodium in the medium. 20 µM of nifedipine induced a depolarization of 6.9±0.8 mV (n=21). Dose response curve with nifedipine gave an EC50 in the 10µM range. We conclude that MDCK cells exhibit a dihydropyridine-activated cationic channel. Experiments are now undertaken to precise the nature of this permeability.

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P02-21

THE CNS CATECHOLAMINERGIC CELL LINE CAD EXPRESSES TTX-SENSITIVE VOLTAGE-GATED SODIUM CHANNELS

Harvey V., Smith K., Garner C., McDonald R.L.

Voltage-gated sodium channels (VGSCs) play a central role in signal transmission in the central nervous system (CNS). Mutations of the alpha-subunits of neuronal VGSCs are implicated in several disorders including generalised epilepsy with febrile seizures (GEFS+) and familial autism. The molecular physiology of VGSCs in the catecholaminergic CNS cell line CAD has been investigated using the whole-cell patch-clamp technique and reverse transcription-polymerase chain reaction (RT-PCR).

Transient inward currents were evoked by 15ms step depolarisations from a holding potential of –60mV, following a 100ms hyperpolarising prepulse to –100mV. The transient inward current activated at -37 +/- 1mV, peaked at 0 +/- 1mV and measured 57.4 +/- 6.5pA/pF (n=13). The reversal potential (Erev), 49 +/- 2mV (n=13) was similar to the theoretical Erev for a Na^+ - selective conductance under these conditions. Bath perfusion with 300nM tetrodotoxin (TTX) reduced the transient inward current at all test potentials; where currents measured at 0mV were reduced from 46.4 +/- 3.0 to 1.73 +/- 0.3pA/pF ($p<0.001$; 96.4% +/- 0.5; n=5). Following the identification of VGSC current, the candidate subtypes were further investigated. Consequently, the expression of TTX-sensitive VGSC mRNA was examined using RT-PCR. Total RNA was isolated from CAD cells using the Promega SV Total RNA isolation system. Upstream and downstream oligonucleotides specific for the alpha-subunits encoding Nav1.1, 1.2, 1.3, 1.6 & 1.7, were designed to anneal in different exons. Using the Promega Access RT-PCR system, only the alpha-subunits encoding Nav1.2, 1.3, 1.6 & 1.7 transcripts of the correct size were detected.
These results show that CAD cells express functional TTX-sensitive VGSCs and may provide a unique tool for their further study.

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S3 ENVIRONMENTAL PHYSIOLOGY

ORAL SESSION

S3-1

TRANSPORT AND ENERGETICS OF EXTREMOPHILES

Konings W.N., Albers S.V., Koning S., Driessen A.J.M.

The ion permeability of cytoplasmic membranes play crucial roles in the bioenergetics of micro-organisms. The proton and sodium-ion permeabilities were measured in liposomes prepared from lipids isolated from psychrophilic, mesophilic, thermophilic and hyperthermophilic bacteria and archaea and from halophilic archaea. In all membranes the proton and sodium-ion permeabilities increased with temperature. Membranes from psychrophilic and mesophilic bacteria and from mesophilic,(hyper)thermophilic and halophilic archaea have similar proton permeabilities at their respective growth temperatures. These observations indicate that micro-organisms are capable of adjusting the lipid composition of their membranes in order to maintain the proton permeabilities constant (homo-proton permeability adaptation).
Thermophilic bacteria are an exception in this respect and are unable to maintain a constant proton permeability at their high growth temperatures. As a result their membranes are very leaky for protons and a significant proton motive force cannot be build up in these organisms. The sodium-ion permeabilities were found to be very low and similar in all micro-organisms studied. Thermophilic bacteria make use of this low sodium-ion permeabilities to generate a sodium motive force which is subsequently used as a driving force for energy-requiring membrane processes such as secondary solute uptake systems.
In thermophilic arcaea such as Pyrococcus furiosus and Sulfolobus solfataricus several binding-protein dependent ABC-transporters have been found to catalyze the transport of their carbon and energy sources. These binding proteins have high affinity for their substrates. Interestingly, P.furiosus possesses ABC transporters that catalyze the uptake of oligomers of maltose and cellobiose.

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S3-2

PHYSIOLOGICAL ADAPTATIONS OF ANIMALS TO CLIMATIC CHANGES AND LIMITATIONS IN RESOURCES

Le Maho Y.

Evidence is now accumulating, suggesting that the climate of past decades may be anomalous compared with earlier climatic variations. Numerous models moreover predict an increase in these climate anomalies, which may induce limitations in resources.

Investigating how animals may face climatic conditions is therefore an important issue. However, until recently, there was a severe limitation in our ability to get detailed information on free-ranging animals. Thanks to the prodigious progress in micro-electronics and computers it is now possible to obtain physiological and behavioural data of animals diving far at sea, wandering into the oceans, flying over deserts or mountains. Not only we are now able to get data on the biology of animals under natural conditions, but through ultra-miniaturized instruments animals deliver us detailed information on their environment.

This lecture will therefore provide examples of new discoveries in the quickly developing field of ecophysiology.

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OC03-1

DNA REPAIR CAPACITY OF IRRADIATED HUMAN LYMPHOCYTES EVALUATED BY COMET ASSAY

Cardile V., Renis M., Scifo C., Bellia M., Lombardo L., Perciavalle V.

DNA is frequently damaged by endogenous and environmental agents, which provoke cellular deleterious consequences. Cells, however, have evolved sophisticated systems in response to DNA damage which constitute crucial defence systems against cytotoxicity, mutagenesis and carcinogenesis induced by DNA damaging agents. Therefore, DNA repair is regarded as one of the essential events in all life forms. Ionizing radiation, interesting for its environmental and clinical implications, is a potent inducer of DNA damage because it causes single- and double-strand breaks, alkali-labile sites, base damage, and crosslinks. This study was aimed to determine whether the alkaline COMET assay (single cell gel electrophoresis) can be used to evaluate DNA repair after damage induced by ionizing radiation. Resting peripheral blood lymphocytes, isolated from fresh buffy coats of 8 healthy blood volunteers by means of Lymphoprep gradient centrifugation, were analysed after exposition to four different doses (0.5, 1.0, 1.5 or 2.0 Gy) of X-ray radiations, followed by incubation at 37°C in a mixture 5% CO_2/95% air, for 2, 4, 8, 24, 48 or 72 h. For each incubation time, exposed and control (unexposed) lymphocytes were scored by fluorescence microscopy using Scion Image software, and COMET tail length, percentage of fragmented DNA (TDNA), and tail moment, expressing the product of the tail/head and TDNA, were measured. The results indicated that irradiated cells, examined 2 or 4 h after the treatment, showed a dose-dependent increase of DNA single and double-strand breaks. DNA repair was observed in 1.5 and 2.0 Gy treated lymphocytes examined 8 and 24 h after the treatment, compared to controls. Both treated and untreated lymphocytes showed drastic DNA damage 48 and 72 h after the treatment, because of their known short life time. Our results confirm COMET assay as a rapid, simple and sensitive technique for visualizing and measuring DNA damage leading to strand breakage in individual cells.

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OC03-2

CARDIAC NATRIURETIC PEPTIDE AND URINE FLOW IN HYPOXIC TROUT

Tervonen V., Vuolteenaho O.*, Nikinmaa M.

When acutely exposed to hypoxia, vertebrates show a rapid hemoconcentration resulting from a decrease in plasma volume and release of erythrocytes from the spleen. Reduced plasma volume is at least partly due to increased renal water excretion, a common transient response to acute hypoxia in vertebrates. One of the potential endocrine mechanisms mediating the diuretic response in hypoxia is cardiac natriuretic peptides. These peptide hormones have potent diuretic and natriuretic effects and they play an important role in modulating intravascular volume homeostasis. To elucidate the role of cardiac natriuretic peptides in hypoxic diuretic response, we used rainbow trout (Oncorhynchus mykiss) and a recently cloned salmon cardiac natriuretic peptide (sCP) as a model. In aquatic environment large variations in oxygen tension may occur and thus fishes encounter hypoxia regularly. To study the effect of hypoxia on cardiac natriuretic peptide plasma levels and urine flow, adult freshwater trout, kept in 12 ºC, were cannulated in the dorsal and ventral aorta and in the urinary bladder. The trout were exposed to hypoxia (3 mg O_2/l) for three hours. The urine flow increased almost immediately in trout exposed to hypoxia and remained at an elevated level for the following two hours. Simultaneously, the plasma immunoreactive sCP (ir-sCP) concentration showed a significant increase in both the ventral and the dorsal aorta. Thus, in trout, an increased plasma ir-sCP level occurs as an immediate response to hypoxia and increase coincides with hypoxic diuretic response. Supported by the Academy of Finland.

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OC03-3

MICROARRAY ANALYSIS OF CIRCADIAN GENE EXPRESSION IN MOUSE LIVER

Lacoche S., Gréchez-Cassiau A., Teboul M., Azmi S., Laudet V., Taneja R., Delaunay F.

Circadian rhythms in physiology are observed in most living organisms from cyanobacteria to humans. These rhythms are generated by a self sustained endogenous clock that is reset by the light/dark cycle and which in turn regulates rhythmically downstream pathways. Biochemical and genetic studies have established that a small group of genes termed clock genes generates a molecular oscillator through a transcriptonnal/translational feedback loop mechanism. Circadian oscillators are present not only in the suprachiasmatic nuclei of the hypothalamus but also in most peripheral organs. To understand how peripheral circadian oscillators regulate rhythmic physiological processes we have analysed circadian gene expression in mouse liver using high density oligonucleotide microarrays. We have

identified ~ 250 rhythmic transcripts that regulate a wide variety of biological processes including metabolism, transcription, transport and signal transduction. Peaks of expression are found at all circadian times yet with a majority of transcripts peaking at dusk and dawn. Several transcriptonnal regulators have been identified suggesting that clock-controlled gene expression is mainly indirect in mammals. We show that the bHLH transcriptonal repressor Stra13 is rhythmically expressed in most peripheral tissues and that its promoter is regulated by the clock gene products CLOCK and BMAL. These data show that circadian gene expression is extensive in liver and suggest that Stra13 may be an important link between peripheral oscillators and physiological outputs.

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OC03-4

EFFECT OF AEROBIC TRAINING ASSOCIATED WITH HYPOXIC EXPOSITION DURING SLEEP ON ANTIOXIDANT CAPACITY
Pialoux V., Mounier R., Gueux E., Mazur A., Rayssiguier Y., Coudert J., Fellmann N.

The aim was to determine, on high-level nordic skiers, the impact of physical training associated with hypoxia during sleep on the antioxidant capacity evaluated by two direct methods and on the production of a lipoperoxidation (malondialdehyde, MDA) marker following an in vitro oxidation.
Eleven subjects were divided in 2 groups. The first group (H) (n=6) trained at low altitude (1,100m) and slept in normobaric hypoxic room during 3 weeks: simulating 2,500 m during the first week, 3,000 m the second week and 3,500 m the third week, and the second group (N) (n=5) were submitted to the same training but slept at 1,100 m of altitude. Venous blood samples were collected in pre-training state (I), immediately at the end of training session (II) and 2 weeks after (III).
Plasma Trolox equivalent antioxidant capacity (TEAC), ferric reactive antioxidant potential (FRAP) were analysed in I, II and III periods. The difference between MDA induced in vitro (MDA-i) and non-induced (MDA-ni), considered as an indirect evaluation of plasma antioxidant capacity, was measured in I and III. TEAC and FRAP were decreased by training (I vs II) in both groups (H=-21%, p=0.03, N=-13%, p=0.04 for TEAC and H=-20%, p=0.01, N=-17%, p=0.03 for FRAP). Only TEAC returned to its basal value in III whereas FRAP values remained significantly lower than in I. For each periods, the FRAP and TEAC values were highly correlated (r=0.85, r=0.63 and r = 0.80 for I, II and III respectively). The difference between MDA-i and MDA-ni were higher in III than in I (H=+70%, p=0.04 and N=+59%, p=0.006). No significant differences were found between H and N for each methods.
Regardless the methods used, an intense aerobic training was responsible for an antioxidant capacity decrease, which persisted after 2 weeks of recovery and the associated hypoxia did not worsen the deficiency.

This study was funded by the Olympic Committee and the "Direction Régionale de la Jeunesse et des Sports Auvergne" - France

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OC03-5

ROLE FOR Na^+/H^+ EXCHANGER 1 IN XENOBIOTIC-INDUCED APOPTOSIS IN LIVER EPITHELIAL CELLS
Huc L., Sparfel L., Rissel M., Fardel O., Lagadic-Gossmann D.

Polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene B(a)P, are ubiquitous environmental pollutants to which humans are commonly exposed. They are responsible for important carcinogenic and apoptotic effects, whose mechanisms are still poorly understood. Among these mechanisms, perturbations of H^+ homeostasis may be involved. This work has been carried out in order to test the effects of B(a)P (50nM) on pHi in rat liver F258 epithelial cell line, using carboxy-SNARF-1 as pH-sensitive fluorophore. B(a)P induced biphasic pHi changes, with first an alkalinization (at 48h) followed by an acidification (at 72h). Determinations of pHi recovery following an acid load showed an increase of acid efflux at 48h. By using cariporide, a specific Na^+/ H^+ exchanger 1 inhibitor, we demonstrated that NHE1 was activated upon B(a)P treatment and was responsible for pHi changes at 48h. The alpha-naphtoflavone (NF), a CYP1A1 inhibitor, as well as the antioxidant thiourea prevented any pHi variation induced by B(a)P, thus indicating a dependence of NHE1 activation upon reactive oxygen species (ROS) produced during B(a)P metabolism. When analysing B(a)P-induced apoptosis, we found that cariporide significantly reduced both DNA fragmentation and caspase-3 like activity. Using flow cytometry and the fluoroprobe dihydroethidium, we further showed that NHE1-dependent early alkalinization affected the mitochondrial ROS production detected during the apoptotic cascade.Altogether, our results suggest that B(a)P, via metabolism-dependent ROS production, induces an early activation of NHE1, thus leading to an alkalinization that might play a significant role in the subsequent induction of mitochondria-dependent apoptosis.

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S3-3

MOLECULAR MECHANISMS OF GENETIC ADAPTATION TO XENOBIOTIC COMPOUNDS
Feyereisen R.

Organisms are permanently exposed to the environment and their response to this environment will determine their survival (short term) or their evolutionary success (long term). Adaptation to xenobiotics is a specific case of adaptation to the myriad of chemicals of natural origin, but it is of particular importance to human well being, because our food or our health is often protected or restored by xenobiotics - pesticides, drugs and antibiotics. This lecture will focus on resistance as an example of genetic adaptation to xenobiotics. The widespread use of insecticides has amounted to a large scale "experiment' in natural selection of insects by chemicals which are often of toxicological importance to humans as well. The biochemical and physiological mechanisms of resistance can be categorized as target site insensitivity, increased metabolic detoxification and sequestration or lowered availability of the insecticide. At the genetic level, the mutations in receptors, transporters or enzymes may be classified into those that alter binding or catalysis by structural changes, up regulation including gene amplification, or down regulation including gene disruption or silencing. Regulation can be altered either by cis- or trans-acting control of expression. Genomic approaches greatly acelerate the discovery of resistance mutations. In several cases, the selection of a precisely homologous mutation has been observed in different species, indicating that resistance is an extreme case of genetic adaptation.

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S3-4

CIRCADIAN CLOCKS: FROM GENE EXPRESSION TO PHYSIOLOGY AND DISEASE
Schibler U., Gachon F., Ripperger J., Brown S.A., Gos P., Le-Minh N., Preitner N.

Circadian pacemakers were originally believed to exist only in a few specialized cell types, such as neurons of the suprachiasmatic nucleus (SCN). However, in recent years, this view has been challenged by the discovery that circadian clocks may exist in most peripheral cell types, and even in immortalized tissue culture cells. Nevertheless, these subsidiary oscillators have to be synchronized periodically by the central pacemaker in the SCN. Our studies suggest that this is accomplished mostly via indirect ways. In fact, feeding time is the most dominant Zeitgeber for peripheral clocks. Thus, the SCN entrains the phase of circadian gene expression and physiology in the periphery primarily by setting the phase of rest-activity cycles, which in turn determines the time of feeding. In addition, body temperature rhythms and cyclically secreted hormones also participate in the synchronization of peripheral clocks.

We have explored biochemical and genetic approaches to identify and study transcriptional regulatory proteins that translate the oscillations generated by the molecular clockwork into overt rhythms in physiology and behavior. TEF, HLF, and DBP, the three members of the PAR bZip protein family, strongly oscillate in liver and other peripheral tissues and thereby regulate the cyclic expression of several target genes (e.g. cytochrome P450 enzymes). In brain regions other than the SCN, however, the levels of these transcription factors fluctuate with only a small amplitude, and never fall below 30% to 50% of maximal circadian values. Our genetic loss-of-function experiments may offer a plausible explanation for why high amplitude cycles of Dbp, Tef, and Hlf gene expression cannot be tolerated in the brain. In fact, these transcription factors protect the mice from lethal seizure attacks (epilepsies) and thus have to be present throughout the day in the central nervous system.

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POSTER SESSION

P03-01

THE EFFECTS OF EXOGENOUS PROSTAGLANDINS AND CYCLOOXYGENASE INHIBITOR ON APOPTOSIS IN RAT HEPATOCYTES

Korniychuk G.M., Khabatyuk N.G., Makogon N.V., Alexeyeva I.N.

In addition to the fact that hepatocytes mainly take part in degradation of eicosanoids, they also produce small amounts of prostaglandins (PG) I2, E2, F2α, TxA2, which act primarily in cell-to-cell communications. The aim of the study was to investigate the influence of cyclooxygenase inhibitor (acetylsalicylic acid – AA) and exogenous PGE2 and PGF2α on apoptosis and necrosis in isolated rat hepatocytes under normal and pathological conditions, by method of fluorescent light microscopy after double cell staining with Hoechst 33342 and propidium iodide. It has been demonstrated, that under normal conditions neither AA nor exogenous PGE2 and PGF2α in a wide range of concentrations (0,1 microM - 3 microM) did not significantly change the ratio of vital, necrotic and apoptotic cells. Carbon tetrachloride (CCl4) administration (10 mM) caused a decrease in amount of vital cells and an increase of necrotic and apoptotic cells. Treatment of hepatocytes with AA (20 microM) before CCl4 adding led to a decrease in apoptotic cells count, whereas treatment with exogenous PGE2 and PGF2α (0,9 microM and 3 microM) on the contrary, increased the number of apoptotic cells. These results suggest that cyclooxygenase pathway of arachidonic acid metabolism is an important modulator of hepatocyte viability and death.

Bogomoletz Institute of Physiology of NAS Ukraine, Kyiv

P03-02

ANTIOXIDANT EFFECT OF A, E, C VITAMINS, TOPICALLY ADMINISTERED AFTER UVB RADIATION EXPOSURE

Filip A.

Ultraviolet radiation lead to reactive oxygen species occurrence in skin and decrease local antioxidant capacity.
We intended to highlight the antioxidant role of A,C, E vitamins, topically administered, after UVB exposure (2,4 J/cm2). Vitamins were applied on skin rats Wistar race, on a surface of 2cm2, previously shaved. We assessed lipid peroxides, total SH groups and non-proteic thiol from tegument at one hour, respectively 24 hours after exposure.
Topically administration of A and C vitamins significantly decreased the lipoperoxidation processes ($p<0.01$) after one and 24 hours, while E vitamin only after 24 hours. Total SH groups significantly raised at one hour and 24 hours after alpha-tocopherol treatment. A vitamin was efficient after 24 hours ($p<0.001$). Topically treatment with C vitamin had a defensive effect proved through the increase of total SH groups, one hour after exposure.
Our data demonstrates that topically treatment with vitamins decreases lipoperoxidation reactions due to UVB and leads to total SH groups and non-proteic thiol regeneration in skin.

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P03-03

CORRECTION OF METABOLIC DISORDERS AT HYPOXIA BY NEW PHARMACOLOGICAL PREPARATIONS

Gonchar O., Klyuchko E., Seredenko M., Oliynyk B.

We studied substances that may be potential medicines for hypoxia disorders treatment: yackton and sufan - derivatives of succinic acid, and splenoside – non- protein factor of spleen with nucleoside complex as active base. Investigations were carried on homogenates, cytosol and mitochondria fractions of liver, heart, lungs, brain tissues of Wistar rats during acute hypoxias: hypoxic, hemic (after the injection of sodium nitrite (6 mg / 100 g/rat weight) and circulatory hypoxia (after bleeding – 2,5 ml / 100 g/rat weight). During the hypoxic syndrome development we registered an increase of lipid peroxidative oxidation (LPO) in all studied tissues, disorders in enzymatic and non- enzymatic antioxidant system activities, acidosis development, depression of electron transport and mitochondrion functions of energy synthesis. Degree of expression for these processes depended from the type of hypoxia and tissue specificity.
Preliminary administration of any preparation (before the extreme influence)– yackton, sufan or splenoside caused the decrease of LPO,

increase of activity of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase as well as increase of reduced glutathione content in comparison with hypoxia state. NAD/NADH rate increased and decreased lactate/ piruvate rate and lactate dehydrogenase activation.
After the splenoside injection we registered an activation of glucoso-6-phosphatedehydrogenase (with the slight changes in succinate dehydrogenase activity (P<0,5) that evidences about the comparative priority of pentose- phosphate pathway. After the yackton injection in hypoxia conditions we registered in mitochondria an increase of succinate dehydrogenase activity (P<0,01) that leaded to the reduction of electron transport and recovery of energy synthesis functions in mitochondria. So, all studied preparations demonstrated antioxidant effect at hypoxia and may be potential medicines.

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P03-04

REGULATION OF ATP-SENSITIVE K^+ CHANNELS BY ACUTE CHANGES IN TEMPERATURE IN FISH CARDIAC MYOCYTES
Paajanen V., Vornanen M.

Sarcolemmal potassium efflux through ATP-sensitive channels (IKATP) is assumed to protect cardiac myocytes in ischemia and hypoxia by reducing the duration of action potential and hence cardiac contractility. The hypoxic opening of IKATP is mediated by the depletion of intracellular ATP which tends to balance ATP supply and demand. Here we show that in ventricular myocytes of the fish heart the opening and closing of IKATP is induced by acute temperature changes.
Using whole-cell, cell-attached and inside-out configurations of the patch-clamp method, we studied IKATP in ventricular cardiac myocytes of an extremely eurythermic and anoxia-tolerant fish species, the crucian carp (Carassius carassius). Under normoxic (O2 = 8.6 mg/L) conditions and in the presence of 5 mM ATP in the pipette solution, IKATP was induced by a gradual rise of temperature above the physiological body temperature of 5°C. Once induced, the amplitude of the IKATP was proportional to the extent of temperature rise (95.3 ± 9.7 pS/pF at 18°C), fully reversible by cooling and inhibited with 10 mM glibenclamide, a blocker of IKATP. The temperature-induced increase of the IKATP was only partly explained by the increase in single channel conductance from 32 pS at 5°C to 50 pS at 18°C (Q10=1.43). Acute temperature changes had no effect on the kinetics of the IKATP in inside-out patches. IKATP of the crucian carp cardiac myocytes were characterized by extremely low sensitivity to inhibition by ATP (ID50= 1.35 mM).
These results indicate that in ventricular myocytes of the crucian carp heart, the opening and closing of IKATP is regulated by acute temperature changes. By this means, the IKATP may modify the duration of ventricular AP and hence regulate cardiac contractility in temperature-dependent manner. The molecular mechanisms by which temperate regulates the opening and closing of the ATP-sensitive K^+ channels remains to be shown.

University of Joensuu - FINLAND

P03-05

OXIDATIVE MECHANISM IN THE TOXICITY OF CADMIUM.
Krichah R., Chater S., Ben Rhouma K., Tebourbi O., Favier A., Sakly M.

Cadmium (Cd) an abundant nonessential element is widely used in electroplating and galvanizing. Soluble cadmium salts accumulate and result in toxicity to various tissues: liver, brain, thymus and central nervous system. In the present study we assessed the ability of acute Cd-exposure to induce an oxydatif stress through the determination of the total antioxydant state (TAS, Kit Randox), glutathion peroxidase activity (GPX) and tissue metallothioneins (MTs) levels. Male wistar rats, weighing 100-150g were injected with a single dose of 1.5 or 3mg Cadmium chloride/kg body weight (bw)/(ip). Control animals received the same volume of saline. All animals were sacrified by decapitation 24 or 48 hours later. Cd-administration induced a decrease of the total antioxydant status (TAS) 24 hours later (0.56 ± 0.08 and 0.63 ± 0.06 mmol/l respectively for 1.5 and 3mg Cd/kg bw vs 0.77 ± 0.03 mmol/l) and a depletion of plasma GPX activity (2.27 103 ± 0.08 and 2.43 103 ± 0.12 vs 5.04 103 ± 0.16 U/l). After 48 hours a partial recovery of this activity was observed (3.19 103 ± 0.06 and 3.52 103 ± 0.04 vs 5.04 103 ± 0.16 U/l), while cytochrom c reduction was more important in the treated rats thymus. The same treatment stimulated MTs biosynthesis in thymus and liver 24 hours later and this effect was more important after 48 hours. These results indicate that acute Cadmium chloride administration induced an oxidative stress state that may contribute to its toxic effects.

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P03-06

DDT CYTOTOXICITY IS NOT MEDIATED BY CORTICOSTEROIDS
Tebourbi O., Ben Rhouma K., Krichah R., Hallegue D., Chater S., Sakly M.

DDT (1,1 bis (p-chlorophenyl) 2,2,2 trichloroethane)is an organochlorine insecticide. DDT is very neurotoxic, hepatotoxic and has an endocrine effect. However, the mechanism of action of this pesticide is not clear. The purpose of the present study is to evaluate the effect of DDT on adrenal gland,the thymic glucocorticoid receptors and serum corticosterone levels. Male Wistar rats received 50 or 100mg of pesticide/kg b.wt. (i.p) during ten consecutive days. After adrenalectomy, the relative weight of the thymus increased confirming the thymolytic effect of glucocoticoids. Administration of DDT induced a thymic atrophy in both normal and adrenalectomized rats. The adrenalectomy also led to an hepatic atrophy. But, after subacute exposure of DDT, the liver weight increased significantly in normal and in adrenalectomized animals. This data associated to the unchanged relative weight of adrenal gland in DDT-injected rats suggest that the pesticide has a direct toxic effect indepentamently of the corticotropin axis. The normal architecture was seen in rats treated with 50mg of DDT while those treated with 100mg of pesticide developped necrosis and cytoplasmic vacuolization in the reticularis and fasciculata zona. The density of glucocorticoid receptors treated with 100mg/kg of DDT increased slightly (552.8 ± 5.1 vs 456.2 ± 4.9fmol/mg protein). Serum corticosterone levels were decreased with the dose of 100mg. This decrease may explain the elevation of thymic glucocorticoid receptor density induced by the higher dose of pesticide. These results suggest that DDT exerted cytotoxicity action directly on somatic cells and didn't activate the adrenal secretion. Nevertheless, the high dose of pesticide decreased the secretion of corticosteroids probably by inhibiting gland streoidogenesis pathways.

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P03-07

INCREASED EXPRESSION OF THE IKR IN CARDIAC MYOCYTES OF THE COLD-ACCLIMATED TROUT
Vornanen M.

Delayed rectifier K currents provide major repolarising power during the phase 3 of cardiac action potential (AP). To prevent temperature-dependent changes in AP duration and cardiac excitability, seasonal temperature adaptation of the heart in ectothermic animals is expected to involve modification of K currents. Hence we studied the effects of thermal acclimation on the rapid component of the delayed rectifier current, IKr, in atrial myocytes of the trout heart. The density of the IKr at 0 mV was 12.7±1.3 and 13.1±2.9 pA pF-1 for cold-acclimated (CA, 4°C) and warm-acclimated (WA, 18°C) trout, respectively, indicating an almost perfect thermal compensation of the current amplitude. On the other hand, deactivation and inactivation kinetics of the IKr were not changed by temperature acclimation, even though they were strongly affected by acute temperature changes. To address the physiological importance of the IKr, currents were elicited by using physiological APs as the command waveform. In agreement with the square wave stimuli, the densities of the IKr were equal for CA trout at 4°C and WA trout at 18°C. More surprisingly and in apparent conflict with the strong temperature-dependency of the IKr, the activation of the IKr was much faster in CA trout at 4°C than in WA trout at 18°C. This is, however, explained by the slow deactivation kinetics of the IKr in the cold as it prevents the closing of the activation gate at physiological heart rates (40/min) at 4°C. The open (non-deactivated) channels generate an immediate outward current when driving force is restored by the depolarisation of the next AP. In conclusion, incomplete deactivation of the channels at low temperatures and cold-induced increase in the density of the IKr generate fast and large repolarising currents in atrial myocytes of CA trout. These properties of the IKr maintain the duration of AP short for adequate cardiac function in the cold environment.

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P03-09

MECHANISMS OF AMINO ACID TRANSPORT BY THE ISOLATED INTESTINE OF THE FROG RANA ESCULENTA
Saïdane D.

This work was aimed at studying the mechanisms of intestinal transport of aminoacids in consideration of their interactions with the transport of major inorganic ions. The study was performed on the isolated intestine of the green frog Rana esculenta by using in vitro approaches: Ussing chambers, membrane vesicles from apical and basolateral membranes of enterocytes, apical short-term uptake of labelled solute (Schultz, Curran, Chez and Fuisz, 1967).
Combination of these techniques allowed us to characterize the following transport mechanisms for aminoacids that we defined by analogy with systems already described in mammals and in certain fish :
1- A neutral aminoacid transporter close to the B (previously NBB) system
2- A Phenylalanine system transferring long chain aminoacids (phenylalanine and methionine)
3- A Chloride-dependent transporter for methionine and phenylalanine, like the mammalian Bo,+ system
4- A Glycine system sensitive to chloride, comparing to the Gly one of mammals
5- A Sodium-dependent mechanism carrying alanine and serine, equivalent to the ASC system
6- Another sodium-dependent transferring lysine and possibly all basic aminoacids, reminiscent of the Y+ system of the rat
7- A Sodium-independent system for the uptake of phenylalanine and very similar to the L system which ensures the sodium-dependent transport of long chain neutral aminoacids
8- Another sodium-independent system for alanine, sharing these properties with the b system described in mammals, now called b0,+

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P03-10

NITRIC OXIDE IN EXPERIMENTAL HEPATIC FIBROSIS
Parvu A.E., Parvu M., Plesca-Manea L., Hoteiuc O.

The role of nitric oxide (NO) in the pathogenesis of liver diseases has been extensively studied. Hepatocellular damages initiate an inflammatory reaction. Elevated nitric oxide production is assumed to be responsible for the pathological changes in many inflammatory conditions.
The purpose of this study was to assess the effects of the chronic stimulation or inhibition of nitric oxide synthesis in male Wistar rats with CCl4-induced hepatic fibrosis. Plasma levels of production of citrulline from arginine (Boyde method), nitrite and nitrate (Griess reaction) were determined in rats before and after intraperitonealy administration of L-arginine (L-ARG), NG-nitro-L-arginine methyl ester (L-NAME), pentoxifiline, dexametasone and methilen blue. We analyzed the relationships between the levels of NO synthesis and some hepatic parameters (ASAT, ALAT, LDH, gamma-GT, AP, bilirubin0. the resulta showed that animals with toxic hepatic fibrosis had correlated elevation of plasma citrulline and nitrite/nitrate levels, most due to an increase stimulation of the iNOS and a lower stimulation of the cNOS types. The hepatic parameters had a parallel increase with the induced form of NO.
CONCLUSION:The most important source of NO in hepatic fibrosis is the induced one. Citrulline and nitrite/nitrate levels may indicate the rate of hepatic damage as do other plasmatic parameters.

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P03-11

APPLICATION OF INHALATED PHOSPHOLIPID LIPOSOMES IN HCL-INDUCED LUNG INJURY
Steneva J., Jordanova A., Lalchev Z., Ninio S., Neicheva T., Petkova D.

The aim of this study was to evaluate the inhalatory application of phosphatidylcholine multilayer liposomes (PL) in HCl – induced acute respiratory distress sindrome (ARDS) in rabbits.
ARDS was induced by administration of 0.2 N HCl via intratracheal instillation for 45 min. After induced ARDS animals under artificial lung ventilation were retreated with PL in saline (25mg/kg body weight) for 60 min. Arterial blood gas (ABG) analysis was performed at 15, 30, 45 and 60 min after PL application. Untreated animals were ventilated for the same time during all the experiments and ABG analysis was performed as well. Animals were killed with thiopental and bronhoalveolar lavage fluid (BALF) was investigated for phospholipids, cholesterol and specific surfactant protein content. The equilibrium surface tension of monolayers obtained from BALF was determined by Wilhelmy balance.
HCl- lung injury caused decrease of arterial PO_2 to the fraction of inspired O_2 (PaO_2/FiO_2) ratio more than 50% compared to the control. We obtained high respiratory acidosis – increase of arterial CO_2 ($PaCO_2$) and decrease of blood pH. An increase of alveolae – arterial PO_2 (A-a pO_2) was also detected. The inhalation of PL led to reversion of gas exchange even at 30 min after application, saturation of arterial blood, decrease of A-a pO_2. Changes in blood pH we obtained at 45 min after the application of PL and at 60 min pH value returned to the control value. HCl- lung injury caused significantly increase of total protein and cholesterol content, decrease of total phospholipids and percent participation of phosphatidylcholine (PC) and increase of that of sphingomyeline in BALF compared to the control. These changes correlated with biophysical parameters. The sample surface tension was decreased. The application of PL led to reverse of the percent participation of PC , biophysical parameters to the control value and lung function as well.

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P03-12

EFFECT OF EXPOSURE TO MAGNETIC FIELD ON SOME BLOOD PARAMETERS IN MALE RATS
Amara S., Abdelmelek H., Ben Rhouma K., Sakly M.

The present work was undertaken in order to investigate the effects of magnetic field (MF) on hematopoiesis, serum lactate dehydrogenase and transaminases activities in male rats. The exposition of rats 1hour/day for 5 consecutive days to MF of 128 mT (m tesla) had a weak effect on hemoglobin and hematocrite levels. The same treatment increased significantly serum lactate dehydrogenase activitie, whereas alanine aminotransferase and aspartate aminotransferase activities remained unchanged. MF exposure for 30 consecutive days increased significantly hemoglobin, the red , the white blood cells and the platelet number. It was concluded that MF expopsure might induce hemoglobin conformational change causing an hypoxia-like state that stimulated hematopoiesis. Sub-chronic exposure to MF increased also serum lactate dehydrogenase and transaminases activites suggesting hepatic damage.
Key words: Magnetic field, LDH, transaminases, hematopoiesis, rat.

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P03-13

LOCUS COERULEUS MODULATES THE HYPOXIC VENTILATORY RESPONSE IN ADULT RATS
Soulage C., Perrin D., Cottet-Emard J.M., Pequignot J.M.

Upon exposure to hypoxia, the initial and most important response is an increase in alveolar ventilation. There is now growing evidence that the medullary catecholaminergic cell groups (A1C1, A2C2, A5, A6) participate in the ventilatory response to hypoxia. The A6 cell group, also referred to as locus coeruleus (LC), is the largest cluster of noradrenergic cell bodies of the brain. The present study was designed to assess the involvement of A6 noradrenergic cell group, for the establishment of the ventilatory response to short-term hypoxia. The breathing response to acute hypoxia (10%O2) was measured in awake and unrestrained rats by barometric plethysmography 15 days after a unilateral lesion of LC with 6-hydroxydopamine (6-OHDA). The 6-OHDA infused “in situ” caused a major loss of noradrenergic neurones in A6 area assessed in histology and in neurochemistry. The unilateral lesion fails to alter minute ventilation, tidal volume or frequency under basal conditions (room-air, 21%O2). During a 12 minutes hypoxic challenge (10%O2) whereas minute ventilation remains unaffected, 6-OHDA treated rats exhibit a lower tidal volume (-67%) than sham-operated ones. This blunted response in tidal volume is however counter-balanced by an enlarged response in frequency. Our results strongly suggest that separated mechanisms and distinct structures are acting in the regulation of tidal volume and respiratory frequency. We concluded that i) LC noradrenergic neurones are not essential for breathing modulation under normoxia, ii) noradrenergic neurones of the LC and catecholaminergic mechanisms are involved in regulation of tidal volume under hypoxia.

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P03-14

INVOLVEMENT OF CGRP BUT NOT TACHYKININS IN LOCAL PRESSURE-INDUCED VASODILATION

Merzeau S., Fromy B., Abraham P., Saumet J.L.

Capsaicin-sensitive afferent neurones are connected to cutaneous receptors which enable them to detect noxious stimuli that are potentially or actually harmful to the tissue. Neurokinins and calcitonin gene-related peptide (CGRP) are released from these peripheral nerve terminals following their activation. Local pressure-induced vasodilation (PIV) is a neural vasodilator response to non-nociceptive externally applied pressure in the skin. The first aim of the present study was to determine whether cutaneous PIV exists in rats and is dependent on capsaicin-sensitive fibres as it is in humans. We then examined whether CGRP and neurokinin receptors are involved in this reflex. Cutaneous blood flow was measured by laser Doppler flowmetry during 11.1 Pa.sec-1 increases in local externally applied pressure in untreated anaesthetised rats. The involvement of capsaicin-sensitive fibres in this mechanism was tested in rats treated neonatally with capsaicin. Separate groups of adult rats were treated with CGRP8-37 (100 µg.kg-1, i.v.), SR140333 (200 µg.kg-1, i.v.), SR48968 (4 mg.kg-1, i.v.) or SR142801 (1 mg.kg-1, i.v.) to antagonise CGRP, NK1, NK2 or NK3 receptors, respectively. PIV was found in rats, as in humans. It was abolished by neonatal treatment with capsaicin and intravenous administration of CGRP8-37 but remained unchanged with SR140333, SR48968 and SR142801 treatments compared to their respective vehicles. These results suggest that PIV depends on capsaicin-sensitive fibres in rats, as in humans. Furthermore, it appears that CGRP plays a major role in this capsaicin nerve mediated vasodilation in rat skin, whereas neurokinins appear to have no role in PIV.

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P03-15

THE EFFECT OF ISOFLURANE ON THE SKIN PRESSURE-INDUCED VASODILATION

Fizanne L., Fromy B., Preckel M.P., Sigaudo-Roussel D., Saumet J.L.

Since general anesthesia has shown to attenuate endothelium-dependent vasodilation, it was of interest to verify whether general anesthesia would modify a skin vasodilation in response to local pressure application, which is endothelium-dependent. To study the effect of general anesthesia on pressure-induced vasodilation development, we examined the effects of isoflurane in light and deep states. Skin blood flow was measured by laser Doppler flowmetry during 11.1 Pa sec-1 increases in locally applied pressure in anesthetized rats. Rats were treated with low or high doses of isoflurane. Following the administration of low doses of isoflurane, skin blood flow increased from baseline, with increasing local pressure application (+37±10% at 2.0 kPa). The increase in skin blood flow disappeared in treated rats with high doses (-20□5% at 2.0 kPa), even when the anesthesia-induced hypotension was corrected by gelofusine infusion (-20□10% at 2.0 kPa). Whereas sodium nitroprusside-induced vasodilation was developed with low and high doses of isoflurane, acetylcholine-induced vasodilation was impaired with high doses compared to low doses. These data show that pressure-induced vasodilation is abolished with high doses of anesthetic. It is not the anesthesia-induced hypotension, but the depth of anesthesia, which can lead to the disappearance of pressure-induced vasodilation by an alteration of endothelial function.

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P03-16

EDHF INVOLVEMENT IN SKIN PRESSURE-INDUCED VASODILATION

Garry A., Merzeau S., Fromy B., Saumet J.L.

At least three different vasodilator agents are synthesised by the endothelium upon exposure to mechanical forces or to receptor-dependent agonists: nitric oxide (NO), prostaglandins and the endothelium-derived hyperpolarising factor (EDHF). Skin pressure-induced vasodilation (PIV) is a neuronal response to locally applied pressure discovered in humans and in rats. This new mechanism results from a complex response originating from capsaicin-sensitive skin sensory fibres and local secretion NO and prostaglandins. No information has been published on EDHF in this mechanism. The aim of the present study was to examine the EDHF role in the PIV development in treated rats with a combined infusion of charybdotoxin and apamin and in controls. Skin blood flow was measured by laser-Doppler flowmetry in response to a progressive local pressure applied to the skin. In treated rats as in controls, the skin vascular conductance increased with increments of local pressure (56.5±11.1% vs 59.5±8.4%, P>0.05). We report here that the vasodilator capacity was not altered in rats treated with charybdotoxin+apamin compared to controls. In conclusion, our study indicates that when NO pathway is intact, there is no or little implication of EDHF in the cutaneous PIV development in rats.

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P03-17

CUTANEOUS PRESSURE-INDUCED VASODILATION IS DEPENDENT ON ENDOTHELIAL NO AND PROSTAGLANDIN RELEASE

Fromy B., Merzeau S., Abraham P., Saumet J.L.

A significant transient increase in cutaneous laser Doppler flow during local external pressure application (11.1 Pa sec-1) was studied in the skin of rats, and defined as pressure-induced vasodilation (PIV). The aim of the present study was to examine the mechanisms involved in the efferent pathway of PIV, by testing whether the resultant vasodilation is endothelium dependent. The involvement of prostaglandins was tested in rats treated with indomethacin (5 mg kg-1, i.p.). Separate groups of adult rats were treated with either NG-nitro-L-arginine (20 mg kg-1, i.v.) or 7-nitroindazole (50 mg kg-1, i.p.) to inhibit nitric oxide synthase (NOS) activity and specific neuronal NOS, respectively. Prostaglandin inhibition resulted in a significant decrease in PIV (P<0.001 vs. vehicle). Inhibition of NOS abolished PIV (P<0.001 vs. vehicle), whereas specific inhibition of neuronal NOS showed diminution in PIV (P<0.001 vs. vehicle). These data suggest that PIV involves a contribution from prostaglandins and is dependent on endothelial NO, whereas neuronal release of nitric oxide has a smaller role.

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P03-18

AMBIENT TEMPERATURE AFFECTS THE CUTANEOUS PRESSURE-INDUCED VASODILATATION IN HUMANS

Koitka A., Saumet JL., Abraham P.

We have previously shown that during progressive moderate pressure strain a transient pressure-induced vasodilation (PIV) exists at the hand in normal subjects, but we failed to find a comparable response at the foot level. Previous works have found a significant sensitisation of vertebrate mechanoreceptors by temperature. The aim of our study was to examine the putative influence of the thermoregulatory state on skin blood flow responses to non noxious mechanical stimulus. We studied 10 healthy human subjects exposed to different ambient temperatures: cool (25.1±0.2°C), intermediate (27.0±0.4°C) and warm conditions (30.6±0.3°C). We measured skin blood flow using laser Doppler flowmetry on the head of the first metatarsus in response to a progressive local pressure increased of 5.0 mmHg/min. Progressive pressure strain led to an almost linear cutaneous laser Doppler flow (LDF) decrease in both cold and intermediate conditions, whereas in warm conditions subjects responded with a PIV. Indeed, mean resting LDF was 57.1±6.8 arbitrary units (a.u.), 75.2±7.7 a.u., 100.6±9.7 a.u at cool, intermediate and warm conditions respectively. In cold, intermediate and warm conditions, compared to baseline mean LDF at 30 mmHg was decreased to 33.4±6.1 a.u. and 50.9±6.2 a.u. and was increased to 134.3±16.7 a.u. (P<0.05 vs. baseline respectively). The data indicate that at PIV exists at the foot level in healthy subjects but the thermoregulatory state profoundly influences the extent and direction of vasomotor response to non-noxious pressure strain which is initiated by capsaicin-sensitive nerve terminals in the human skin. The results of these experiments suggest that the ambient temperature will affect discharge in the mechanical C-fibers involved in the PIV. Furthermore, there were no differences between responses in human hands and feet, suggesting an ubiquitary organisation of this original protective cutaneous reflex.

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P03-19

EARLY DECREASE OF SKIN BLOOD FLOW IN RESPONSE TO LOCALLY APPLIED PRESSURE IN DIABETIC SUBJECTS

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Pressure ulcers are common debilitating complications of diabetes due to tissue ischemia. The skin blood flow in response to locally applied pressure might be impaired in diabetic patients due to combined effects of a typically low skin temperature and alterations of microcirculatory function, which could be worsen by neuropathy. We measured skin blood flow by laser Doppler flowmetry over the internal anklebone in response to a local pressure applied at 5.0 mmHg.min-1 in three groups of diabetic patients (with clinical, with sub-clinical and without neuropathy) and in healthy matched controls at usual room temperature. Compared to matched controls with comparable skin temperatures (29.3±0.4°C vs. 28.7±0.4°C), the skin blood flow response to locally applied pressure was further impeded in diabetics even without neuropathy. Indeed, skin blood flow decreased significantly from baseline at much lower applied pressure in diabetics, even without neuropathy (7.5 mmHg), than in controls (48.8 mmHg). The large difference between these pressures could partially explain the high risk of the occurrence of decubitus and plantar ulcers in diabetes.

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P03-20

EFFECTS OF NATURAL AND ENVIRONMENTAL ESTROGENS ON CELL SIGNALLING IN MUSSEL IMMUNOCYTES

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There is increasing evidence that immune cells represent preferential targets of the rapid, non genomic effects of both natural and environmental estrogenic compounds.
In bivalve molluscs, such as the mussel Mytilus galloprovincialis Lam., circulating hemocytes, that resemble the monocyte/macrophage lineage, are responsible for innate immunity. In this work the effects of the natural estrogen 17beta-estradiol on hemocyte cell signalling were evaluated, and the results were compared with those of synthetic estrogens (such as DESB), estrogenic chemicals (such as BisphenolA, PCBs) and plant estrogens (genistein). The results indicate that low nM concentrations of 17beta-estradiol induced a rapid, dose dependent increase in cytosolic [Ca^{2+}] in Fura2/AM-loaded mussel hemocytes. Moreover, Western Blot analysis show that 17beta-estradiol affected the phosphorylation state of components of both the MAPK (Mitogen Activated Protein Kinase) and of the STAT (Signal transducers and Activators of Transcription) family, whose activation has been demonstrated to play a crucial role in mediating the transduction of bacterial signals in mussel hemocytes. Higher concentrations of 17beta-estradiol were toxic to hemocytes, resulting in significant destabilisation of lysosomal membranes. Both synthetic and environmental estrogens mimicked the effects of 17beta-estradiol; however, similar effects were observed at concentrations 1000 times higher (microM) than those of the natural estrogen. Overall, our data demonstrate that in invertebrate cells both natural and environmental estrogens can act through rapid, non genomic mechanisms affecting both Ca^{2+}- and tyrosine kinase-mediated cell signalling involved in mediating the innate defence response to bacterial stimuli.

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P03-21

CANDIDA ALBICANS PROMOTES ICAM-1 AND E-SELECTIN EXPRESSION THROUGH A NFKB MECHANISM

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Candida species are the most frequent cause of life-threatening invasive fungal infections in the immunocompromised host and are responsible for 10% of all nosocomial bloodstream infections. The leading cause of candidiasis is Candida albicans. Adhesion to the epithelium is the first step toward colonisation of the oral mucosa by this fungus. Oral epithelial cells hence play a crtitical role in maintaining the equilibrium between host cells and C. albicans. The aim of this study was therefore to investigate the early events following contact between epithelial cells and C. albicans. To this end, the expression of ICAM-1 and E-selectin by oral epithelial cells and the signal transduction pathways following C. albicans infection have been studied. In this context, an engineered human oral mucosa has been produced, then infected with C. albicans (10ex5 yeast/cm2). At the end of each appropriate contact period, total mRNA and proteins were extracted from oral epithelium and then used for RT-PCR, immunohistochemistry and Western blot analyses. Our results demonstrate that C. albicans significantly up-regulates the mRNA expression of ICAM-1 and E-selectin after 24 h of infection. ICAM-1 and E-Selectin were promoted by mitogen-activated protein (MAP) kinase cascade (ERK1/2, JNK1/2, p38, cJUN, ATF-2). Indeed, C. albicans modulates the phosphorylation pattern of these MAP kinase proteins and the expression of EGFR and NFkB. In conclusion, this study made some light on the mechanism involved in C. albicans adhesion to oral epithelial cells. (Funded by the FRSQ, NSERC and CIHR)

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P03-22

Hg^{2+}-DEPENDENT CELL SIGNALLING IN TROUT HEPATOMA (RTH-149) CELLS

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The study of ligand-independent cell signalling is a promising field for an understanding of cellular responses to stress. We report here different signalling events triggered by Hg^{2+}, a strong reagent of sulfhydryl and imidazole groups, on the RTH-149 trout hepatoma cell line.
Confocal imaging of fluo 3-loaded cells showed that Hg^{2+} triggered [Ca^{2+}]i transients and Ca^{2+} waves. The [Ca^{2+}]i rise was reduced by the Ca^{2+} channel blocker verapamil and abolished by extracellular glutathione (GSH), but it was almost unaffected by cell loading with the heavy metal chelator TPEN or with esterified GSH. In Ca^{2+}-free medium, Hg^{2+} induced a lower [Ca^{2+}]i transient, that was abolished by manoalide, a PLC inhibitor, or by cell loading with GDP-□S or heparin. Also, cells loaded with heparin and exposed to Hg^{2+} in the presence of external Ca^{2+} showed a drastic reduction of [Ca^{2+}]i rise. Data indicate that Hg^{2+} induces both extracellular Ca^{2+} entry and InsP3-dependent intracellular Ca^{2+} release. These two processes are not independent, as Ca^{2+} release is amplified by Ca^{2+} entry through Ca^{2+}-induced Ca^{2+} release.
Western immunoblotting of cell lysates and the use of antiphosphotyrosine showed that Hg^{2+} induced an increase of tyrosine phosphorylation. Pre-incubation with genistein did not abolish this effect but only reduced it, probably due to tyrosine kinase stimulation coupled to phosphatase inhibition. The use of phosphospecific antibodies against MAPKs, representing stress-activated tyrosine kinase signalling pathways, showed strong activation of ERK and p38, and a lower activation of JNK.
Our results indicate that short-term effects of Hg^{2+} consist in the activation of both calcium and phosphotyrosine signalling, possibly due to Hg^{2+} interactions with plasma membrane receptors. Hg^{2+}-dependent signalling could play a role in the activation of defense mechanisms able to protect cells after metal upload.

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P03-23

EFFECT OF ANTIOXIDANT ADMINISTRATION ON LIVER FUNCTION AFTER COLD PRESERVATION

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One of the main causes of the grafts loss after liver transplantation is due to the ischemia-reperfusion injuries. In that case, it is clinically important to elucidate the mechanism of cellular damage during hepatic preservation and reperfusion and to improve a new preservation solution and a rinse solution. Our aim was therefore to evaluate the usefulness of enriched solutions for rinsing liver after cold preservation.
The isolated perfused rat liver (IPRL) model was used to assess organ recovery. After 24h of cold preservation in university of Wisconsin (UW) liquid and 30 min of warm ischemia, livers were perfused for 2h at 19 °C with Krebs-Henseleit solution (KH, n=7) or KH+antioxidants (n=6) or KH+nifedipine (n=6). The results showed that antioxidants addition to KH induced an increase of bromosulfoptalein (BSP) clearance ($p<0.05$ vs KH and KH+nifedipine) and a decrease of aspartate aminotransferase ($p<0.05$ vs

KH and KH+nifédipine) and alanine aminotransferase ($p<0.05$ vs KH) release into perfusate. In addition, intrahepatic resistances are improved ($p<0.05$ vs KH and KH+nifedipine) with antioxidants.
In conclusion, antioxidants given during the early post-preservation period improve liver graft function. During cold storage, energy depletion leads to an impairment of cellular homeostasis and several toxic mediators are released. The use of an enriched solution with antioxidants to rinse grafts after cold storage allows loading the organ with protective compounds, which could be essential for recovery after reperfusion.
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P03-24

EXERCISE PERFORMANCE IN SUBJECTS WITH SICKLE CELL TRAIT IN HOT CLIMAT
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The sickle cell trait is a genetic abnormality of the red blood cell. It is due to the mutation of a parental gene, one amino-acid of the chain ß of the globin a glutamic acid which is substituted by a valin on the haemoglobin (HbAS). For subjects with sickle cell trait (SCT). The possibility to display any disturbance during predominantly anaerobic and aerobic exercises is unclear. 19 subjects with sickle cell trait and 19 subjects control have been studied during incremental exercise test on cycloergometer and on aera. They are all students of the Institut National Supérieur d'Education Populaire et Sportive of DAKAR. The environmental temperature mean has been 26°C. After haematological analysis an incremental exercise has been performed during 15 or 20 mn for one group. For another group a sub maximal muscular exercise for one hour with 75% of maximal heart rate has been done. We have determined VO2max , heart rate, blood pressure, rectal and skin temperature during exercise.
Haematological analysis has shown any different between the two groups. Any difference was found in VO2max and cardio circulatory variables during maximal exercise in cycloergometer between control group and sickle cell trait group VO2max was at mean 44.7±8.1 vs 44.6±6.9 ml/mn/kg respectively. The two groups have done sub maximal exercise during 1 hour without difficulty. We have not observed any difference between the two groups in cardiovascular, thermal variables and developed mechanic power. Rectal temperature for control group and SCT group was at mean 37.86±0.23 vs 37.93±0.27°C respectively and 33.92±1.91 vs 32.5±2.1°C for skin temperature
These results show that subjects with SCT have exercise performance comparable with control subjects during incremental maximal exercise and sub maximal exercise for 1 hour. We can assure that subjects with SCT in our country may participate in sports competition, as well as subjects with normal HbAA

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P03-25

THE QUALITATIVE CHANGES OF RED BLOOD IN HYPOXIA
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The quantitative and qualitative changes of red blood in hypoxia were complexly studied.
The objects of research were white rats. The hypoxia was equivalent to seven thousand meters above the sea level. It was achieved by rats' incubation in the barochambers during seven days (6 hours daily). We estimated the level of hemoglobin, concentration of erythrocytes, reticulocytes in the blood and erythroid cells in the bone marrow. The size of erythrocytes was measured. Two methods was used to determine the hemoglobin's pattern: electrophoresis in polyacrylamid gel and acid elution method.
After the seven day of hypoxia the blood level of five and six hemoglobin's fraction (these are fetal isoforms of rat's hemoglobin) was increased. It was accompanied with the large cells appearing in the blood circulation. The measurement of erythrocytes with acid stable fractions of hemoglobin (five and six isoforms) showed that these were the largest. It is known that the size of erythroid cells is decreasing during it maturation. So it can be supposed that the higher level of five and six hemoglobin's fractions characterize one of the erythroid cell maturation stage. However the hemoglobin's pattern of extracted reticulocytes didn't differ from the one of other age cells. Besides we ascertained that old rats with depressed erythropoiesis had the high level of five and six hemoglobin's isoforms in the blood circulation.
Therefore the macrocytes with the higher level of five and six fractions of hemoglobin is not the stage of erythroid maturation. Probably there are some ways of red blood's formation. The first way is inherented to adult healthy animal. Another way prevails in fetal and neonatal period, in old age and in hypoxia. So hypoxia leads to qualitative erythropoiesis' changes consisted in production of macrocytes with higher level of five and six hemoglobin's isoforms (fetal isoforms).

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P03-26

EFFECTS OF COLD-RESTRAINT STRESS-INDUCED LIPID PEROXIDATION ON RAT PERITONEAL MACROPHAGES FUNCTIONS
Tan R., Bulbul M., Izgut-Uysal V.N.

The aim of this research was to study the effects of cold restraint stress-induced
lipid peroxidation on the phagocytic and chemotactic capacities of peritoneal macrophages from rats. Macrophages were obtained with peritoneal lavage from control and stress groups. The rats in stress group were exposed to cold and restraint stress for 4 hours at +4ºC. TBARS formation and catalase activity were measured in peritoneal macrophage suspensions. Phagocytosis of macrophages was evaluated according to the mean number of phagocyted particles, and chemotaxis in a Boyden chamber. In stress group, catalase activity and TBARS production were higher than the control animals but this difference was not significant. Chemotactic and phagocytic capacities of macrophages reduced significantly in the stress group. In conclusion cold-restraint stress decreased the phagocytic and chemotactic activities of peritoneal macrophages from rats.

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P03-27

EFFECT OF THYROID HORMONE IN A STUDY ON ADULT AND FETAL SYNGENEIC HEPATOCYTE TRANSPLANTATION
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AIM:The aim of this work is to assess the survival and functionality of syngeneic adult and fetal hepatocytes transplanted into Gunn rats, an experimental model of the syndrome of Crigler-Najjar type I, and to evaluate the ability of thyroid hormone (T3) to stimulate liver repopulation.METHODS:Hepatocytes were isolated by collagenase digestion and transplanted into spleens of Gunn rats. These were divided into four experimental groups: I) Animals receiving adult hepatocyte transplantation (THC) without T3. II) Animals receiving fetal hepatocyte transplantation (THF) without T3. III) Animals receiving THC and the same day and every 10 days thereafter, T3 (Sigma; 400 mg/100 g body weight) injected subcutaneously. IV) Animals receiving THF and treatment with T3. Controls consisted of untreated rats and treated with T3 but without transplantation. Rats were killed from 1 to 15 days after implants and livers and spleens were processed by histological methods. Also, bilirubin levels were assessed from blood and bile at the end of the study. RESULTS:Although all groups show a significant decrease in serum total bilirubin, the percentage of decrease in animals transplanted with either adult or fetal hepatocytes and treated with T3 is significantly greater. In both THF and THC stimulated with T3, there is a dramatic decrease of total serum bilirubin by day 15 after the implant which is coincident with an increase of conjugated bilirubin in bile. Light microscopy study shows that transplanted cells migrate to the liver of recipient animals.
CONCLUSION:In short, it seems that repeated injections of T3 might provide a strong stimulus for transplanted cells to repopulate the liver as indicated by the decrease in bilirubin being the effect most remarkable 15 days after two injections of thyroid hormone. The use of proliferating agents could be an alternative to repeated implants for correcting a metabolic diseases. (This work was financed by grants FIS 01/0001-01 and 02)

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P03-28

PLASMA REPRODUCTIVE STEROID AND MORPHOLOGY OF THE GONAD IN THE FLOUNDER (PLATICHTHYS FLESUS)

Durán B., Damasceno-Oliveira A., Coimbra J.

The objective of this study was to establish the different stages of the gonadal development and the associated steroid hormone levels in adult males and females of a commercially important flatfish species, Platichthys flesus, during its annual reproductive cycle and its migrations from feeding grounds to spawning grounds, from estuarine waters (fresh and brackish water) to coastal waters (sea water).
The animals were captured in the estuary of the river Douro (Porto-Portugal) and adjacent coastal waters during a period of time of seven months, starting from Setember until November. The sampling times were chosen to coincide with the period of gonadal recrudescence and spawning. Blood was collected from the caudal vein for sexual steroid analysis with a radioimmunoassay (RIA), 17b-estradiol in females and testosterone in males. Gonadal tissue samples were collected and taken for histological examination. These were fixed with Smith´s fixative, embedded in paraffin, stained with Gill hematoxylin and eosin and examined by light microscopy.
The results obtained in this study allow to describe and to interpret the different phases of the gonadal development that take place in different enviromental conditions. It was observed that this species has an annual reproductive cycle, beginning in autumn and with spawning in spring. The variations in plasma reproductive steroid concentrations are correlated with the morphologic changes of the gonad.

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P03-29

EFFECT OF MAXIMAL MUSCULAR EXERCISE ON TNF-α : ATHLETE – SEDENTARY COMPARISON

Tabka Z., Denguezli M., Debbabi H. ., Ben Jabrallah M., Gaied S., Chouchane L.

Tumour necrosis factor-alpha (TNF-α) is a multi-functional cytokine that arouses a particular interest at the fundamental level as well as at the clinical one. Initially described for its anti tumour property, this agent reveals it self as an important mediator of inflammation, besides, it has been shown that it is associated to many different auto-immune infectious and tumoral diseases.
The aim of this study is to identify the effect of maximal muscular exercise on the plasma concentration of TNF-α.
The experiences concerned healthy volunteers : seven athletes and eight sedentary, each of them underwent a triangular effort exercise on an ergometer bicycle.
Two blood samples were taken, one before, and the other immediately after the exercise. The plasma concentrations of TNF-α were measured using an enzyme-linked immunosorbent assay (ELISA).
This measurement showed a significant increase of TNF-α plasma concentration both for the athlete and sedentary subjects (before 20.37 pg/ml ± 25.39 ; after 123.57 pg/ml ± 120.35) (before 18.33 pg/ml ± 19.74 ; after 54.59 pg/ml ± 67.92). This increase is much more significant as for athletes. It is estimated at 500% versus 200% for the sedentary.
During an intense physical activity, the muscle is mechanically injured and this process stimulates the liberation of inflammatory cytokines by tissular macrophages.
A continual production of TNF-α engenders an excessive inflammatory response followed by an increase of the prostaglandin and of the stress hormones secretion, modifying, by the same way, the cytokinic responses profile and this results in a less efficient immunitary responses, a process which increases upper respiratory tracts infect a few hours or days after the exercise.

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P03-30

ION TRANSPORT AND BIOMINERALIZATION IN SCLERACTINIAN CORALS

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Whereas scleractinian corals are one of the major calcifying groups of organisms in the living world, calcification processes largely remain a biological enigma. Within the organic matrix, calcification consists of the precipitation of $CaCO_3$ according to the equation $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$.
Calcification occurs in a "biologically-controlled medium" at the innermost margin of the ectodermal (calicoblastic) cells of the aboral layers, consequently, to reach the skeleton, ions have to cross oral and then aboral cell layers.
Experiments were conducted on Stylophora pistillata microcolonies.
We demonstrate that Ca^{2+} ions cross the oral layers by a diffusive, paracellular pathway. Experiments with channel inhibitors show that Ca^{2+} transepithelial transport involves at least one transcellular pathway across the calicoblastic cells. This pathway involves L-type Ca^{2+} channel proteins which alpha1 subunit has been cloned and immuno-localized on the calicoblastic cells. Identities and conservative substitutions between the rabbit alpha1C-subunit and the coral Ca^{2+} channel are 52.5% and 86% respectively, demonstrating the conservation of ionic supports through evolution. Concerning Ca^{2+} export from the calicoblastic cells to the skeleton, we have cloned a Ca-ATPase gene. Phylogenetic reconstruction shows that the pump is closely related to the PMCA family found in vertebrates. By fluorescence in situ hybridization we show the preferential expression of the pump within the aboral tissue.
Regulation of biomineralization also implies the fine control of pH. Using an antibody raised against a P-type proton-ATPase of yeast, we observe a specific staining of the calicoblastic epithelium. We hypothesize that this protein is necessary for the elimination of H^+ resulting from the deprotonation of HCO_3^- during $CaCO_3$ precipitation. We actually purify this carrier.
We thus propose a model for ion transport and outline the role of calicoblastic cells in coral calcification.

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P03-31

THE INFLUENCE OF NITRIC OXIDE ON PHYSICAL EFFORT CAPACITY AND OXIDATIVE STRESS

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Numerous studies support the implication of nitric oxide (NO) in diverse physiological processes but there are few accounts of the influence of NO in physical exercise capacity (POC). There is though a well-documented correlation between NO metabolism and reactive oxygen species production (ROS). We investigated the influence of NO on POC of adult rats, through administration of L-arginine and methylen blue, a precursor and an antagonist of NO activity, respectively, and we measured its effects through quantification of two reliable oxidative stress markers, that is, malondialdehide (MDA) and ceruloplasmine. We used a pretest-postest experimental design according to which we preliminary measured the POC of 24 adult Wistar rats. The animals were thereafter injected intravenous (i.v.) either with L-arginine (n = 12; 300 mg/kg) or methylen blue (n = 12; 0.3 mg/kg). Two hours after the experimental manipulation, we quantified again the POC of rats, using the data from pretest as control for comparison. We dosed the level of glucose, proteins, lipids, MDA, and ceruloplasmine from blood. Our results indicated that L-arginine determines a significant reduction of both distance, and duration of physical effort, correlated with significant reductions of glucose and protein levels. Only when associated with physical exercise L-arginine determines a significant reduction of MDA and ceruloplasmine concentrations. In contrast, methylen blue increases the POC of rats, associated with non-significant metabolic modications, but with a significant reduction of MDA concentration, and a significant increase of ceruloplasmine concentration. These results led us to conclude that NO reduces PEC, possibly acting as a ROS scavenger, although its participation as a reactive form of O_2 to muscular fatigue cannot be definitely excluded. Methylen blue increases PEC along with antioxidant activity, probably through a different mechanism.

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P03-32

EFFECTS OF WEAK ELECTROMAGNETIC FIELD ON CUTANEOUS VASOMOTOR RESPONSES

Tretjakovs P., Jurka A., Stefanovska A., Aivars J., Pirags V.

The purpose of our study was to evaluate by means of laser Doppler fluxmetry (LDF) the effect of weak electromagnetic field (wEF) on cutaneous vasomotor activity. The subjects were 22 diabetic patients (D) without late diabetic micro- or macro-vascular complications and 20 healthy volunteers as controls (C). The groups were matched for age, sex, and body mass index.We recorded cutaneous blood flow and changes in the flow induced by wEF (0.9+/-0.6 mT, 80+/-34 Hz, 60 min) by alfa-PULSAR (Electronic Ltd) on the pulp and on the dorsum of the big toe using LDF (PeriFlux 4001, Perimed). We evaluated changes in the dynamics of the LDF signal by spectral analysis (SA) based on wavelet transformation. The vasoconstrictor response to deep inspiration was measured (on the pulp) before and after 60 min of wEF. The results showed a significant increase in LDF maximum compared with resting LDF on the dorsum in both groups (D, $p<0.05$; C, $p<0.0001$), but on the pulp - only in the control group ($p<0.001$). On the dorsum, the data of SP showed an increase in the mean amplitude of oscillations of LDF in the frequency interval from 0.009 to 2.3 Hz compared to values at rest (D, $p<0.01$; C, $p<0.001$). Comparing the mean amplitude from 0.009 to 0.018 Hz (reflect metabolic activities), an increase was only in controls on the pulp ($p<0.05$). After 60 min of wEF influence, the vasoconstrictor response to deep inspiration increased in both groups ($p<0.05$ and $p<0.001$), but the effect was lower in the diabetic group compared to the control group ($p<0.001$). In conclusion, our findings indicate that wEF induces functional alterations (endothelial vasoactive production), which can improve circulatory performance. The alterations in metabolic activity due to wEF may improve control of the autonomic sympathetic neurovascular system in diabetic patients.

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P03-33

SEASONAL TESTICULAR CYCLE IN MERIONES SHAWI (GERBILLIDAE). EFFECTS OF TEMPERATURE AND PHOTOPERIOD.

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Seasonal reproductive cycle of male gerbils, Meriones shawi shawi (desert rodent, Gerbillidae) was determined in animals reared under natural temperature and photoperiod conditions. There was no statistically significant variation in plasma testosterone values measured monthly during the eighteen months of experiment. Testis volume reached a maximum in spring and summer. Minimal values occurred during November-December, in the colder months and in short photoperiod (winter solstice).
The effect of cold temperature (1 month at 10°C) on testicular activity in animals maintained at 25°C before and after this "cold experiment" was investigated. There was no statistically significant variation in plasma testosterone values measured during these cold or hot temperature conditions. After the 10°C period the testis volume was found to be decrease and come back to normal values when brought back to 25°C.
The effect of photoperiod, short days (8L:16D) versus long days (16L:8D) was studied. There was no statistically significant difference between plasma testosterone values measured in animals stabulated in short days and in long days. The testis volume increased in animals maintained in long days compared to animals maintained in short days.
These observations show an apparent dissociation between exocrine and endocrine testicular activity in this species. This species exhibits a constant endocrine activity through the whole year. On the other hand, the testis volume (more representative of the exocrine activity) appears dependent on the photoperiodic and thermic conditions.

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P03-34

POLYMORPHISM OF THE ROD VISUAL PIGMENT BETWEEN ALLOPATRIC POPULATIONS OF THE SAND GOBY

Donner K., Jokela M., Vartio A., Fyhrquist-Vanni N., Paulin L., Merilä J.

The rod visual pigments of four populations of sand goby (Pomatoschistus minutus) living in spectrally different light environments were studied by micro-spectrophotometry and opsin sequencing in a quest for adaptive differences within the same species. The populations were that of the Baltic Sea at the SW coast of Finland (maximal transmission of the water 550-575 nm), Kattegat at the west coast of Sweden (500-550 nm), the English Channel at Plymouth (500-525 nm) and the Adriatic Sea near Venice (450-475 nm). Small but statistically significant differences between the absorbance maxima of
the rhodopsins (varying from 508.3 nm in the Baltic to 503.0 nm in the Adriatic population) correlated with the differences in the light environment (except in the English vs. the Swedish population). Opsin gene sequences were compared, on one hand, to reveal functional amino acid substitutions that may underlie the spectral differences, on the other hand to be related to cytochrome B phylogeny.

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P03-35

COPPER ZINC SOD IN ANEMONIA VIRIDIS, AN ANIMAL SUBMITTED TO DAILY HYPEROXIC CONDITIONS

Plantivaux A., Richier S., Merle P.-L., Furla P., Garello G., Zoccola D., Tambutté S., Tambutté E., Allemand D.

Animal tissues submitted to hyperoxic environment usually display severe damage, mainly due to radical oxygen species (ROS) overproduction. However, some animals, such as sea anemones and corals, are adapted to hyperoxic conditions. These animals harbor symbiotic protists, named zooxanthellae, which possess photosynthetic capacities. Using O_2 microelectrodes implanted within the sea anemone Anemonia viridis (cnidarians), we confirmed that, under light condition, zooxanthellae rapidly photosynthesize oxygen at levels toxic for many cells (3-times normoxia). In these conditions, no apparent tissue damage occurs, suggesting that these animals are good models to study ROS detoxifying strategies. Moreover, the symbiosis is sometimes disrupted, leading to a phenomenon called bleaching, responsible for massive worldwide cnidarian death episodes. Although the cellular signaling remains to be identified, it has been proposed that ROS could play important roles in the processes leading to bleaching. This explains why the study of ROS detoxification in these cells is also of environmental interest.
We focused our interest on superoxide dismutases (SOD), which are the first actors of the ROS enzymatic defenses. Using native-gel electrophoresis and inhibitors, a great variety of SOD was distinguished in Anemonia viridis: CuZn, Mn and also Fe SOD, with a specific tissue distribution. We further characterized CuZnSOD, which activity appeared restricted to animal tissues. By a molecular approach, two CuZnSOD isoforms were cloned (AY164663 & AY164664), having 40% of homology and distinct 5' regions. Using in situ hybridization, we confirmed that both CuZnSOD transcripts were immunolocalized in animal tissues, but not in zooxanthellae.
These results are a first step toward the understanding of the mechanisms of resistance against oxidative stress in the partners of this particular symbiosis.

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P03-36

CARDIAC ADAPTATIONS IN THE TIME COURSE OF AN ALTITUDE TRAINING CAMP IN A RAT MODEL

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Introduction : Recent studies have reported controversial results on the ability of altitude training to induce increase in aerobic performances in athletes. Although cardiovascular system is playing a key role in aerobic performances, there are very few reports on cardiac morphological and functional adaptations following altitude training, and conflicting results have been found. The aim of this study was to investigate the cardiac modifications induced by 5 weeks aerobic training at altitude.
Methods : Twenty six rats were randomly assigned to 3 groups : N (n=9, living in normoxia, 80 m, PIO2= 159 mmHg), NT (n=9, living and training in normoxia, 80 m, PIO2= 159 mmHg) and CHT (living and training in hypoxia, 2800 m, PIO2= 105 mmHg). CHT and NT were subjected to the same relative training endurance program for 5 weeks (45 min per day, 80% maximal aerobic velocity, 5 days per week). Morphological and functional cardiac parameters were evaluated by Doppler-echocardiograhy, catheterization and aortic pulsed-Doppler transducer.
Results : In CHT group, the main findings were a right ventricular hypertrophy [right ventricular mass: (CHT)=130±14.7 mg/100g and (NT)=118±11.8 mg/100g; $p<0.05$] associated with a reduced pulmonary

right ventricular flow peak velocity [(CHT)=58±7 cm/s and (NT)=74.9 cm/s; p<0.001]. Moreover trained rats, CHT and NT presented a concentric and eccentric left ventricular hypertrophy respectively (NT and CHT versus N; p<0.01). However, the resulting increase in systemic cardiac output (Qc) remains lower in CHT rats when compared to NT [(CHT)=16±2 ml/min/100g and (NT)=18±2 ml/min/100g; p<0.05].
Conclusion : Our results suggest that both training and hypoxia could induce changes in loading conditions, leading to the specific cardiac adaptations reported here. Moreover, those modifications suggest that cardiovascular system could, in part, directly be involved in the limitation of the beneficial effects of altitude training.

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P03-37

MUSCLE AND BRAIN OXYGENATION DURING EXHAUSTIVE CYCLING IN THE CIRRHOTIC PATIENT
Bay Nielsen H., Henry Secher N., Ott P.

In cirrhotic patients exercise capacity is reduced and we hypothesised that the hyperdynamic splanchnic circulation induces insufficiently elevated or even reduced O_2 availability of muscle and brain as evaluated by near-infrared spectrophotometry (NIRS). Eight cirrhotic patients underwent semi-supine cycling at light (29 ± 3 W, mean with SEM), moderate (57 ± 5 W) and exhaustive (84 ± 11 W) intensities for 10 min each. Arterial and hepatic venous blood blood samples were obtained and also hepatosplanchnic (HSP) blood flow was determined using constant infusion of indocyanione green. Resting heart rate and mean arterial pressure were 70 ± 4 b/min and 89 ± 2 mmHg, respectively, and they increased to 140 ± 6 b/min and 117 ± 4 mmHg, respectively, during exhaustive exercise. Cardiac output increased from 6 ± 1 to 14 ± 1 l/min, while HSP became reduced (from 1.0 ± 0.1 l/min to 0.7 ± 0.1 l/min). Arterial lactate reached 2.7± 0.6 mmol/l compared to 0.3 ± 0.1 mmol/l at rest with enhanced HSP lactate uptake (0.3 ± 0.1 vs. 1.4 ± 0.2 mmol/min) and glucose output (0.6 ± 0.1 vs. 1.5 ± 0.2 mmol/min) during cycling. Cerebral oxygenation increased from 67 ± 6% to 71 ± 4% during exercise. An elevated concentration of oxygenated and deoxygenated hemoglobin of muscle during exercise reflect that muscle blood flow more than compensated for an increase in O2 extraction. The results suggest a cellular impairment rather than an attenuated availability of substrate and O_2 to muscle and brain during exercise in the cirrhotic patient.

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P03-38

ELECTROENCEPHALOGRAM IN CHILDREN WITH DIABETES MELLITUS
Sfredel V., Trăilă A., Iancu I., Dănoiu S. Matcas H., Sfredel D.

Objectives. The purpose of this study was to find the relation between diabetes mellitus (DM) at children and the electroencephalogram (EEG) changes, the correlation between these changes and the stage of this disease or the number of the severe hypoglycemic attacks.
Material and methods. The standard EEG was obtained from 15 children with DM, mean age 15.2 years, 5 boys and 10 girls. Patients were randomized in two groups by the criteria stage of DM and the number of the severe hypoglycemic attacks (convulsions). We have also recorded EEG at 15 normal children, same mean age and sex.
We have analyzed the basic parameters of EEG (amplitude, frequency, index), the placement of the alpha and beta waves and the lesional and irritative waves.
Results. Discussions. The analyze of the results took into account the specific of the EEG in children and specially the high index of irritative waves. We found 38.4 % children with convulsive hypoglycemic attacks and a high correlation between these attacks and the historic of DM.
The standard EEG was normal at 40,5 % of patients. 55 % of patients reveal a high index of unspecify irritative waves (spikes, polyspikes, slowly, hypervoltated waves), most of them syncronisated, bilateral and unsistematised. We also recorded a relative high index of slowly waves in theta and delta bands, with a mean voltage. In 54.5 % patients we found a positive correlation between the changes of EEG and the number and the severity of the hypoglycemic attacks.
Conclusions. 55 % of the children with DM reveal changes of EEG in different degrees of severity in correlation with the historic of DM or the number of convulsivante hypoglycemic attacks.

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P03-39

SERUM LEPTIN, INSULIN AND CORTISOL LEVELS AFTER A SUBMAXIMAL EXERCISE IN SEDENTARY AND TRAINED MEN
Zaouali Ajina M., Bouassida Chikhaoui A., Charfeddine B., Miled A., Gharbi N., Tabka Z., Zbidi A.

The aim of this study is to determine the effect of a submaximal exercise and recovery on leptin, cortisol and insulin levels.
The experimental protocol consists in a submaximal exercise on 2 sessions of 21min each 70% maximal oxygen uptake (VO2 max) for the first exercise and to 85% VO2 max for the second exercise separated by a passive recovery period (40 minutes) or active recovery at 30% of VO2max. Blood samples are taking before and after every session and after 2 hours and 24 hours of recovery period.
After a submaximal exercise of 21 minutes leptin concentration doesn't change for either sedentary or trained subjects. After 2 hours of recovery, a significant decrease in two groups is noted compared to the value of the end of the first session. There's a significant difference on leptin level between the two groups at all steps and during the two protocols. The sedentary presents more elevated leptin values that those of trained men.
The cortisol level increases significantly of 21,5 % at the sedentary following the first session of the exercise. However, a is noted after 2 hours of recovery compared to the end of first session. No remarkable difference is noted between the two groups concerning cortisol level either in protocol with passive or active recovery. A significant reduction of 52% at the sedentary in insulin level is found at the end of the first session of exercise. This value is re-establishes at the end of the passive recovery. However, a significant reduction is also noted at the end of the second session and remains after 2 hours of recovery to reach after 24 hours a value that is not different of the basis value. No significant difference is noted between the sedentary and trained subjects in two protocols.
Leptin responses to submaximal exercise appears therefore during recovery period and precisely after 2 hours and its during this period that appears the effect of other regulating hormones as insulin and cortisol.

Service De Physiologie Et Des Explorations Fonctionnelles Faculte De Medecine. Sousse. Tunisie

P03-40

IL-1b EXPRESSION AND SECRETION BY HUMAN ORAL EPITHELIAL CELLS IN RESPONSE TO C. ALBICANS INFECTION
Mostefaoui Y., Claveau I., Rouabhia M.

Oropharyngeal candidiases are the most common form of mucosal fungal infections and are primarily caused by Candida albicans, a dimorphic fungal commensal organism of the gastrointestinal tract. Clinical and experimental observations suggest that, through cytokines and chemokines, oral epithelial cells play key role in host defense against candidiasis. In this context we sought to investigate whether oral epithelial cells convey IL-1b as a pro-inflammatory cytokine against C. albicans infection. To reach our goal, we engineered human oral mucosa, and then infected the tissue with live or dead C. albicans (10ex5 yeast/cm2). At the end of each appropriate contact period, we measured the expression of IL-1b at the mRNA and protein level. We also evaluated the effect of the tissue on C. albicans adherence and growth. Only live C. albicans modulate IL-1b expression and secretion. In deed, our results showed that IL-1b mRNA expression was significantly increased at early infection stage, and then deceased at later infective phase. The modulatory effect of C. albicans on IL-1b expression was confirmed by an increased amount of inactive form (33 kDa) of IL-1b at early infection period and significant decrease at subsequent contact periods. When active form (17 kDa) was measured in the supernatant, it showed a significant and time dependent increase of IL-1b secreted by epithelial cells infected with live C. albicans. These results indicate that IL-1b is involved in the local defense against C. albicans infection. On the other hand, we showed that oral epithelial cells down-regulate the growth of live C. albicans. Taken together, these results provide additional evidence for the contribution of oral epithelial cells to local defenses against exogenous stimulation such as C. albicans infection. (Funded by the FRSQ, NSERC and CIHR).

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S4 BLOOD PRESSURE REGULATION

ORAL SESSION

S4-1

RENAL SODIUM HANDLING AND BLOOD PRESSURE CONTROL

Burnier M.

The association between dietary sodium intake and blood pressure and its cardiovascular complications has been recognized for several decades. The evidence that sodium plays an important pathophysiological role in the development of hypertension comes from various sources including epidemiological, physiological and pathophysiological studies. The recent report of the molecular mechanisms involved in the pathogenesis of some rare forms of hypertension with a simple Mendelian inheritance pattern, i.e the Liddle's syndrome, the glucocorticoid-remediable hypertension and the apparent mineralocorticoid excess (AME) has revived the interest for salt-induced hypertension since the reported genetic defects decrease the ability of the kidneys to excrete sodium. Whether the same mechanisms apply for highly prevalent forms of essential hypertension is still unknown but it is very likely that other mechanisms contribute to the increase in blood pressure in essential hypertension. In fact, there is also increasing experimental and clinical evidence that an increased sodium reabsorption in the renal proximal tubule could contribute to the development of hypertension. Using lithium clearance as a marker of renal sodium handling by the proximal tubule, we have demonstrated in rats as well as in humans that hypertension is associated with an impaired modulation of sodium excretion in the proximal tubule. More recently, we have also found that sodium handling by the proximal tubule is an independent determinant of the sensitivity of blood pressure to salt in hypertension. The development of new molecular techniques and possibly also new physiological tools to investigate in greater details the renal response to sodium should offer the opportunity to revisit the still partly puzzling association of salt and blood pressure.

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S4-2

COMPARATIVE PHYSIOLOGY OF THE CARDIOVASCULAR SYSTEM

Westerhof N. (1), Segers P. (2), Stergiopulos N. (3)

Blood pressure and Cardiac Output, result from the interaction of heart, including venous return, and arterial system. Using simple descriptions of heart and arterial load we can quantitatively describe how pressure and flow arise. We then derive ventriculo-arterial coupling parameters and compare them in different mammals.
The heart is described by the varying elastance model. Parameters: slopes of the diastolic (Emin) and end-systolic (Emax) pressure-volume relations, and their intercept with the volume axis (Vd). Ventricular filling pressure is Pv. The arterial load is modeled with the 3-element windkessel model: peripheral resistance (R), arterial compliance (C) and aortic characteristic impedance (Zc), with RC = τ the time constant of diastolic aortic pressure decay. This overall model allows evaluation of the contribution of the individual parameters to pressure and flow. Application of dimensional analysis leads to nondimensional ventriculo-arterial coupling parameters: τ/T and CEmax.
Comparative physiology makes use of the allometric equation: P = P0 Me, with P the parameter of concern (reference value P0), M body mass, and e exponent. A double logarithmic plot of T and τ, against body mass shows that the ratio of τ/T is similar in all mammals. Since PP/Pmean = τ/T it follows that with similar mean pressure also pulse pressure is the same in mammals. An analogous reasoning holds for CEmax. The ratio of characteristic impedance and peripheral resistance is similar in mammals too. This implies that systemic arterial input impedance, when normalized, is similar in mammals, suggesting similar shapes of pressure and flow waves. While pressures are similar, Cardiac Output and whole body metabolism are proportional to body mass (i.e., exponent e = 1).
We conclude that comparative physiology of cardiovascular parameters explains the similarity in pressure and flow wave shapes.

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OC04-1

PROTECTION OF HEART FROM REPERFUSION INJURY BY THE MITOCHONDRIAL PERMEABILITY TRANSITION INHIBITORS

Sagach V., Shymanskaya T., Nadtochiy S.

Last years it have been shown the postreperfusion disturbances of cardiac contractility will be due to the different metabolites that release from mitochondria under an opening of mitochondrial permeability transition pore. These agents effect on heart function and vessels tone. In experiments on isolated hearts of guinea-pigs, perfused under Langendorff preparation, possible protection of hearts from reperfusion injury by the known inhibitors of mitochondria permeability transition pore - cyclosporin A, and trolox - water-soluble vitamin E - was studied. It has been shown that cardiac reperfusion was followed with an increase in an oxygen cost of myocardial work by 83% from control level in 40 min of reperfusion, in addition to the disturbances of cardiac contractility, tone of the coronary vessels and heart rate. The heart and oxygen metabolism disturbances, stimulated by global 20 min ischaemia and reperfusion, were significantly decreased by a preliminary application of investigated agents. Trolox improved cardiac recovery both when it was perfused in vitro and after its administration per os before the heart removing. In this case in 40 min of heart reperfusion left ventricular developed pressure was 79% as compared to 51% in that at control; dP/dtmax and dP/dtmin by 88% and 85% accordingly against 66% and 45% in control; oxygen cost of myocardial work didn`t change reliably. Conclusion: postreperfusion disturbances of cardiac contractility, tone of the coronary vessels and heart rate, as well as noneffective oxygen utilization by the heart tissue were due to an opening of mitochondrial permeability transition pore. Cyclosporin A and trolox protected the heart from reperfusion disturbances.

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OC04-2

ANP HINDERS POST IMMERSION VASOMOTOR ADJUSTMENTS

Mourot L., Wolf J.P., Robinet C., Galland F., Bouhaddi M., Courtiere A., Meliet J.L., Regnard J.

Changes in vasomotor tone and hemodynamics were compared before and after dehydration linked and not to water immersion. 10 highly fit divers underwent two similar 6 h exposures (periods of alternate rest and exercise) performed in a dry ambience (DY) and, 4 weeks later, with immersion up to the neck in 15°C water (WI). Venous blood was taken thirty minutes before and after each exposure, to assess hematocrit (Hct), hemoglobine (Hb), plasma concentration of total proteins and vasomotor agents: noradrenaline (NA), arginin vasopressine (AVP) and atrial natriuretic factor (ANF). Heart rate (HR), stroke volume (SV; cardiac impedance), systolic (SAP), diastolic (DAP), and mean (MAP) arterial pressures were also measured. Htc and Hb were used to estimate changes in plasma volume (PV) and to correct plasma concentration of vasoactive mediators. Total peripheral resistance (TPR) was calculated as the ratio of MAP/CO (cardiac output). Weight loss was similar after both WI and DY (mean 2.4 kg; $p<0.05$ with baseline on each day) but PV reduction was greater after WI than DY (-14.7 ± 1.6 % and -9.7 ± 1.6 %; $p<0.05$). CO and MAP were maintained, but HR was reduced only after DY ($p<0.05$) whereas SV was reduced only after WI ($p = 0.07$). Vasoconstrictor agents were released ($p<0.05$) in both dehydration conditions with NA and AVP higher ($p<0.01$) after WI than DY. After DY, DAP (NS) and TPR ($p<0.05$) were increased, but not after WI. Thus, dehydration prompted the release of vasoconstrictor mediators. After DY, CO was maintained with an increased TPR, triggering in turn a baroreflex decreased HR. Conversely, within one hour after WI a marked trend to SV decrease was present, but HR and TPR were unchanged despite the twofold larger increase in plasma vasoconstrictor mediators than after DY. We submit that the amount of ANF released during WI impeded the action of NA and AVP, causing in turn the supine unchanged arterial pressures and TPR.

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OC04-3

DETERIORATION IN CAROTID BAROREFLEX DURING CAROTID ENDARTERECTOMY

Sigaudo-Roussel D., Evans DH., Naylor AR., Panerai RB., London NL., Bell P., Gaunt ME.

Blood pressure instability after carotid endarterectomy (CEA) has been associated with a disturbance of the baroreflex control mechanism caused by the surgery on the carotid sinus region. The purpose of this study was to identify if a deterioration in carotid baroreceptors occurs during the surgery. Heart rate (HR) and blood pressure (BP) were recorded continuously in 60 patients undergoing CEA as well as pre-operatively and post-operatively at 2 days and 6 weeks. The baroreflex sensitivity was determined by cross-spectral analysis of HR and SBP. During the surgery, three tests were used to assess the baroreflex response. The first test simulated a sudden fall in systemic blood pressure by clamping the common carotid artery. The second test simulated a rise in systemic blood pressure by applying a pressure using a rubbing action on the luminal surface of the carotid sinus region. The rub test was performed twice, once with the atheromatous plaque in situ and once when the plaque had been removed. The third test is the clamp removal and restoration of blood flow through the carotid sinus. Carotid cross-clamping increased systolic blood pressure (SBP) from 117±3 mmHg before clamping to 125±3 mmHg ($P<0.05$) at 30 beats after clamping. The first rub test with the plaque in situ decreased SBP from 121±3 mmHg to 117±3 mmHg ($P<0.01$) at 10 beats after the rub test, indicating a functioning baroreceptor reflex. The second rub test increased SBP from 126±3 mmHg to 128±3 mmHg, ($P<0.05$). SBP dropped ($p<0.01$) when unclamping suggesting a selective alteration of the baroreflex sensitivity. The baroreflex sensitivity was significantly reduced 2 days post-operatively than pre-operatively ($P<0.05$). These findings suggest that the act of plaque removal could be associated with a partial disruption of baroreceptor mechanism in the carotid artery. This could affect type I baroreceptors.

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OC04-4

MECHANISMS OF ACETYLCHOLINE AND FLOW-INDUCED VASODILATATION IN MESENTERIC ARTERY
Lopez V., Thorsgaard M., Buus NH., Simonsen U.

The purpose of the present study was to investigate whether endothelium-dependent vasodilatation evoked by acetylcholine and flow is mediated by the same mechanisms in isolated rat mesenteric small arteries. Mesenteric small arteries were mounted in a pressure-myograph for the measurement of internal diameter. The segments were stretched to 110% of passive length and pressure kept at 80 mmHg. Vessels were contracted with the tromboxane analogue, U46619 (10-7M) and flow was applied by a peristaltic pump resulting in shear stress levels of 4 and 16 dyn/cm2. Indomethacin was present throughout the experiment. In endothelium-intact vessels low (5.1±0.6 dynes/cm2) and high (19±2 dynes/cm2) shear stress evoked vasodilatations which were reduced by endothelial cell removal, 68±11 and 68±8% respectively ($P<0.05$, $n=7$), while acetylcholine vasodilatation was abolished. A nitric oxide synthase inhibitor, asymmetric dimethylarginine (ADMA, 1 mM), reduced low and high shear stress-evoked vasodilatation, but it did not change acetylcholine-evoked vasorelaxation. Inhibition of Ca^{2+}-activated K^+ channels with the combination of apamin (0.5*10-6M) and charybdotoxin (0.1*10-6M) did not change shear stress and acetylcholine-evoked vasodilatation, but in combination with ADMA, they abolished acetylcholine-evoked vasodilatations while shear stress-induced vasodilatation was unaltered. The presence of an Src tyrosine kinase inhibitor, herbimycin A (10-6M) had no effect on acetylcholine vasodilatations, but it abolished low and high shear stress-evoked vasodilatation, respectively, 32±12 and 68±14% ($P<0.05$, $n=8$). The present study suggests that Ca^{2+}-activated K^+ channels are involved in acetylcholine–evoked vasodilatation, while a Src tyrosine kinase is involved in flow-induced nitric oxide (NO)-mediated vasodilatation in rat mesenteric small arteries.

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OC04-5

CONTRIBUTION OF NO-SYNTHASE AND ARGINASE TO MEDULLARY CARDIOVASCULAR CONTROL IN RATS
Shapoval L.N., Sagach V.F., Pobegailo L.S., Dmytrenko O.V.

In acute experiments on anaesthetized normotensive and spontaneously hypertensive rats we attempted to analyze the contribution of neuronal NO-synthase (nNOS) and arginase to the activity of the cardiovascular neurons within the medulla oblongata. Unilateral injections of both L-arginine, substrate for NO synthesis, and NO donor sodium nitroprusside into the medullary cardiovascular nuclei (NTS, DVN, AMB, LRN) in most experiments resulted in lowering the SAP level mainly due to reducing the peripheral vascular resistance. Their effects were more pronounced in spontaneously hypertensive rats as compared to normotensive ones. The data obtained give evidence for an uneven distribution of NO-producing neurons within the medulla in dorso-ventral direction. Preliminary inhibiting nNOS with 7-nitroindazol attenuated the haemodynamic responses on L-arginine injections into the medullary structures. Injections of either nNOS inhibitor L-NNA or antagonist for arginase norvaline into the medullary nuclei induced mainly an elevation of the SAP which was similar in both normotensive and spontaneously hypertensive rats. Both enzymes are known to use L-arginine as a substrate for their metabolism, so they can compete for it in some cases. Distribution of arginase- containing neurons within the medulla oblongata seems to be also uneven and corresponds to that of NO-producing ones. We suggest that arginase participates in the mechanisms of the central cardiovascular control together with nNOS perhaps modulating the activity of nNOS

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OC04-6

PHYSIOLOGICAL AND METABOLICAL STUDIES OF HYPERTHYROID RAT HEART
Revnic C.R., Revnic F., Botea S., Voiculescu V.

Introduction: A better understanding of action mechanisms of Thyroid hormone on peripherial vascularisation is essential for the recognising T3 as a therapeutic agent.The aim of our study was to investigates the effect of T3 administred in excess on physiological and biochemical parameters of rat myocardium Material and methods:16 Wistar rats have received injections with T3 4.5 mg/kg body weight for four weeks.The hearts has been mounted in Langendorf retrograde perfussion using Krebs Hanseleit buffer for 30 minutes followed by 60 minutes reperfussion. Heart rate,coronary flow and left ventricle developed pressure have been recorded at the end of stabilisation period and during reperfussion in order to see the heart capacity to recover after a medium ishemia. Biochemical parameters:LDH,CK,SOD,CT,LDL and HDL fractions ,total lipids and lipid peroxides have been determined using standard methods.Results: During reperfusion,cardiac frequency is unchanged while LVPD is very much amplified and coronary flow exibits a decrease in its values in hyperthiroid hearts comparatively with contols. There is an increase in myocardium and plasma CK and LDH is four times higher than in contols, accounting for the increase in anaerobic glycolisis.A decrease in SOD in hyperthiroid myocardium accaunts for the onset of oxidative stressTotal lipids are significantly increased serving as energetic support for accelerated metabolism Conclusion: Following a medium ischemia hyperthiroid heart has a good capacity to recover ,the only modified parameter is LVDP which is very much amplified. Due to low plasma cholesterol levels and of the absence of a significant modification of plasma lipid peroxides ,hyperthiroid rats are not the target for atherogenic factors which are present in other forms of arterial hypertension.

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S4-3

GENETICS OF FAMILIAL HYPERKALAEMIC HYPERTENSION
Delaloy C., Hachouel J., Jeunemaitre X.

Familial Hyperkalaemic Hypertension (FHH), also called Gordon's syndrome or pseudohypoaldosteronism type II, is a rare mendelian autosomal dominant form of hypertension associating hyperkalaemia, low renin and aldosterone plasma levels. The study of a large family from the North of France showed no strong relationship between the severity of the metabolic disorders and blood pressure, which exhibited a positive relation with age as in the normal population. Affected patients are very sensitive to small doses of thiazide diuretics. FHH is the mirror image of Gitelman's syndrome, caused by mutations in the thiazide-sensitive sodium-chloride cotransporter (NCC). In addition to phenotypic heterogeneity, genetic heterogeneity has been demonstrated. Three loci have been identified at 1q, 17q and 12p, and there is evidence for of at least a fourth locus.
The first two genes found as responsible for the disease, WNK1 and WNK4, belong to a new family of serine-threonine kinases. The causing-disease mutations in WNK4 are missense mutations clustering in highly conserved domains close to the coiled-coil domains. The mutations in WNK1 are large deletions in intron 1, which could increase the expression of the gene. The

regulation of WNK1 expression is complex with at least two isoforms, expressed ubiquitously or specifically in the kidney (mainly in the distal tubule). The two kinases are localized either in the cytoplasm (WNK1) or at the tight junctions (WNK4) of the distal tubular cells where they could increase sodium reabsorption by a mechanism that might involve an increase of NCC activity and/or chloride transport through tight junctions. A decrease of NCC-mediated sodium flux by WNK4 has recently been demonstrated in Xenopus oocytes, while WNK1 prevented WNK4 inhibition of this transporter. These results could explain the positive effects of WNK4 mutations or increased expression of WNK1 on sodium transport in the distal tubule.

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S4-4

INTERACTIONS BETWEEN POSITIVE AND NEGATIVE FEEDBACK IN THE REGULATION OF BLOOD PRESSURE
Malliani A.

The neural regulation of arterial blood pressure is traditionally attributed to the interaction of two main mechanisms: central integration and peripheral negative feedback reflexes. Numerous experimental observations, however, have clearly demonstrated the existence of peripheral cardiovascular reflexes, usually mediated by sympathetic afferent fibers (Rev Physiol Biochem Pharmacol 1982; 94: 11-74), that are unequivocally excitatory in nature. For instance, it has been demonstrated that the distension of a short segment of the thoracic descending aorta, i.e. a stimulus mimicking the effects on aortic wall of an increase in pressure, induces a reflex pressor response through a sympathetic excitatory reflex initiated by aortic receptors (Circ Res 1974; 34: 78-84 and 1982; 50: 125-132). Accordingly, the existence of positive feedback reflex mechanisms was proposed. Furthermore, during the stretch of the thoracic aorta the gain of the reflex bradycardia response to an increase in arterial pressure was markedly blunted. On the other hand it is well-known that quadriplegic patients can undergo marked hypertensive crises during gentle stimulation of the abdomen. The new scheme of neural regulation of arterial pressure that we have been proposing during the last two decades is based on the continuous interaction of three mechanisms: 1) central integration, 2) peripheral negative feedback and 3) peripheral positive feedback reflex mechanisms. The interaction of opposing principles, besides being a fundamental biological property, would ensure a more adequate control of cardiovascular variables in terms of stability and different velocities of changes (i.e. instability) according to the various behavioral needs. Finally, the excitatory cardiovascular reflexes mediated by sympathetic afferents may become particularly important in several pathophysiological conditions of paramount importance (Hypertension 2002; 39: 63-68).

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POSTER SESSION

P04-01

BIPHASIC EFFECTS OF ANGIOTENSIN (1-7) AND ITS INTERACTIONS WITH ANGIOTENSIN II IN RAT AORTA
Haulica I., Bild W., Mihaila CN., Ionita T., Boisteanu CP., Neagu B.

Objective: to investigate the effects and possible relationship between ang (1-7) and angiotensin II at the vascular level.
Method: Experiments were performed on isolated rat aortic rings perfused with Krebs-Henseleit saline, using isometric force transducers.
Results: Angiotensin (1-7) induced well-known endothelium-dependent relaxation and vasodilating effects in preparations precontracted with phenylephrine. Without preconstriction, angiotensin (1-7) in high doses (10-6 – 10-5 M) produced either a significant inhibition of angiotensin II vasoconstriction or a nontachyphylaxis vasopressor response. While losartan (a selective AT1 receptor antagonist) inhibited the vasoconstriction induced by angiotensin (1-7), A779 (a selective ang (1-7) receptor antagonist) blocked only its relaxation. Unlike losartan, blockade of AT2 receptors with PD 123319 remained without effect.
Conclusion: Taking into account the biphasic effects of angiotensin (1-7), we proposed that it is one of the active components of the renin-angiotensin system, which is involved as a modulator both in the counter-regulatory actions of angiotensin II and in the self-regulation of its own vasodilating effects.

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P04-02

ANTIHYPERTENSIVE AND DIURETIC EFFECTS OF THE LEAF EXTRACT OF ANNONA MURICATA (ANNONACEAE) IN RAT.
Dimo T., Mbuyo Pami E., Nguelefack T. B., Panjo Yewah M., Njamen D., Talla E.

In Cameroon, concoctions of Annona mirucata leaves are used in the treatment of arterial hypertension by traditionnal healers. The antihypertensive effects of the methanol extract of A. muricata leaves were evaluated in normotensive Wistar rats (NTR) and Salt-Loaded Hypertensive Rats (SLHR) using the direct cannulation method. Acute changes in urine volume and urinary excretion of Na^+ and K^+ were also studied. Intravenous administration of the extract induced a significant dose-dependent fall in mean arterial blood pressure (MABP). At the lowest dose of 5 mg/kg, the extract reduced MABP in NTR and SLHR by 27% and 34%, respectively. At 50 mg/kg, decreases of 37% and 53% were obtained in NTR and SLHR, respectively. The antihypertensive effect of the extract was more remarkable in hypertensive than in normotensive rats. A. muricata did not provoke significant changes in urine volume and excretion of Na^+ and K^+ in NTR at the dose of 150 mg/kg. Oral administration of the extract at 300 mg/kg, resulted in a significant increase in urinary volume in NTR and SLHR by 76% and 201%, respectively. Urinary excretion of Na^+ was increased by 804% in SLHR, whereas change in K^+ excretion was not significant. The extract of A. muricata is thus a promising source of antihypertensive and diuretic pharmaceuticals.

University of Yaounde I - Cameroon

P04-03

EFFECTS OF METOPROLOL ON ISCHAEMIA-INDUCED "COMPENSATORY" FLOW CHANGES IN ANAESTHETISED DOGS
Ványi J., Papp J.Gy., Parratt J.R., Végh Á.

In anaesthetised dogs occlusion of one of the main branches of the left coronary artery results in an increase in blood flow through the other main branch of the same artery. The underlying mechanisms of this "compensatory" flow increase occurring within the normal, non-ischaemic area are not known. In the present study we examined whether b1-adrenoceptors, which are present in both the myocytes and large coronary arteries in this species, are involved in this phenomenon. In 10 chloralose-urethane anaesthetised dogs ischaemia was induced by four 5 min occlusions of the left anterior descending coronary artery (LAD) with 10 min reperfusion intervals between the occlusions. One hour later, the selective b1-receptor antagonist metoprolol was infused in a dose of 1 mg kg-1min-1 in a side branch of the LAD, 20 min prior to, and then throughout, the

repeated occlusion / reperfusion cycles. Coronary blood flow was measured both on the LAD and the circumflex (LCX) coronary arteries by Doppler and electromagnetic flow probes, respectively. Coronary flow reserve was assessed as the maximum increase in flow velocity following increasing doses of intracoronary adenosine (from 12.5 to 200 mg). LAD occlusions resulted in significant increases in diastolic blood flow in the LCX (of 24 ± 3, 23 ± 3, 23 ± 3 and 22 ± 4 ml min-1 during occlusions 1-4 respectively). These "compensatory" flow increases
were significantly attenuated to 9 ± 2, 10 ± 1, 8 ± 1 and 9 ± 2 ml min-1 ($P < 0,05$) when the occlusions were repeated in the presence of metoprolol. Metoprolol did not modify baseline coronary flows in either artery nor did it modify the hyperaemic coronary flow velocity changes which resulted either from reperfusion or adenosine administration. Since metoprolol was, at least in part, able to reduce the "compensatory" flow increase we conclude that catecholamines might play a role in this phenomenon. However, metoprolol did not influence coronary flow reserve.

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P04-04

THE EFFECT OF ESTROGEN REPLACEMENT THERAPY ON ACCUMULATION OF FOAM CELLS IN DIABETIC MALE RABBIT
Sharifi A., Sharifi M., Fesharaki M., Allai H.

The risk factors for cardiovascular diseases are related directly or indirectly in men as in women, to lipid and lipoproteins plasma level. But women have the advantage of the beneficial effects of either endogenous or exogenous estrogen on these levels throughout most of their lives. Disease such as diabetes interfere with the favourable lipids and lipoproteins plasma levels seen in men, and heart disease risk is significantly increased. The aim of this project is to study the effect of estrogen on accumulation of foam cells, cholesterol, triglyceride, Low Density Lipoprotein, High Density Lipoprotein, in diabetic male rabbits. Diabetes was induced in 21 male white rabbits by injecting alloxan (200mg/kg) into the lateral air vein. Diabetic rabbits were divided in to three groups and were given nutrition as following: 1- high cholesterol diet, 2-high cholesterol diet +estrogen (1mg/rabbit/week), 3-high cholesterol diet + estrogen (5 mg/rabbit/week). Their blood sample was taken before and after of the study and the plasma level of cholesterol, triglyceride, HDL, LDL, and was measured. Blood pressure was measured directly in all groups of experimental animals. The cholesterol, LDL and accumulation of fatty streak reduce in groups that received estrogen ($p<.05$). HDL in group three is lower than group two($p>.05$). Triglyceride in group two and three is lower than group one ($p>.05$). Data have been shown mean ± standard error and for comparison of interagroups used T-paired-test and ANOVA for comparison intergroups. The $P<.05$ is significant.
It seems that estrogen with high and low dose can reduce cholesterol, LDL, triglyceride and accumulation of foam cells in diabetic animal as well as non-diabetic one. Estrogen inhibits HDL reduction and does not change triglyceride value. According to this research estrogen replacement therapy is suggested, but more study is needed to confirm the absolute effect.

Medical University of Kurdistan - Sanandaj - IRAN

P04-05

SYMPATHETIC MODULATION OF CARDIOVASCULAR ACTIVITY IN NORMAL AND STRESSED FEMALE AND MALE RATS
Glushkovskaya-Semyachkina O., Anishchenko T.
The aim of study was to investigate the sex particularities in cardiovascular responses to adrenaline (Ad) and propranolol (P) in normal and stressed rats.
The rats of both sexes (n=40) was instrumented with catheters for measuring of arterial pressure (AP), heart rate (HR) and for P (0.1 mg/1000g) and Ad (10mg/1000g) administration. The study of AP, HR was performed during: 1) control conditions; 2) 60-min immobilization stress (IS); 3) Ad or P injection; 4) IS+Ad or P.
In females compared with males IS resulted in more significant tachycardia (32% against 24%, $P<0.05$) but less significant hypertension (11% against 18%, $P<0.05$). The short-lasting hypertensive effect of Ad didn't differ between females and males (39% and 31%, respectively). However, females vs. males demonstrated higher compensatory decrease in HR (74% against 30%, $P<0.001$). Ad caused the increase in AP and decrease in HR responses to IS, more pronounced in females than in males. The usual gender difference in HR and AP responses to IS were reversed. So during IS+Ad females vs. males exhibited lesser tachycardia (11% against 18%, $P<0.05$), but more significant hypertension (45% against 31%, $P<0.05$). P injection caused bradycardia that was exspressed in a greater degree and in the greater number of females vs. males (by 58% in 67% of females and 10% in 58% of males). The decrease in HR induced by P was accompanied by compensatory increase in AP (21% in females and 15% in males). The IS of rats injected by P was accompanied by bradicardic response in females and small tachycardic response in males. P didn't depress stress-induced elevation in AP that was similar in males and females. Our data suggest that sympathetic modulation of cardiovascular activity is more pronounced in females than in males both under normal and stress conditions. Partly supported by grand CRDF (REC-006) and Grand of Ministry of Education of Russia RD 02-1.4-261.

State University of Saratov- Russia

P04-06

INTESTINAL BLOOD FLOW IN RATS KEPT ON LOW SODIUM DIET: ROLE OF NO, ALPHA-1 AND AT1 RECEPTORS
Sipos A., Bartha J., *Vág J., *Keremi B., *Csillag M., Hably Cs.

Background: According to our previous studies low sodium diet results in elevation of intestinal blood flow. The question is if activation of vasodilator mechanisms and/or decrease of vasoconstrictor effects result in the increase of blood flow? Method: In rats kept on low (LS) or normal (NS) sodium diet for six weeks the blood pressure (BP), the cardiac output (CO) and the intestinal blood flow (IBF) were measured (by 86Rb-accumulation technique) in anaesthesia. NO synthesis was inhibited by L-NAME (15 mg/kg), angiotensin II type 1 (AT1) receptors were blocked by Candesartan (1,0 mg/kg), and alpha1-adrenergic receptors by Prazosin (0,5 mg/kg).
Results (x+/-S.D.): Sodium depletion failed to influence the CO, but IBF (ml/min/100g tissue) increased (219 +/- 66 vs. 174 +/- 49 $p<0.01$). L-NAME increased the BP and decreased the CO both in NS and LS rats, and IBF decreased both in NS (117 +/- 38 vs. 174 +/- 49, $p<0.001$) and LS (106 +/- 42 vs. 219 +/- 66, $p<0.001$) rats; the decrease was higher in LS rats than in NS ones ($p<0.05$). Candesartan decreased the BP and CO both in NS and LS rats, but IBF decreased only in LS rats (126 +/- 35 vs. 219 +/- 66, $p<0.001$). Prazosin decreased the BP and CO both in NS and LS rats, but had no effect on IBF either in NS or LS rats. Conclusion: In case of decreased sodium intake the vasoconstrictor effect of angiotensin II is partially counterbalanced by increased NO production.

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P04-07

THE ROLE OF ALPHA ADRENERGIC RECEPTORS IN VASOCONSTRICTOR EFFECT OF EPINEPHRINE IN RAT GINGIVA
****Keremi B., **Sipos A., **Hably C., *Vág J., *Fazekas Á.***

Background: Epinephrine is widely used in dental practice. However the role of adrenergic receptor subtypes in the mediation of vasoconstrictor effect is not well-known. Thus, the aim of the present study was to investigate the effect of alpha1 or/and alpha2 adrenergic receptor blockade on the gingival vasoconstriction evoked by locally applied epinephrine (0,01%). Method: Experiments were carried out on anaesthetized female Wistar rats. Prior to locally applied epinephrine animals received iv. injection of physiological saline (Group A, n=9); prazosin (0.5mg/kg, group B, n=12); yohimbine (10mg/kg, group C, n=12); prazosin + yohimbine (group D, n=9). Gingival blood flow was measured by laser doppler flowmetry. Blood pressure (mmHg), heart rate (1/min), gingival blood flow (BPU) were registered continuously and gingival vascular resistance was calculated (GVR, mmHg/BPU). Results: Epinephrine did not influence blood pressure (102+/-8 vs. 98+/-8) and heart rate (369+/-17 vs. 368+/-18). Epinephrine produced GVR elevation in group A (0.52+/-0.05 vs. 0.22+/-0.02, $p<0.001$), in group B (0.28+/-0.02 vs. 0.24+/-0.02, $p<0.01$) and in group C (0.25+/-0.03 vs. 0.22+/-0.02, $p<0.05$), but not in group D (0.19+/-0.02 vs. 0.18+/-0.02). The increment of GVR in group B and C was practically the same (17% vs. 17%). In contrary the alteration in group A was significantly higher (139%, $p<0.001$). Conclusion: The vasocontrictor effect of locally applied epinephrine is mediated by both alpha1- and alpha2-adrenerg receptors.
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P04-08

IS BARORECEPTOR REFLEX HEART RATE CONTROL CHANGED IN BORDERLINE HYPERTENSION?
Voita D., Vitols A.

It is showed that baroreceptor (BR) heart rate (HR) control is diminished in mild hypertension, but there are contradictory data about BR function at borderline hypertension (BH). The aim of the present study is to analyze the BR reflex bradycardic reaction in BH at rest and during pressor reaction accompanying static muscular exercise.
In 56 patients with BH (men, aged 18-24 yrs) and 27 age and gender matched controls (C) were studied at rest and during static muscular exercise with force 50% MVC and duration 60s. Beat-to- beat HR and finger mean arterial pressure (MAP) were monitored non-invasively. Carotid baroreceptors were stimulated applying neck suction (-60 mmHg for 5s) at rest and during handgrip. The obtained data showed that BR reflex bradycardic reaction revealed high variability in the patients group - from 2 to 20 bpm, but in C the bradycardic reaction to BR activation was relatively stable - it varied from 16 to 22 bpm. HR at rest was increased ($p<0.05$) in patients comparing to C and the correlation between HR and BR bradycardic reaction was found ($p<0.01$). In BH whom HR didn't differ significantly from the same aged healthy C, the bradycardic reaction to BR activation and its dynamics during the static exercise also was not different. The bradycardic reaction at rest and decrease of the bradycardic reaction amplitude during the pressor response accompanying static exercise was faster and more expressed in patients with significantly ($p>0.01$) higher HR at rest comparing to C. Although MAP didn't differ significantly in comparing patients groups. The present data showed that BR reflex control is changed only in those patients with BH which have elevated HR at rest comparing to controls. We hypothesis that these patients, probably, may develop the more stable hypertension. Further study will be performed in future.

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P04-09

RAT RETINAL TISSUE RELEASES A VASORELAXING FACTOR
Van de Voorde J., Boussery K., Delaey C.

The present study aimed to investigate whether the retina of the rat exerts a vasodilatory influence by the release of a relaxing factor (as was previously observed with bovine retina) and to characterise the retinal relaxing factor (RRF). The relaxing influence of the rat retina was investigated by placing the retina in close proximity of a precontracted isolated rat carotid artery ring segment, mounted for isometric tension measurements. Application of rat retina relaxed the precontracted artery in a reliable and reproducible way (33.3 + 1.7 % relaxation). The NO-synthase inhibitor nitro-L-arginine (0.1 mM), the soluble guanylyl cyclase inhibitor ODQ (1 μM) and removal of the endothelium of the artery all failed to affect the RRF-response. The RRF-response was not decreased, in contrast rather increased, after treatment with a cyclooxygenase-inhibitor (indomethacin or sodium diclofenac). Acute hypoxia largely enhanced retina-induced relaxation. Several potential mediators of hypoxia-induced vasodilation (glutamic acid, GABA, aspartic acid, taurine, glycine, adenosine, lactic acid) were excluded from being the RRF, and from mediating the enhanced response to RRF in hypoxia. Inhibition of the plasma membrane Ca^{2+}-ATPase with vanadate (1 mM) significantly affected the RRF-response. It is concluded that (an) as yet unidentified relaxing factor(s) is (are) continuously released from the rat retina. Acute hypoxia profoundly enhances the RRF-response. None of the known mediators of hypoxia-induced vasodilation, nor NO, prostanoids or endothelial factors mediates the RRF-response. Activation of the plasma membrane Ca^{2+}-ATPase seems to be involved in the RRF-response.

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P04-10

CHRONIC TREATMENT OF INTERFERON-ALPHA REDUCED THE ENDOTHELIUM-DEPENDENT RELAXATION
Yao H., Cao C.M., Jin H.F., Shan Q.X., Wang L.L., Xia Q.

Objective: To investigate the vascular effect of acute and chronic treatment of interferon-alpha (IFN-alpha) in rat aortic rings.
Methods: The isolated thoracic aortic rings were mounted on the organ bath and the tension of the vessel was recorded.
Results: IFN-alpha (100~10000 U/ml) caused concentration-dependent relaxation of aorta rings preconstricted with PE (1 microM) in endothelium-intact rings. Removal of the endothelium, or pretreatment with L-NAME (100 microM) or methylene blue (10 microM) or AMG (100 microM) inhibited the relaxation of IFN-alpha, respectively. Pretreatment with IFN-alpha (1,000,000 U/d, i.p.) for five days markedly inhibited the endothelium-dependent relaxation of the aortic rings to acetylcholine. But the endothelium-dependent relaxation to acetylcholine was not changed by pretreatment of IFN-alpha (10000 U/ml) with the isolated aorta rings for 2 h.
Conclusion: The results indicate that the vasorelaxation induced by IFN-alpha in rat aorta rings is endothelium dependent and possibly mediated by inducible nitric oxide synthase. Chronic treatment of IFN-alpha may impair the enodothelium or NO-sGC pathway.

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P04-11

THE BENEFIT OF ANTICOAGULANT TREATMENT IN PROLONGUED IMMOBILIZATED PATIENTS FOR HIP FRACTURES
Ion I., Ceamitru N., Adumitresi C.

The objective of our study is to emphasize the advantage of using low molecular anticoagulant treatment in patients with limb fractures who underwent orthopedic surgery.
In this study, were examined patients with fractured hips, hospitalized in the Orthopedic surgery department of the County Hospital – Constanta, between November 16th and December 7th, 2001. During the whole period of hospitalization, have been administrated to the patients, subcutaneously, once daily, low molecular weight heparin's (LMWH). The blood tests were performed before surgery, immediately after it and 7 days latter. From plasma obtained after blood centrifugation, collected on sodium citrate, were determined Fibrinogen (FIB), Activated Partial Prothrombin Time (APTT), Prothrombin Time (PT), and Thrombin Time (TT). From the whole blood collected on EDTA, thrombocyte number was counted.The obtained data were statistically analyzed and values of $P < 0.05$ were considered significant.
There was observed the increase of thrombocytes number and fibrinogen values in patients with hips fractures as a reactive response to the inflammation and surgical intervention. Also, under LMWH treatment APTT was maintained in the normal range and prolongation of PT and TT were noticed.
Because LMWH administered subcutaneously (SC) once daily are at least as effective and safe as low dose unfactionated heparin (UFH) administered SC two or three times daily, LMWH has become the anticoagulant of choice for the prevention of venous thrombosis following major orthopedic surgery.

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P04-12

ROLE OF NITRIC OXIDE IN REFLEX SELF-REGULATION OF BLOOD PRESSURE
Datsenko V., Moybenko A., Pavlyuchenko V., Maisky V.

The main goal of the present study was to elucidate the possible nitric oxide participation in the reflex self-regulation of circulation.
Experiments (n=20) was carried out on the anaesthetized closed-chest dogs. We used the unique method of double-lumen catheterization and autoperfusion of the left coronary artery in the dogs and stimulation of cardiac receptors in order to reproduction cardiogenic reflexes. Excitation of cardiac receptors were reproduced by intracoronary injection of veratrine and catecholamines. In order to estimate reflex vasomotor reactions in the peripheral vessels, performed autoperfusion of the hindlimb arterial vessels by a constant flow pump. Other part of experiments (n=9) was performed on the anaesthetized rats under similar conditions. We carried out the especial series of experiments with NADPH-d-staining to evaluate the distribution of NOS-containing neurons in the medulla of dogs and rats.
Veratrine and cahecholamines injections resulted in a reflex decrease of mean arterial pressure and relaxation of coronary and peripheral vessels.
After NOS inhibition by L-NNA (30 mg/kg i.v.) reflex coronary and peripheral vessels vasodilatation and depressor reaction decreased or disappeared, while reflex cardiogenic vasoconstrictor responses significantly intensified. Species differences were shown: the depressor reflexes decreased after NOS inhibition in dogs, but they increased or were not changed in rats.

Morphological analysis showed species differences in the distribution of NOS-containing neurons in the medulla. In comparison to rats, the rostral and caudal ventrolateral medulla and subdivision of nucleus tractus solitarius in dog contained more NO-generated neurons.
Our data suggest that NO-dependent mechanisms play an important role in the realization of cardiogenic reflexes, predominantly related to n.vagus. These NO-dependent reflex reactions decrease a cardiac afterload, therefore they could be admitted as compensatory reactions.

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P04-13

INCREASE OF BAROREFLEX AND HRV WITH TRAINING IN THE ELDERLY
Pichot V., Roche F., Denis C., Garet M., Costes F., Barthélémy J-C

Purpose: Autonomic nervous system activity decreases continuously with age and appears as a powerful predictor of disease and death. Thus, in the intent of improving health, attempts are made to reincrease autonomic nervous systemactivity. Methods: We assessed autonomic nervous system activity by heart rate variability and cardiac spontaneous baroreflex sensitivity in eleven elderly men (73.5±4.2 years) before and after a 14 weeks of cycloergometer sustained interval training program. Heart rate variability indices were calculated using time domain, Fourier and wavelet analysis over 24-hour Holter recordings. Baroreflex sensitivity was calculated from 15-minute recordings of blood pressure and RR interval spontaneous variations using sequences and cross spectral methods. Results: After the training period, VO2peak increased by 18.6% (26.8±4.4 to 31.8±5.2 ml.kg-1.min-1, $p<0.01$). Total power and high frequencies of heart rate variability increased up to +73.8% ($p<0.05$) and the BRS indices increased up to +52.5% (6.9±2.2 to 10.5±3.7 mmHg.ms-1, $p<0.05$). Conclusion: Intensive endurance training in the elderly increased the spontaneous cardiac spontaneous baroreflex sensitivity and more generally the parasympathetic activity. Physiological mechanisms and long-term clinical benefits on health status should be further investigated.

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P04-14

NEW CARDIAC HORMONE ASSAYS FOR THE ASSESSMENT OF HEART FUNCTION
Hirvinen M., Ruskoaho H., Vuolteenaho O.

Natriuretic peptides are cardiac hormones regulating blood pressure and fluid homeostasis. A- and B-type natriuretic peptides (ANP and BNP) and their N-terminal prohormones (NT-ANP and NT-BNP) are upregulated in heart failure and they appear to serve as excellent markers of the cardiac performance. In this study we prepared recombinant peptides, raised antisera and set up several specific immunoassays for N-terminal prohormones. We tested these assays in healthy persons and cardiac patients.
We set up two competitive immunoassays for NT-ANP (epitope specificities 1-20 and 46-79) and five for NT-BNP (1-22, 5-24, 10-29, 52-70, 57-76). Recombinant human NT-ANP1-98 and NT-BNP1-76 were used as tracers and calibrators. The sensitivities of the assays allowed direct measurement from plasma or serum. The normal values of NT-ANP (250 pmol/l) and NT-BNP (85 pmol/l) increased parallelly with the NYHA classification (6-fold and 17-fold, respectively at NYHA4). The two NT-ANP assays (1-20 and 46-79) showed good correlation ($n = 230$, $r2 = 0.8$) indicating that these assays recognize the same peptide. RP-HPLC analyses from human plasma and serum samples revealed a single immunoreactive peak corresponding to NT-ANP1-98. On the other hand, immunoreactive NT-BNP in human blood consisted of several components. The NT-BNP concentrations obtained by different assays (5-24, 10-29, 57-76) correlated extremely well ($r2 = 0.75$-0.83), although the absolute levels varied between the assays. Apparently the different antisera recognize circulating fragments of NT-BNP with varying lengths and half-lives.
In conclusion, we set up sensitive immunoassays for NT-ANP and NT-BNP. The assays utilize native calibrators and can measure the various circulating forms of the N-terminal prohormones. The assays can be used to monitor the cardiac status in physiological and pathophysiological situations.

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P04-15

EFFECTS OF ENDOTHELIN-1 IN SALMON CARDIAC PEPTIDE SECRETION AND CARDIAC CONTRACTILE FUNCTION
Vierimaa H., Ronkainen J., Ruskoaho H., Vuolteenaho O.

We have recently cloned from salmon (Salmo salar) a novel heart-specific hormone, salmon cardiac peptide (sCP), related to mammalian natriuretic peptides. sCP has natriuretic, diuretic and vasodilatory effects. sCP has turned out to be an useful model for studying general biology of natriuretic peptides. We have previously shown that major stimulant for sCP secretion is mechanical load of the heart. Now, we have studied effects of endothelin-1 (ET-1) in sCP secretion and characterized its inotropic effects to salmon myocardium. We have set up specific radioimmunoassays for sCP and its aminoterminal fragment (NT-pro-sCP) and used them to characterize their secretion patterns.
Human ET-1 injection to the dorsal aorta of salmon caused a clear (1.5-fold) increase in serum NT-pro-sCP levels in vivo. The increase was abolished by pre-treatment with the endothelin type A receptor antagonist (BQ-123 and BQ-610). In vitro ET-1 caused a moderate increase in sCP release from isolated salmon ventricle ($p < 0.01$). The ET type A receptor antagonist attenuated the response induced by load ($p < 0.001$). ET-1 exerted a positive inotropic effect in salmon myocardium preparation which could be inhibited by ET type A receptor antagonist.
Thus, endogenous ET-1 appears to play a significant role in the maintenance of salmon cardiac endocrine and contractile function. The effects are mediated by ET type A receptors. ET-1 may have a paracrine role in regulating the release of the sCP peptides. The effects of ET-1 in vivo may be both direct and indirect (ET-1-induced increase in blood pressure). The role of ET-1 in salmon is therefore similar as in mammals. Thus, the regulation of the cardiac endocrine and contractile function appears to be highly conserved over the great phylogenetic distance between fish and mammals.

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P04-16

COMPARISON OF PATHOPHYSIOLOGICAL SYMPTOMS IN ATHEROSCLEROTIC HEART DISEASES IN DURING OF LIFETIME
Nobahar M., Vafaei AA.

Atherosclerotic Heart Diseases (AHD), [Unstable angina (UA) and Acute myocardial infarction (AMI)] are very prevalence in aging and are mainly factor for create mortality in elderly (>65 years old). Although many studies have described symptoms associated with AHD, few, if any, have examined symptom predictors of AHD and whether they differ by patients' age. Aim of this study to compare of pathophysiological symptom predictors of AHD in younger (< 45 years), 46-65 and older (> 66 years) patients. This research was a retrospective cross-sectional descriptive study that analysis of observational data gathered by checklist (demographic data, history, sign and symptom) on 570 patients hospitalized in coronary care unit during one year in Fatemiah hospital in Semnan. Data indicated that UA more prevalence in women and AMI more prevalence in men. Diaphoresis and Pain (presence, spread and sort) have more prevalence in younger patient than older ($P<0.05$). Don't significantly different between another symptoms and age. In compare symptoms between UA patient and AMI, result indicated that pain, diaphoresis, nausea and vomiting in AMI more prevalence than UA ($P<0.05$). Finding above shown that typical AHD symptoms are lower predictive in older age. Therefore early and correct diagnosis of sign and symptom in elderly are very helpful for control of AHD.

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P04-17

COMPARISON OF PATHOPHYSIOLOGICAL RISK FACTORS THAT INDUCED OF AHD IN DURING OF LIFETIME
Nobahar M., Vafaei AA.

The main cause of Atherosclerotic Heart Diseases (AHD), [Unstable angina (UA) and acute myocardial infarction (AMI)] is risk factors (Smoking, Hypercholesterolemia, and Hypertension, Diabetes, Obesity, History of heart diseases and family history). Evidence indicated that presence of this factors dependent of age that differentially in younger and older patient. The aim of

this study to compare of pathophysiological risk factors that may be induced AHD in younger (< 45 years), 46-65 and older (> 66 years) patients. This research was a retrospective cross-sectional descriptive study that analysis of observational data gathered by checklist (demographic data and risk factors) on 570 patients hospitalized in coronary care unit during one year in Semnan Fatemiah hospital. Data indicated that hypertension is more prevalence in older ($P<0.05$). But hypercholesterolemia, smoking, diabetes and history of heart diseases more prevalence in moderate age but less prevalence in younger and older. Family history was more prevalence in younger ($P<0.05$). Don't significantly different between another risk factor and age. In compare of risk factors between UA and AMI, result indicated that smoking and diabetes more prevalence in AMI and history of heart diseases and hypercholesterolemia more prevalence in UA than the AMI ($P<0.05$). Finding above shown that the risk factors that may be induced of AHD are differs in during of life time. Therefore early diagnosis of risk factors and modulating those are very helpful for prevention of AHD.

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P04-18

HEART RATE AND BLOOD PRESSURE VARIABILITIES IN YOUNG DIABETICS

Javorka M., Javorka K., Javorková J.

The primary aim of the study was to compare the parameters of the heart rate and blood pressure variabilities quantified by application of the Poincaré and sequence plots and sample entropy parameter in the group of young subjects with diabetes mellitus type 1 (DM1) and in healthy controls. The patients included 17 subjects (10 females, 7 males, mean age 22.4 ± 1.0 years) with an average duration of DM1 of 12.4 ± 1.2 years. Controls (17 subjects) were matched for age, sex and BMI. RR intervals were telemetrically transmitted through the VariaCardio TF4 system (Sima Media,Olomouc,Czech republic) to a receiver and PC, peripheral beat-to beat blood pressure values were registered by the volume-clamp method (Finapres, Ohmeda, USA) and PC.
Conventional parameters of the heart rate variability (HRV) in high and low frequency bands obtained by spectral analysis (FFT) were lower in diabetics in comparison to controls. In DM1 group, the length and widths of the Poincaré plot constructed from resampled RR intervals were significantly shorter; the percentage of the points in the 3rd quadrant of the sequence plot was decreased; the number of the RR interval sequences with a minimal RR length changes was higher. Complexity of the heart rate evaluated by the sample entropy parameter (SampEnRR) was not different between the groups. HRV parameters correlated neither with the DM1 duration nor with the values of glycated hemoglobin. Blood pressure variability at rest in the young patients with DM1 was not different from controls.
The results using non-conventional mathematical methods confirm the heart rate dysregulation in young diabetics primary by the insufficient parasympathetic regulatory output without a disorder of the BP regulation at rest.

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P04-19

EFFECTIVENESS OF OVERALL BAROREFLEX REGULATION: ITS MEASUREMENT BY A TWO-POINT METHOD

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The aim of the study is to quantify the overall baroreflex effectiveness, which results from the combined action of baroreflex regulation and the counteracting effect of short-term (minutes) total systemic autoregulation, by means of the effective overall open-loop gain, Goe. This gain is estimated by a method which requires two measurements of cardiac output, CO, and mean systemic arterial pressure, MAP: one in the reference state (set-point) and the other in a steady-state reached 1 to 3 minutes after a small CO perturbation. Defining ΔP and ΔPi as the steady state changes in MAP, with and without resistance regulation, respectively, Goe is computed as $\Delta Pi/\Delta P-1$. A small decrease of CO by partial occlusion of the inferior vena cava in anaesthetised cats and by cardiac pacing in anaesthetised dogs yielded mean (±SEM) Goe values of 1.4±0.2 in the cat (n=8) and 1.5±0.4 in the dog (n=5). The real baroreflex open-loop gain, Gor, was calculated by correction for total systemic autoregulation, which was quantified using the relation between autoregulation resistance gain and initial (control) peripheral resistance, normalised for body weight (Burattini et al., Am. J. Physiol. 1994;267:R1182-9). Mean Gor was 3.3±0.4 in the cat and 2.8±0.8 in the dog. The ratio of Goe to Gor indicated that total systemic autoregulation masks ~55% of the baroreflex open-loop gain. A model based analysis showed that without autoregulation, i.e. with Goe larger than 2 to 3 units, the transient response of MAP to a stepwise perturbation in CO may result in sustained and, eventually, undamped oscillations with a ~0.1 Hz rhythm, most likely caused by characteristics of the resistance vessels. We conclude that autoregulation reduces the effectiveness of the baroreflex gain and prevents baroregulation from instability. Our two-point method for estimation of Goe in closed-loop conditions might become a practical tool for quantifying baroreflex effectiveness.

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P04-20

THE EFFECTS OF EXERCISE ON CARDIOCIRCULATORY AND ENDOCRINE PARAMETERS IN ELDERLY PATIENTS WITH EAHT

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The lack of physical activity in elderly represents a major risk factor in the onset of cardiovascular pathology.Cardiovascular disases and esspecialy arterial hypertension occupy the first place among pathologies found in the elderly.
The aim of our study was related with the evaluation of a 30 minutes standard physical effort of moderate intensity upon cardiovascular parameters(heart rate and systolic and diastolic blood pressure and upon metabolical and clinicofunctional parameters in elderly male patients.24 patients aged between 46-78 years old admitted in the Rehabilitation Clinique for osteoarticular and posttraumatic pathologies divided into two groups of 21 patients each:group A of adults patients with normal physical activity and group B sedentary and obese patients with EAHT.Before and after training program, cardiocirculatory parameters and EKG have been determined , as well as the levels of CK,CKMB .HGH , hTSH,T3, T4 and Cortisol have been determined with a DELFIA 1234 Research Spectrofluorimeter.After each day of training,the EMG of biceps and triceps muscle have been evaluated with an EMG Schwartzer-Picker 2000. Results:Our data have pointed out that after standard physical effort of moderate intensity the sedentary and hypertensive subjects have adapted very well to the effort as the clinicofunctional parameters of cardiorespiratory apparatus have shown. The subjects were able to perform movements with a better neuromuscular coordination. Conclusion:The decrease of blood pressure values after the standard physical effort in sedentary patients with hypertension accaunts for a good degree of their adaptability to physical effort of moderate intensity being well tolerated constituting as an alternative to the classical drug treatment with increased risk by its nephrotoxic effects.

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P04-21

DIFFERENT PATTERN OF CONDUIT ARTERIES OF ADULT RATS AND THEIR NEWBORNS AFTER NITRIC OXIDE INHIBITION

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We studied morphological characteristics of thoracic aorta (TA) and carotid artery (CA) of adult nitric oxide deficient (NODH) rats and their newborns. Adult rats were administered NG-nitro-L-arginine methyl ester (L-NAME) in drinking water (40 mg/kg/day) for 6 weeks. The newborns were 4 weeks old born from these NO deficient parents (parents were administered L-NAME for 5 weeks before fertilization and females continued 7 weeks during pregnancy and breast feeding). Both groups had own age matched controls. Blood pressure (BP) was measured weekly noninvasively on tail artery, using the plethysmographic method. At the end of the experiment the cardiovascular system was perfused with glutaraldehyde fixative under the pressure 120 mm Hg. TA and CA were processed according to standard electron microscopic procedure. Geometry of the arteries was measured on semithin sections in light microscopy. Volume densities of smooth muscle cells (SMC) and extracellular matrix (ECM) in the arterial wall (tunica intima + tunica media) of CA were determined in electron microscopy. Adult rats: BP of NODH rats (172±1.7 mmHg) was higher then in controls (102.8±1.1 mmHg). The inner diameter (ID) in TA was increased, not in CA. Increased wall thickness (WT) of both arteries was due to increase of both SMC but mainly ECM. WT/ID was higher in NODH rats then in controls.

Newborns: in spite of increased BP of NODH newborns (150±2.3 mmHg vs. 105±2.1 mmHg in controls) the ID increased in TA only, not in CA. Surprising was decline of WT of both TA and CA comparing to control arteries. The decline of WT was accounted for by the pronounce decline of volume density of SMC. WT/ID ratio in vessels from NODH newborns was lower too. In conclusion: The known WT and WT/ID ratio increase in NODH adult rats was contradictory to decrease of both these parameters in NODH newborns.

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P04-22

PENTOXIFYLLINE REDUCES ENDOTHELAEMIA, VWF AND HPA-AXIS ACTIVATION IN RATS IN IMMOBILIZATION STRESS

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Stress is generally considered to be a risk factor of several diseases including cardiovascular diseases. The direct evidence on stress-induced damage to the endothelium is still lacking. Therefore, the model of immobilization stress used in previous studies was exploited as a suitable model for neuroendocrine activation and possibly for endothelial damage.
The aim of present study was to verify the following hypotheses: (1) a single exposure to an intensive stressor is followed by a damage to the endothelium, (2) potential stress-induced endothelial cell damage is reduced by repeated administration of pentoxifylline (PTX) and (3) PTX treatment modifies neuroendocrine activation under stress conditions through changes in hypothalamic-pituitary-adrenocortical (HPA) axis activation.
Rats were treated with saline or PTX (20 mg/kg, s. c.) once daily for 7 days. In saline pretreated rats, a single exposure to immobilization stress for 120 min was followed by an increase in endothelaemia, von Willebrand factor (vWf) concentrations, adrenocorticotropic hormone (ACTH) and corticosterone release, as well as by enhanced gene expression of hypothalamic corticotropin releasing factor (CRH). Pretreatment with PTX significantly reduced endothelaemia, plasma ACTH and corticosterone concentration in the adrenals.
The obtained results have shown that a single exposure to an intensive stressor associated with significant HPA-axis activation caused damage to the endothelium. PTX pretreatment reduced markers of endothelial injury as well as stress-induced rise of hormone levels. These data provide the evidence on protective action of pentoxifylline under stress conditions.
This study was supported by grants of EC ICA1-CT-2000-70008, VEGA 2/20007 and 1/9302/02.

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P04-23

ENDOTHELIUM-PROTECTIVE EFFECTS OF POLYPHENOLIC COMPOUNDS FROM RED WINE

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Protective effects of red wine polyphenols (RWP) on cardiovascular system have been documented in numerous human as well as animal experimental studies. These effects involve improvement of vascular relaxation mediated by increased production of nitric oxide (NO) by vascular endothelium. In the present work, the model of endothelial damage by chronic administration of carbon tetrachloride (CCl4) was used for evaluation of endothelium-protective effect of RWP.
The aim of this study was to investigate the effect of RWP and CCl4 on vascular responses and endothelaemia as the marker of endothelial cell injury in vivo in rats. The polyphenolic extract was administered orally 40 mg/kg/day for 8 weeks, CCl4 parallel 0.5 mg/kg intraperitoneally twice a week. After 8 weeks animals were sacrificed, leaving 2 groups of animals with spontaneous regression and regression with polyphenols administration during 3 weeks.
It was found that CCl4-pretreatment did not change vasoconstrictor responses of isolated renal arteries to standard doses of noradrenaline (0.1; 1; 10 μg) but relaxations to acetylcholine (at dose 20 μg) were diminished if compared to controls. Combination of RWP with CCl4 decreased vasoconstrictor responses to noradrenaline and opposed unfavorable effect of CCl4 itself on vascular relaxation.
CCl4-pretreatment increased 3-fold endothelaemia when compared with controls (2.47±0.28 cells/10 μl). Polyphenolic compounds themselves did not lead to significant changes, but pretreatment with them decreased significantly CCl4-induced rise in endothelaemia. Spontaneous regression did not affect significantly elevated endothelaemia. Administration of polyphenols during 3-week regression significantly decreased the number of cells in blood. These findings supported histologically suggest protective effects of red wine polyphenols on vascular endothelium.
This study was supported by grants VEGA 1/9302/02 & 1/9303/02.

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P04-24

CONTRIBUTION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE 2 AND 4 IN ANGIOGENESIS

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The proliferation and migration of endothelial cells induced by play a major role in angiogenesis. Physiological angiogenesis is tightly regulated by growth factors such as vascular endothelial cell growth factor (VEGF) which stimulates endothelial cells to migrate, proliferate and differentiate to form new vessels. Several pathological conditions such as atherosclerosis and tumor growth are associated to an excessive angiogenesis in which vessels develop in an uncontrolled or disorganized manner. Elevation of cAMP in endothelial cells has been shown to inhibit cell proliferation and migration. Our hypothesis was that inactivation of cAMP-specific phosphodiesterases (PDEs) would inhibit angiogenesis. The effect of PDE inhibitors on in vitro and in vivo angiogenesis, using human umbilical vein endothelial cell (HUVEC) and chick chorioallantoic membrane (CAM) models, were studied. Treatment of HUVEC by VEGF increased the global cAMP-PDE activity and PDE2 and PDE4 activities. VEGF acts at the transcriptional level since it increased the expression of PDE2, PDE4A, PDE4B and PDE4D at mRNA level. Treatment of VEGF-stimulated HUVEC by EHNA (PDE2 selective inhibitor) and RP73401 (PDE4 selective inhibitor), resulted in an increase of intracellular cAMP level and an inhibition of proliferation and migration. PDE2 inhibition merely decreased the S to G2/M phase transition, whereas PDE4 inhibition prevented the G0/G1 to S phase transition. Western blot analysis indicated that treatment of VEGF-stimulated HUVEC by EHNA and RP73401 was modulating the expression of MAP kinases, cyclin A, cyclin D1, p21waf1, and p27kip1. Moreover, the effect of PDE inhibitors were investigated on in vivo angiogenesis. Treatment of CAM with PDE2 and PDE4 inhibitors reduced dose-dependently the density of capillary vessels. Altogether, these results indicate that PDE2 and PDE4 represent new potential therapeutic targets for angiogenesis and that PDE2 and PDE4 are implicated in angiogenesis.

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P04-25

LACK OF DYSTROPHIN REDUCES NO-DEPENDENT VASCULAR FUNCTION: TOTAL RECOVERY AFTER GENTAMICIN TREATMENT

Loufrani L., Dubroca c., Li Z., Levy bi., Paulin D., Henrion D.

Mutations in the dystrophin gene causing Duchenne's muscular dystrophy (DMD), lead to pre-mature stop codons. In mdx mice, a model for DMD, they can be suppressed by aminoglycosides such as gentamicin. Dystrophin is likely to play a role in flow (shear stress) mediated endothelium-dependent dilation (FMD) in arteries. Thus we investigated the effect of gentamicin on vascular, structure and function in mdx mice.
Mice carotid and mesenteric resistance arteries (450 and 85μm diameter, respectively) were mounted in vitro in arteriographs allowing continuous diameter measurements. In mdx mice, NO-dependent FMD and endothelial NO-synthase expression were lower than in control mice. In mdx mice treated with gentamicin, dystrophin was recovered in vascular cells, FMD and NO-synthase expression were identical to control in mdx mice treated with gentamicin. Smooth muscle-dependent contractions as well as dilation to acetylcholine (endothelium-dependent) and sodium nitroprusside (endothelium-independent) were not affected by the absence of dystrophin and/or by gentamicin. FMD, attenuated in vimentin-null mice, was not restored by gentamicin.
These findings open important perspectives in the mechanism involved in the pathophysiology of genetic diseases related to pre-mature stop codons such as DMD.

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P04-26

COMPARISON BETWEEN THE EFFECTS OF ICV OR IV INFUSION OF HYPERTONIC NaCl DURING HAEMORRHAGE
Hjelmqvist H., Frithiof R., Ullman, J., Eriksson, S., Rundgren, M.

Effects of treatment with intravenous infusion of hypertonic (1.2 M, 4 mL / kg) NaCl (IHTNa) or intracerebroventricular (ICV) administration of (0.5 M 0.02 mL / min) NaCl (CHTNa) on tolerance to haemorrhage were investigated in conscious or anaesthetized sheep. All treatments were started 30 min before commencement of a slow (0.7 mL / kg / min) venous heamorrhage, which was continued until the mean systemic arterial pressure (MSAP) suddenly dropped to < 50 mm Hg. Corresponding bleeding during ICV infusion of artificial cerebrospinal fluid (aCSF) served as control.
To reach the distinct fall in MSAP the following amount of blood had to be withdrawn in a) conscious animals: aCSF 13.9 ± 0.5 mL / kg (n = 7), CHTNa 24.0 ± 1.8 mL / kg (n = 6), IHTNa 22.4 ± 1.4 mL / kg (n = 6) and b) anaesthetized animals: aCSF 10.2 ± 0.9 mL / kg (n = 7), CHTNa 10.4 ± 0.9 mL / kg (n = 6), IHTNa 15.1 ± 0.2 mL / kg (n = 5). Significantly more blood ($p < 0.001$) had to be removed from conscious CHTNa than in anaesthestized CHTNa compared to the much lower difference between conscious IHTNa and anaesthetized IHTNa.
In conclusion, CHTNa as well as IHTNa treatment were found to improve the tolerance to haemorrhage in conscious animals. However, the effect of CHTNa was abolished during iso-flurane-anaesthesia, suggesting that the mechanism for the beneficial effect of ICV infusion of hypertonic saline is different from the peripheral effect of hypertonic saline.

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P04-27

THE EFFECT OF CYSTATIN C ON ADP-INDUCED PLATELET AGGREGATION
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Cystatins are natural inhibitors of cycteine proteinases. Cystatin C is an inhibitor of cathepsin B, L and H. Because of the widespread extracellular distrubution there is a growing interest about the physiological effects of cyctatin C and its roles on several pathological processes. The aim of the present study was to investigate the effect of cystatin C on ADP-induced platelet aggregation in whole blood.

Venous blood samples were obtained from healthy, non-smoker, male volunteers who did not take any medication preceeding two weeks (n=16). Samples were collected in siliconized tubes containing 3.8% sodium citrate. 1, 1.75 and 3µg/ml of cystatin C was added into the blood samples and incubated 2 minutes at 370C. ADP (10µM) induced platelet aggregation was evaluated by impedance technique in whole blood. Maximal intensity of platelet aggregation (MIA) was calculated. The data was analysed statistically by using Friedman test.

Cystatin C reduced maximal intensity of ADP-induced platelet aggregation 30.9 % in concentration of 1 µg/ml, 73.2 % in concentration of 1.75µg/ml ($p=0.06$). 3µg/ml of cystatin C caused a 55.6 % decrease in platelet aggregation.

It was shown that cystatin C inhibited platelet aggregation in concentration-dependent manner. Its effect may due to the inhibition of cathepsins. Further researches are needed to clear the underlying mechanisms.

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P04-28

INTRAVITAL MICROSCOPY OF A TUMOR-DRAINING LYMPH NODE
Carrière V., Colisson R., Girard J-P., Amalric F., M'Rini C.

Homing of the whole naive lymphocyte pool into secondary lymphoid organ is a physiological process that is critical to ensure encounter between antigen-presenting cells (APCs) and the unique or few T cell(s) specific to presented antigens. In peripheral lymph nodes (PLNs), this process is initiated by a multi-step sequence of interactions between lymphocytes and endothelial cells lining the node High Endothelial Venules (HEVs). We used intravital microscopy (technic allowing in vivo analysis of circulation blood cells into tissues of an anaesthetized mouse)to study tumor-induced modifications of lymphocyte and other circulating blood cell behaviors into HEVs of inguinal node draining a B16-F10 melanoma at different stages of development. Intravital microscopy studies indicate that proximity of melanoma tumor deeply modifies the behaviors of blood cell when circulating inside the node venular tree. In venules flowing in regard to the node medulla, tumor proximity triggers unusual phenomena of polynuclear cell rolling and firm adhesion whereas in venules flowing in regard to the node paracortex, it decreases the physiological firm adhesion of naive lymphocyte. These tumor-induced modifications are very early starting less than 24 hours after tumor implementation and are sustained as long as last the tumor and the animal survey. Another mechanism set up by tumor to escape the immune surveillance seems to have been identified, mechanism that targets one of the earliest phases of the immune response, the homing of naive lymphocytes inside secondary lymphoid organs.

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P04-29

INFLUENCE OF AGE ON ADRENOCEPTORS AND CATECHOLAMINES IN WOMEN
Litschauer B.

The incidence of cardiovascular diseases is lower in premenopausal women compared to men but this difference is no longer apparent after menopause. This has been ascribed to protective effects of estrogen, involving besides metabolic, vascular and cardiac mechanisms direct influences on the autonomic nervous system.
The aim of our study was to assess sympathetic nervous system activity by measuring adrenoceptors and catecholamines in young and middle-aged women in relation to the estradiol concentration.
In 20 healthy middle-aged women, aged 48 - 64 years, without hormon replacement therapy during the preceding 6 months and 38 healthy female students, aged 19 to 27 years, baseline plasma concentrations of adrenaline and noradrenaline, blood pressure, heart rate and platelet alpha2-adrenoceptor- and lymphocyt beta2-adrenoceptor densities and estradiol concentration were measured.
Middle-aged women had significantly higher noradrenaline plasma concentrations and lower platelet alpha2-adrenoceptor densities compared to values observed for young women, whereas lymphocyt beta2-adrenoceptor density and adrenaline plasma concentrations were similar in both groups. A significant positive correlation between estradiol and noradrenaline and a negative one with alpha2-adrenoceptor densities was found. Blood pressure was significantly higher and heart rate was significantly lower in middle-aged women combared to young women. Blood pressure was positively related to noradrenaline, beta2-adrenoceptor density and negatively to alpha2-adrenoceptor density.
To the extent that platelet alpha2-adrenoceptors reflect the behavior of alpha2-adrenoceptors in other tissues, the findings of the present study indicate that estradiol may modulate sympathetic activity by increasing presynaptic inhibitory alpha2-adrenoceptors.

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P04-30

THE EFFECT OF THE THYROLIBERIN ON THE SYNTHESES OF THE BETA-ENDORPHIN AND CORTICOTROPHIN ROPIN
Hazar H., Yetkin Y., Yetkin A.

Researching of the regulation of the blood circulation has showed the importance of the opioids. The aim is to study the effect of the thyroliberin on the level of b-endorphin and corticotrophin in blood plasma and spino-cerebellar fluid.
The studies were performed on the cats weighting from 3.5 to 4.0 kg under general anesthesia (Nembutal, 40mg/kg, i.v.). The blood from jugular vein and spinal fluids were withdrawn from thoracic region of the vertebra at the 3rd and 20th minutes before and after the test-substance. Thyroliberin was applied in 1 mg/kg of dose. The level of the b-endorphin and corticotrophin were assessed by the radio-immunologic methods
Thyroliberin did not cause any changes on the level of the beta-endorphin in the plasma and spinal fluid. At 3rd and 20th minutes plasma control level was 47±3.3. pk mol/l. After 3 and 20 minutes, they were found 42±8.2 and 45±10.5 pk mol/l respectively. Control level of spinal fluid was found 1.3±0.4. The levels of beta-endorphin were found 1.8±0.4 and 1.8±0.5 pk mol/l.

In plasma, thyroliberin increased significantly the level of the corticotrophin in the average of %75±1.8 at 3 and 20 minutes, whereas in the spinal fluid thyroliberin decreased the level of the corticotrophin at 3 and 20 minutes %41±1.3 and %63±9.1, respectively.
Thyroliberin did not effect on the beta-endorphin level in plasma and spinal fluid is shown that the effect of the thyroliberin is not over the opioid systems. However, increasing in blood plasma and decreasing in the spinal fluid of the corticotrophin by the effect of thyroliberin can express through the negative feedback. If corticotrophin involve at a high level in the plasma, it makes an inhibitory effect on itself synthesis.

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P04-31

AGEING AND HYPERTENSION DO NOT AFFECT GLUCOSE METABOLISM IN THE RAT
Ruggeri P.*., Cogo C.E. *., Brunori A.*., Natalucci S. °., Burattini R. °

The aim of the study is to investigate whether insulin resistance and impaired glucose effectiveness develop with ageing and exposure to high blood pressure in the spontaneously hypertensive rat (SHR). The minimal model of glucose kinetics was applied to insulinaemia and glycaemia data collected by a 12-sample, 120-minutes intravenous glucose tolerance test (IVGTT) from 36 rats under pentobarbital anaesthesia (40mg/kg, i.p.). These rats were divided into four groups (n=9, each group): two groups of young (12 weeks) spontaneously hypertensive (Y-SHR) and normotensive Wistar Kyoto (Y-WKY) rats and two groups of old (40 weeks) spontaneously hypertensive (O-SHR) and normotensive (O-WKY) rats. The glycaemic metabolism of each group was characterised by the estimates of insulin sensitivity, SI, and glucose effectiveness, SG, obtained from fitting the minimal model to data. The SI index quantifies the ability of insulin to promote glucose metabolism, whereas SG quantifies the ability of glucose to promote its own metabolism. The Y-SHR and O-SHR groups were contrasted to the age matched, Y-WKY and O-WKY groups, respectively, to investigate the possible association between insulin resistance and hypertension. The O-SHR and O-WKY groups were contrasted to the Y-SHR and Y-WKY groups, respectively, to address the issue as to whether abnormalities in glucose metabolism develop with age. No significant differences ($p>0.05$) were observed in the mean SG and SI estimates between the Y-SHR and the Y-WKY group, as well as between the O-SHR and the O-WKY group. Moreover, no significant differences ($P>0.05$) were observed in the mean SG and SI estimates between the O-SHR and the Y-SHR group, as well as between the O-WKY and the Y-WKY group. We conclude that, in the genetic model of hypertension considered here, ageing and long-lasting exposure to high blood pressure levels do not necessarily lead to the development of insulin resistance and impaired glucose effectiveness.

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P04-32

SPATIAL DISCONTINUITIES IN TRANSMISSION OF THE AORTIC PULSE WAVE IN THE ANAESTHETISED RABBIT
Sears T.A., Banks D.

Following the classical studies of Murgo the arterial pulse wave has continued to interest Physiologists and Clinicians for the information it contains about cardiac performance, the peripheral vascular bed and local blood flow. More recently interest has centred on the nature and source of reflected waves that can be the cause of, or exacerbate, hypertension. Arterial pulse waves are usually examined in the frequency domain by Fourier analysis or directly in the time domain by applying appropriate hydrodynamic models to measurements of pulse wave pressure gradients. In the study of nerve impulse transmission the changing spatial distribution of the propagating wave with time reveals spatial discontinuities due to nodes of Ranvier. We thought it of interest to examine the aortic pulse wave in an analogous way by using spatial and temporal sampling frequencies commensurate with the probable conduction times of 1-2ms (conduction velocity 4.0 - 5.0 m/s) between, and the spacing of, the segmental arterial branches (0.8 - 2.0cm). In anaesthetised rabbits we have measured aortic blood pressure successively at multiple sites 10 or 5.0 mm apart along the aorta using a high-fidelity transducer and a sampling rate of 1000Hz or higher. Off-line at each site we derived QRS-event- triggered averages of the pulse wave, usually of 250ms duration, and via an Excel spreadsheet and Origin software transposed the data to create spatial plots of the propagating pulse wave at different times, ms by ms. Such spatial profiles have directly revealed reflection sites and discontinuities that correlate with the sites of major branches of the aorta, such as the renal, and mark the transition from the initial branch-free segment of the descending aorta and the first pair of intercostal arteries.

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P04-33

ACTIVE REGULATION OF CORONARY ARTERY DIAMETER IN RESPONSE TO EXTRAVASCULAR PRESSURE ELEVATION.
Azzawi M., Austin C.

Myogenic tone, an important autoregulatory mechanism in vivo, has been demonstrated in vitro using isolated pressurized small arteries by increasing intravascular pressure (IvP). In vivo, however, coronary vessels are subjected to compressive forces of the surrounding cardiac muscle, increasing extravascular pressure (EvP). The effects of increasing EvP on myogenic regulatory mechanisms is unclear. Using novel methodology we studied whether isolated coronary vessels actively regulate their diameter in response to a sustained elevation of EvP. Wistar rats were humanely killed by cervical dislocation. Septal coronary arteries were dissected out and mounted on a modified pressure myograph, superfused with physiological salt solution (pH 7.4, 37oC, 95% air/5% CO_2). A secure lid over the myograph chamber allowed EvP to be elevated (via a 95%air/5%CO_2 source) over sustained periods. The internal vessel diameter was determined using a video dimension analyser. Data given as mean + SEM. At an IvP of 60 mmHg, coronary arteries (mean diameter 184 mm + 14 mm, n=8) developed myogenic tone. Pressure-diameter relationships were studied over an IvP range of 20-100 mmHg at zero EvP. Active lumen diameters were significantly lower than passive diameters at all pressure increments ($p<0.01$), demonstrating evidence of active regulation of diameter over an IvP range of 40-60 mmHg. The influence of EvP elevation was also assessed at constant IvP of 60 mmHg (mean diameter 220+ 15 mm, n=5). Elevation of EvP produced an immediate decrease in diameter over an EvP range of 20-100 mmHg. A sustained elevation of EvP led to active regulation of diameter over an EvP range of 40-60 mmHg, stabilising within 1-9 min. Active diameters were significantly lower than passive diameters at 20, 40, 60 ($p<0.01$), and 100 mmHg ($p<0.05$) EvP. Thus we demonstrate that coronary vessels show active regulation of coronary artery diameter in response to a sustained elevation of EvP.

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S6 SENSORS AND EFFECTORS IN BODY FLUID HOMEOSTASIS

ORAL SESSION

S6-1

MACULA DENSA CELL SENSING OF DISTAL TUBULAR LOAD AND NITRIC OXIDE RELEASE

Liu R., Persson E.G.

Earlier investigations in our laboratory have indicated that Na,K,2Cl co-transport mechanism is involved in sensing the fluid/NaCl load to the macula densa cells. The luminal NaCl concentration ([NaCl]) at the macula densa (MD) controls both tubuloglomerular feedback (TGF) and renin release. In earlier studies we have found that nitric oxide (NO) inhibits TGF sensitivity potently. The NO concentration in the MD cells is not known.
In the present study we measured NO production rate in MD cells with confocal microscopy in the isolated perfused thick ascending limb using a NO-sensitive fluorophore 4,5-diaminofluorescein (DAF-2). Calcein was used to measure cell volume changes. The loop perfusion fluid was a modified Ringer solution containing 10, 35, or 135 mM NaCl with a constant total osmolarity (290 mOsm), while the bath was perfused with the 135 mM NaCl solution.
The results showed that MD cell volume and NO production increased considerably with increasing luminal [NaCl]. Furthermore, we found that 5 mM L-arginine increased (30%) NO production in the MD cells. 7-nitroindazole, an nNOS inhibitor, could totally inhibit the NO production caused by L-arginine and by increased luminal [NaCl].
In conclusion, we could quantitatively measure the MD cell volume changes caused by the changes of luminal [NaCl], and found that increasing the luminal [NaCl] resulted in an increase in cell volume. We also found that NO formation in macula densa cells could be measured with DAF-2 and that NO production was increased through neuronal NO synthase activation with an increased luminal [NaCl]. An increased NO production will inhibit the vasoconstriction induced by the TGF and at the same time will reduce TGF sensitivity.

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S6-2

INTERACTION BETWEEN ANGIOTENSIN II, NITRIC OXIDE AND PROSTAGLANDINS IN THE CONTROL OF RENAL FUNCTION

Salazar F.J., López R., Llinas M.T.

Several studies performed by our group have demonstrated that there is an important interaction between angiotensin II (Ang II), nitric oxide (NO) and prostaglandins (PG) in the acute and long-term regulation of the renal hemodynamic and excretory function. The results obtained suggest that both NO and PG protect the renal vasculature from the hemodynamic and tubular effects of Ang II. Recently we have examined the role of the cyclooxygenase (COX) isoforms in producing the PG involved in modulating the renal vasoconstriction and antinatriuresis induced by acute and prolonged reductions in NO synthesis. It was found that the administration of a non-selective COX inhibitor enhances the renal vasoconstriction and antinatriuresis secondary to a decrease in NO. It has also been found that: A) a reduction in NO synthesis is followed by an stimulation in the production of COX-2-derived metabolites; B) the administration of a selective COX-2 inhibitor enhances the renal vasoconstriction induced by a decrease in NO, and C) this enhancement is similar to that elicited by a non-selective COX inhibitor. These results support the notion that COX-2 play a more important role than COX-1 in producing the PG involved in buffering the renal vasoconstriction secondary to acute and chronic reductions in NO. From the results obtained in our laboratory it can also be proposed that the COX-1 isoform, rather than COX-2, is involved in producing the PG that regulate renal excretory function when endogenous NO synthesis is reduced. In support of this idea, it was found that the administration of a non-selective, but not that of a selective COX-2 inhibitor, enhances the sodium retention elicited by a decrease in NO during acute or prolonged increments in extracelular volume, or during the infusion of a medullary vasodilator (bradykinin). In summary, the results obtained suggest that COX-1 and COX-2 play different roles in the regulation of the renal hemodynamic and excretory function.

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OC06-1

INTERACTIONS BETWEEN EPITHELIAL NITRIC OXIDE SIGNALLING AND PDE ACTIVITY IN DROSOPHILA

Davies S., Broderick K., MacPherson M., Regulski M., Tully T., Dow J.

Signalling by nitric oxide (NO) and guanosine 3', 5'-cyclic monophosphate (cGMP) modulates fluid transport in Drosophila melanogaster. Expression of an inducible transgene encoding Drosophila NO synthase (dNOS) increases both NOS activity in Malpighian (renal) tubules, and DNOS protein in both Type I (principal) and Type II (stellate) cells. However, cGMP content is increased only in principal cells. DNOS overexpression results in elevated basal rates of fluid transport in the presence of the phosphodiesterase (PDE) inhibitor, Zaprinast. Direct assay of tubule cGMP-hydrolysing phosphodiesterase (cG-PDE) activity in wild-type and dNOS transgenic lines shows that cG-PDE is Zaprinast-sensitive and is elevated upon dNOS induction. Zaprinast treatment increases cGMP content in tubules, particularly at the apical regions of principal cells, suggesting localisation of Zaprinast-sensitive cG-PDE to these areas.
Potential cross-talk between activated NO/cGMP and calcium signalling was assessed in vivo with a targetted aequorin transgene. Zaprinast potentiates both neuropeptide- and cGMP-stimulated calcium levels upon dNOS induction. In tubules in which DNOS is overexpressed, the Zaprinast-induced transport phenotype is inhibited by the calcium channel blocker, verapamil.
Molecular genetic intervention in the NO/cGMP signalling pathway has uncovered a pivotal role for cell-specific cG-PDE in regulating the poise of the fluid transporting Malpighian tubule via direct effects on intracellular cGMP concentration and localisation, and via interactions with calcium signalling mechanisms.

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OC06-2

THE IMPORTANCE OF ADENOSINE A1-RECEPTORS FOR RENAL SALT EXCRETION

Brown R., Fredholm B., Persson A.

Adenosine serves as an important modulator of a vast array of physiological functions. The present study was performed to investigate the roll of adenosine A1-receptors in regulating blood pressure and electrolyte balance during variations of dietary salt intake and tubuloglomerular feedback (TGF) mechanism in adenosine A1-receptor (A1AR) knockout (ko) mice. A1AR-ko and wild-type (wt) mice were placed on standardized normal-salt (NS)(0.7%) or high-salt (HS)(7%) diets for a minimum of ten days prior to blood pressure and excretion measurements. The animals were chronically implanted with telemetric blood pressure devices for long-term blood pressure measurement. Blood pressure was continuously recorded in the conscious animals during a 2-week period. Mice were placed in metabolic cages and 24-h urine collections were obtained. On a NS-diet mean arterial blood pressure was approximately 20 mmHg higher in the A1AR-ko (109±3 mmHg) compared to the wt mice (92±4 mmHg). On a HS-diet A1AR-ko blood pressure was 107±4 mmHg compared to 104±3 mmHg in the wt mice. Sodium excretion was elevated in the A1AR-ko compared to the wt mice on NS-diet (0,046±0.009 and 0,027±0.004 µmol/min/10gbw, respectively) and was normalized on HS-diet (0.14±0.02 and 0.12±0.03 µmol/min/10gbw, respectively). In a separate set of experiments TGF mechanism was assessed in mice on a NS-diet. The decrease in tubular stop-flow pressure in response to increased distal tubular flow-rate, found in wt mice (11.4±1.1 mmHg), was absent in the A1AR-ko (0.1±0.8 mmHg) mice. In conclusion, on a NS-diet the A1AR-ko animals lack TGF and therefore lose salt. Earlier results from our lab have shown that the A1AR-ko mice have a significant elevated plasma renin concentration on NS-diet and this could explain the significantly increased blood pressure in the A1AR-ko-animals. During high salt intake the TGF is not essential and salt excretion is essentially normalized compared to the controls.

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OC06-3

TNBS COLITIS SELECTIVELY ALTERS THE NATURE OF MUCOSAL MICROCIRCULATION TO ALLOW INFLAMMATION

Phillipson M., Henriksnäs J., Antoon J*., Perry M*., Holm L.

Inflammatory Bowel Diseases (IBD) cause severe gastrointestinal injury and inflammation of the mucosa. Interaction of leukocytes with endothelial adhesion molecules may initiate the signal, inducing inflammation. We have found that the blood vessels in the superficial gastric mucosa are resistant to leukocyte adhesion and inflammation. Here we studied leukocyte-endothelial (L-E) interactions in the colonic mucosal venules to test the hypothesis that the onset of IBD depends on a breakdown of the anti-inflammatory properties of the superficial mucosal microcirculation. Rats were treated with saline or TNBS (50mg/ml in 50% ethanol, intrarectally) 7-14 days prior to the experiments, then anesthetized with Inactin and L-E interactions in the different colonic layers was assessed either with the dual label antibody technique (ICAM-1 and P-selectin expression) or intravital microscopy. In mucosal venules in the saline treated group, almost no rolling or adherent leukocytes could be detected. However, in the TNBS treated group, rolling in the mucosal venules was 6.9±2.0/min/50µm. In the submucosal and muscularis venules, rolling and adherent leukocytes were observed in both saline and TNBS treated groups without any significant difference between the groups. There was no expression of P-selectin in the control colonic mucosa and only a low expression of ICAM-1 (23% of that expressed per gram in the submucosa and muscularis). After TNBS, P-selectin was significantly increased only in the mucosa, while ICAM-1 was not upregulated. Conclusion: In control animals there is no leukocyte rolling or adhesion in colonic mucosa. However, after induction of colitis, p-selectin is selectively activated in the mucosal venules causing leukocyte rolling, which could be responsible for the onset of inflammation.

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OC06-4

RENAL HYALURONAN ACCUMULATION AND HYALURONAN SYNTHASE EXPRESSION AFTER ISCHEMIA-REPERFUSION INJURY

Hansell P., Johnsson C., Jacobson A., Heldin P., Hällgren R., Göransson V.

Hyaluronan (HA) is a connective tissue component with unique water binding and pro-inflammatory properties. In anaesthetized rats we investigated if renal cortical HA accumulation and the intrarenal distribution and expression of HA synthases (Has 1,2,3) correlate with renal dysfunction after renal ischemia-reperfusion (IR) injury. After 20, 30 or 45 min of unilateral renal ischemia and 72h of reperfusion, renal function and cortical HA content were measured. Has 1, 2 and 3 mRNA were determined using RT-PCR in control and IR kidneys subjected to 45 min ischemia and 72h reperfusion. IR-kidneys had reduced urine concentrating ability, potassium excretion, glomerular filtration rate (GFR) and renal blood flow. On average, IR-kidneys had more than ten times higher amounts of cortical HA than the contralateral control kidney and their water content was elevated while papillary HA was largely unaffected. Has 2 expression in the cortex was heavily upregulated in IR kidneys while Has 3 remained at control levels. Has 1 could not be detected. There was a direct correlation between the amount of cortical HA and the time period of ischemia and also between the cortical amount of HA and depression of functional parameters. In conclusion, IR injury depresses parameters of renal function which coincides with an elevated cortical HA content and Has 2 expression. The enhanced Has 2 expression indicates that the cortical HA accumulation is primarily dependent on increased HA synthesis and not impaired degradation/elimination. The water binding and proinflammatory properties of HA may contribute to renal dysfunction after IR.

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S6-3

THE NEURAL REGULATION OF SODIUM HANDLING BY THE KIDNEY.

Johns E.J.

The kidney receives a very dense innervation from the sympathetic nervous system which represents the autonomic control of its function. The efferent renal sympathetic nerves enter the kidney and pass in close proximity to both vascular and tubular structures. As activity within the nerves increase there is a progressive recruitment of functions; at low levels renin secretion only is raised; thereafter tubular sodium reabsorption increases while it is only at higher levels of activity that renal blood flow and glomerular filtration rate are reduced. In terms of dealing with fluctuating levels of sodium intake, the dynamic responses at the kidney will be exerted via neural influences on renin release and the level of sodium reabsorption. The sensory information flowing into the central nervous system arises from the cardiovascular baroreceptors, somatonsensory receptors, visceral receptors as well as from higher cortical centres. It is therefore important to understand what factors may alter the ability of the central nervous system to sense changes in extracellular fluid balance and thereby determine renal sympathetic nerve activity. It is evident that the brain renin-angiotensin system acts in a neuro-modulatory fashion to determine the sensitivity of the reflex neural regulation of kidney function. Moreover, it is important to be aware of how paracrine and autocrine factors may influence the impact of the sympathetic nerves on the epithelial cells mediating fluid reabsorption. Thus at the cellular level, nitric oxide, superoxide anions and the degree of oxidative stress can influence the effectiveness of transmission at the neuroeffector junction. An understanding of these interactions is important in order to appreciate mechanism underlying pathophysiological states, such as hypertension, where the neural control of the kidney is often abnormal.

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S6-4

LONG-TERM CONTROL OF TOTAL-BODY SODIUM: PRESSURE ESCAPE, RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AND NO

Seeliger E., Reinhardt H.W.

Long-term control of mean arterial blood pressure (MABP) is closely related to control of total-body sodium (TBS). The renin-angiotensin-aldosterone system (RAAS), pressure natriuresis, and nitric oxide (NO) are thought to be important elements of TBS control. Standardized balance studies were performed in freely moving dogs to elucidate their individual contributions as well as their interactions. Long-term 20% reduction of renal perfusion pressure (rRPP) in dogs on high Na intake results in Na retention on day 1 via stimulation of the RAAS, which augments TBS, thus increasing MABP. On the following days, 24-h Na balances become equilibrated again. This resetting of 24-h balances on an elevated level of TBS was termed Pressure Escape in analogy to mineralocorticoid escape. Pressure Escape is mainly accomplished by suppression of aldosterone; low-dose aldosterone infusion during rRPP results in ongoing Na retention. After accomplishing Pressure Escape, the TBS surplus is defended by the body: reduction in Na intake does not reduce surplus of TBS and elevation of MABP, because of a renewed RAAS stimulation. Long-term infusion of the NO-inhibitor L-Nitroarginine (LN; non-pressure dose to prevent pressure effects on renin release and natriuresis) results in TBS deficit via aldosterone suppression. LN does not alter Na retention during rRPP, nor does it compromise the accomplishment of Pressure Escape. A significant contribution of pressure natriuresis to TBS control could only be demonstrated during long-term 20% elevation of RPP as induced by sustained elevation of TBS. Here, pressure natriuresis facilitates Na excretion, which prevents further Na accumulation, but does not restore TBS to normal. A 20% reduction of RPP does not induce Na retention by the putative mechanism of (low-) pressure (anti-)natriuresis, yet only via RAAS stimulation. It is suggested that pressure natriuresis is not operative at lowered, normal, or moderately elevated pressures.

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S6-5

INITIAL DYNAMICS OF WHOLE BODY SDOIUM CONTROL: A PHYSIOLOGICAL WHODUNIT

Bie P.

Normally long term control of sodium metabolism does not imply changes in arterial blood pressure (BP). Therefore, an increase in sodium intake will elicit either an elevation in BP which subsides over time or a neurohumoral tuning of renal function at constant BP. The acute response to sodium loading mimicking sodium intake may help to determine whether regulatory sodium excretion (NaEx) can increase markedly over hours without change in BP. However, the quantitative performance of normal NaEx is easily distorted by drugs leaving conscious, unstressed organisms as the only sources of reliable data.
Recent studies in normal dogs and volunteers have confirmed that slow infusions of saline (0.006-0.04 mmol/min/kg b.wt.) may be performed over hours without changes in arterial blood pressure, but with gradually increasing NaEx to 5-10 times control. Even in subjects on low-salt diet (0.5 mmol/kg/d) a slow infusion of saline (0.02 mmol/min/kg) elicited an

immediate natriuresis rising to a 7-fold increase in NaEx over a few hours. The renal response is explainable by the concomitant decrease in renin system activity (PRA, Ang II and aldosterone). However, BP, glomerular filtration rate, plasma hormones (atrial natriuretic peptide, vasopressin, oxytocin), and intrarenal humoral events (as reflected by excretion rates of nitrates, cGMP, and endothelin) remained constant. Other results indicate that NO generation may be a powerful controller of NaEx capable of overriding the renin system. Taken together, the results leave open several explanations for the sensory mechanism(s) by which the renin system normally seem to dominate the regulation of NaEx: (i) low-pressure receptors, (ii) osmoreceptors, or (iii) concentration receptors responding to filtered load of sodium. In any case, relatively fast components of sodium metabolism exist, demonstrate exquisite sensitivity, operate at constant BP, and may change NaEx by 1-2 orders of magnitude in a matter of hours.

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POSTER SESSION

P06-01

ACUTE EFFECTS OF ACIDOSIS ON PROTEIN AND AMINO ACID METABOLISM IN PERFUSED RAT LIVER

Holecek M., Safranek R., Rysava R., Kadlcikova J., Sprongl L.

Acidosis is frequently associated with protein wasting and derangements in amino acid metabolism. As its effect of on protein metabolism is significantly modulated by other abnormal metabolic conditions caused by specific illness, it is difficult to separate out the effects on protein metabolism solely due to acidosis.
The aim of the present study was to evaluate using a model of isolated perfused rat liver the direct response of hepatic tissue to acidosis.
We have compared the hepatic response to perfusion with solution of pH 7.2 and pH 7.4 (controls). Parameters of protein and amino acid metabolism were measured using both recirculation and single pass technique with 4,5-[3H]leucine, [1-14C]leucine and [1-14C]ketoisocaproate (ketoleucine) as tracers and on the basis of difference of amino acid levels in perfusion solution at the beginning and the end of perfusion. Statistical analysis was performed using Mann-Whitney test.
In the liver perfused with solution of pH 7.2 we observed higher rates of proteolysis, protein synthesis, amino acid utilization, and urea production. Furthermore, the liver perfused with solution of pH 7.2 released a higher amount of proteins to perfusate than the liver perfused with solution of pH 7.4. Enhanced decarboxylation of ketoisocaproate in liver perfused by solution of a lower pH indicates increased catabolism of branched-chain amino acids (leucine, valine, and isoleucine), decreased reamination of branched-chain keto acids to corresponding essential amino acids, and increased ketogenesis from leucine.
The study was supported by a grant of the Grant Agency of the Czech Republic No. 305/01/0578.

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P06-02

THE EFFECT OF AMILORIDE DURING INFUSION OF OXYTOCIN IN MALE SPRAGUE-DAWLEY RATS

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A possible natriuretic mechanism of action of intravenously infused oxytocin was investigated in male Sprague-Dawley rats. The effects of an intravenous bolus injection of amiloride (3.0 mg/kg BW) on urine volume, potassium and sodium excretion and omolality were measured with and without an intravenous infusion of oxytocin in saline solution (1200 ng/h/kg BW). Control values were obtained during infusion of saline solution (0.9% NaCl). To simulate experimental conditions control animals were given an injection of saline solution identical in volume to the injection of amiloride and the following rinsing volume.
The effect of amiloride on urinary flow after administration of oxytocin was an 11-fold increase (from 4.289±0,577 µL/min to 48.827±60694 µL/min), thereby contributing to a 660-time increase in sodium excretion (from 0.025±0,007 to 16.621±2.074 µmol/min). In amiloride-only treated animals, the flow after the bolus dose was 17.731±1.757uL/min and the sodium excretion 4.482±0.795 µmol/min. Administration of oxytocin only resulted in a flow of 8.468±1.555uL/min and a sodium excretion of 1.191±0.317 µmol/min.
Nor was the amiloride-caused change in potassium excretion inhibited by oxytocin. The potassium excretion after treatment with amiloride decreased to 13% of that in the control group (from 3.220±0.387 µmol/min to 0.172±0.080 µmol/min) In the group receiving amiloride and oxytocin both, the decrease was to 4% of control group values (from 3.220±0.387 µmol/min to 0.054±0.019 µmol/min).
Hence, the effects of amiloride were not inhibited by the actions of oxytocin, but rather enhanced. In summary, amiloride administrated after reaching a near steady state effect of oxytocin was found to give rise to an effect far greater than that after administration of oxytocin or amiloride alone. It's therefore concluded that the intrarenal natriuretic mechanisms of oxytocin are likely not to emanate from the amiloride sensitive sodium channels.

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P06-03

THE EFFECT OF CHANGES IN INTESTINAL TRANSIT ON REMOTE SECRETO-MOTOR REFLEXES

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Introduction: The involvement of nervous system in local control of secreto-motor reflexes (SMR) to various secretagogues has already been studied to some extent as well as the importance of enteric nervous system regulating local motility. However, the underlying mechanisms for enteric reflexes to changed intestinal transit (IT) remain to be elucidated. We have therefore designed an experimental model allowing us to study the effects of changes in IT on SMR in vivo.
Methods: Anaesthesia was induced with Nembutal and maintained by intra-arterial infusion with chloralose in rats. Arteria and venae femoralis were catheterised. A proximal jejunal and a distal ileal segment were isolated, cannulated and connected to a pressure transducer and the rest of the intestine was extirpated. In some experiments the extrinsic nervous supply to the segments was cut. The proximal segment was perfused at various perfusion rates (PR).
Results: Increased PR at 6, 64 resp 2ml/h caused a significant reduction of motility Hz in the perfused segment of normal (ND) resp denervated (D) animals. PR over 32ml/h elevated the Hz of the propagating contractions in the distal segment of D animals ($p<0.05$). In the stimulated intestinal segment, PR exceeding 32ml/h augmented the secretion in over 75% of both ND and D rats. An increase of secretion was observed in the more distal segment with PR over 2ml/h in 80% of the ND animals. On the other hand, no secretion appeared in the D animals.
Conclusions: We can conclude that denervation caused an increase in motility of the distal segment indicating a descending inhibitory effect of the nervous system to increased IT. These changes in transit rate result in an elevation of motility by other pathways e.g. hormones. On the other hand, the observed increase in secretion in the more distal segment disappeared after severing the nerves suggesting the involvement of nervous pathways in the development of such secretion to faster IT proximally in the gut.

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P06-04

EARLY CHANGES IN TUBULOGLOMERULAR FEEDBACK IN HYPERTHYROID RATS

Hultström M., Sandgren A., Källskog Ö.

The aim of the present study was to evaluate the tubuloglomerular feedback mechanism (TGF) in hyperthyroid rats. The TGF resets in hypertension. Whether the change in TGF is caused by hypertension or hypertension develops as an effect of changed renal autoregulation is not completely understod. Changes of the TGF mechanism before the onset of hypertension might be indicative of the cause of hypertension in hyperthyroid rats.
Methods: Male Sprague-Dawley rats were treated with either 1000 µg/kg triiodothyroxine (T, n=8) or vehicle (C, n=8) for two days before surgery. Renal blood flow (RBF) and glomerular filtration rate (GFR) was measured for 20 minutes following a one hour stabilization period. Thereafter TGF measurements were performed using micropuncture.
Results: There was no difference in blood pressure but a clear difference in GFR (C:3.48±0.15ml/(min*kg BW) vs. T:3.86±0.13ml/(min*kg BW) $P<0.05$) and RBF (C:6.106±0.52ml/min vs. T:7.77±0.48ml/min $P<0.05$). Micropuncture results show an increase in stop flow pressure (C:38.91±1.27 mmHg vs. T 43.02±1.02 mmHg $P<0.05$) and a decrease in TGF reaction at maximal stimulstion (C: 11.82±0.91mmHg vs.T: 7.017±0.77mmHg $P<0.05$). The turning point was not significantly altered.
Discussion: The increase in RBF and GFR are in accordance with what others have found. The TGF result indicates that an increased proximal absorbtion reduces the signal to the macula densa. This leads to a lower TGF activity and afferent dilation, which is seen as an increase in stop flow pressure. The change in macula densa activation will also trigger the renin angiotensin system that is considered to be the cause of hyperthyroid hypertension.
Conclusion: Hyperthyroidism causes early changes in renal autoregulation which may be responsible for the development of hypertension in hyperthyroid rats.

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P06-05

CHANGE OF BODY FLUID PARAMETERS BY SODIUM INTAKE IN TRAINED CONSCIOUS DOGS

Kjolby M.J., Wamberg S., Bie P.

Background. The effects of daily sodium intake (NaInt) on renal and cardiovascular parameters were measured under steady state conditions. Dogs were fed a commercial low-salt diet plus NaCl to 8 levels of NaInt.
Methods. NaInt were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 8.0 mmol/kg/d for 7 days. Potassium intake was 2.79±0.03 mmol/kg/d. Measurements were made 20 h postprandially after 9 h dehydration. Systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MABP) were measured invasively. Clearance of exogenous creatinine provided glomerular filtration rate (GFR). Plasma volume (PV) was measured by dye dilution and blood volume (BV) determined from PV and arterial hematocrit. Plasma hormones were determined by radioimmunoassay techniques.
Results. SBP, DBP and MABP remained constant irrespective of NaInt at 136.7±1.0, 88.9±0.4 and 107.9±0.4 mmHg, respectively. Heart rate (HR) was constant at 63±1 min-1. PV increased by 0.47±0.04 ml per kg body weight per unit increase in NaInt ($p<0.01$), i.e., 0.47 (ml/kg)/(mmol/kg/d); BV increased by 0.66±0.07 (ml/kg)/(mmol/kg/d) ($p<0.001$). Plasma sodium was constant at 145.2±0.2 mmol/l. Plasma potassium decreased linearly with increasing NaInt by -0.038 (mM)/(mmol/kg/d) ($p=0.001$) while plasma renin (PRA), angiotensin II (AngII) and aldosterone (Aldo) decreased exponentially (=a*exp(k*NaInt)+b, kPRA=-5.5, kAngII=-4.6 and kAldo=-2.4, respectively, all $p<0.05$). Plasma atrial natriuretic peptide, angiotensinogen and vasopressin did not change (69±7 pg/ml, 1008±56 ng/ml and 1.16±0.06 pg/ml, respectively). GFR was constant at 39.1±2.6 ml/min.
Conclusions. Large increases in sodium intake were associated with (i) constancy of blood pressures and HR, (ii) exponential decreases in PRA, AngII and Aldo concentrations, (iii) increase in BV, and (iv) a linear decrease in plasma potassium concentration. BV and plasma potassium may work together to inhibit the activity of the renin system activity during increases in sodium intake.

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P06-06

THE EFFECTS OF ULTRASOUND ON SYNOVIAL FLUID ZINC LEVEL IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Recently, trace element levels such as serum zinc and copper in rheumatoid arthritis patients became important. In some studies synovial fluid zinc levels of rheumatoid arthritis patients were found to be increased.
In this study, the effect of ultrasound on synovial fluid zinc level and synovial fluid leukocyte count was investigated in classical and/or definite rheumatoid arthritis patients, with the application of a dose of 2 watt/cm2 for 5 minutes on the knee joints of twenty patients. Synovial fluid zinc levels were estimated by atomic absorption spectrophotometer.
Synovial fluid zinc levels immediately after application of ultrasound was significantly decreased when compared with that before ultrasound application ($p<0.05$). When the synovial fluid zinc level immediately after ultrasound application was compared with that 24 hours after ultrasound application, the difference was found to be statistically significant ($p<0.05$).
Synovial fluid leukocyte count immediately after ultrasound application was statistically significantly increased when compared with synovial fluid leukocyte count before ultrasound application ($p<0.05$). The decrease of synovial fluid zinc level and increase of synovial fluid leukocyte count immediately after ultrasound application seems to be related to the increase of inflammatory activity due to the ultrasound application.

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P06-07

SUPPRESSED NITRIC OXIDE MEDIATED ARTERIAL DILATION IN RATS WITH ENHANCED RBC AGGREGATION

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The effects of enhanced red blood cell (RBC) aggregation on nitric oxide (NO) dependent vascular control mechanisms have been investigated in a rat model. Rats were exchange-transfused with the suspensions of rat RBC

coated with specially designed and produced co-polymers, resulting in significantly enhanced RBC aggregation during the five day follow-up period. Mean arterial blood pressure increased gradually in five days. Arterial segments of 300 micrometers were isolated from gracilis muscle of rats on the fifth day and mounted between two glass micropipettes in a special chamber equipped with pressure servo control system. Both dose dependent dilation by acetylcholine and flow-mediated dilation of arterial segments pressurized to 30 mmHg and pre-constricted to 50% of the original diameter by phenylephrine were significantly blunted in rats with enhanced RBC aggregation, compared to the control group. Both responses were totally abolished by non-specific NOS inhibitor (L-NAME) treatment of arterial segments, indicating that the responses were NO-related. Additionally, expression of eNOS protein was found to be decreased in muscle samples obtained from hyperaggregating rats. These results imply that enhanced RBC aggregation may result in suppressed expression of NO synthesizing mechanisms, leading to altered vasomotor tonus. This effect can be explained by the decreased wall shear stress due to increased axial accumulation of hyperaggregating RBC.

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P06-08

EFFECTS OF SELECTIVE OPIOID ANTAGONISTS ON HEMODYNAMIC RESPONSES TO HEMORRHAGE

Frithiof R., Hjelmqvist H., Ullman J., Eriksson S., Rundgren M.

Introduction
During a continuous hemorrhage a paradoxical sympathoinhibition causes bradycardia and hypotension. The aim of this study was to investigate the contribution of central opioid mechanisms in initiating and prolonging this decompensated phase in conscious sheep.
Methods
Adult conscious ewes were continuously bled (0.7 ml/kg/min) from a jugular vein until mean arterial blood pressure reached 50 mm Hg. Starting 30 min before hemorrhage either artificial cerebrospinal fluid (aCSF) or one of the following selective opioid receptor antagonists were infused intracerebroventricularly (ICV); ICI 174,864 (delta-rec antagonist, 0.24mg/h), nor-BNI (kappa-rec antagonist, 2.4 mg/h), CTOP (my-rec antagonist, 0.12 mg/h). The infusion was terminated and the shed blood retransfused 60 min after hemorrhage was completed. Cardiovascular parameters were monitored via ultrasonic flow probes and carotid and pulmonary catheters. Data are expressed as mean ± SEM.
Results
Infusion of nor-BNI significantly increased the blood loss necessary to initiate the decompensated phase compared to aCSF controls (18.9 ± 1.0 n=6 vs 13.9 ± 0.5 n=7). Neither ICI 174,864 (16.9 ± 0.9 n=3) nor CTOP (14.2 ± 1.3 n=7) infusion affected the onset of hypotension. There were no apparent differences between experimental groups regarding other measured cardiovascular parameters before, during and after hemorrhage.
Conclusion
ICV nor-BNI delays but does not fully prevent the onset of hypotension and bradycardia during a continuous hemorrhage. This suggests that endogenous opioid kappa-receptor agonists in the CNS are partly involved in initiating the decompensated phase of hemorrhage in conscious sheep. However, studies using localized injections into specified anatomical areas in conscious animals are needed to further investigate the involvement of CNS pathways in the decompensated phase.

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P06-09

ANGIOTENSIN II AT2 RECEPTORS AND DUODENAL MUCOSAL BICARBONATE TRANSPORT IN THE S-D RAT

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Background: Activation of the angiotensin II receptor type 2 receptor (AT2R) has been associated with increased duodenal mucosal alkaline secretion in previous experiments using Sprague-Dawley rats. This effect was absent after changing to another line of S-D rats. The present investigation was undertaken to evaluate if the magnitude of expression of AT2R determined the duodenal mucosal alkaline secretory response to the AT2R agonist CGP42112A.
Methods: Duodenal mucosal alkaline secretion was measured in anaesthetised rats by means of in situ pH-stat titration. Real time PCR and Western blot were used to determine the AT1R and AT2R RNA and protein expression, respectively.
Results: In the previous S-D line CGP42112A elicited a significant 45(8)% net increase of the duodenal mucosal alkaline secretion. In the current line a similar dose of CGP42112A did not elicit any change in duodenal mucosal alkaline secretion (n=11). The response to luminal PGE2 (10-5 M) was similar in the two lines of S-D rats. The RNA expression of AT1aR and AT1bR were significantly lower in tissue from the previous line. The AT2R RNA expression was significantly higher in the previous line. The protein expression of AT1R protein did not differ between the previous and the current line. The AT2R protein expression was significantly higher in tissue from the previous compared to current line. The calculated individual AT1R to AT2R ratios (RNA and protein) were significantly higher in the current line compared to the previous line S-D rats.
Conclusion: A low AT2R expression explains the absence of secretory response to the AT2 agonist CGP42112A.

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S7 CELLULAR AND MOLECULAR ASPECT OF RENAL PHYSIOLOGY

ORAL SESSION

S7-1

TUBULAR CELL FUNCTION : FROM HEALTH TO DISEASE

Prie D., Terzi F., Silve C., Friedlander G.

The general purpose of our group aims to identify the molecular and cellular mechanisms underlying pathological states of renal function. These studies combine in vitro and in vivo approaches, both in aninals and humans, from cell culture to experimental models of renal injury and to clinical investigation. Two examples are given.
The first one concerns the role of vimentin, an intermediate filament which is expressed by mesenchyme-derived cells, but not by epithelial cells, under normal conditions. However, vimentin is re-expressed by proximal tubular cells in culture or, in vivo, during the recovery phase after ischemic injury. Using mutant mice in which the vimentin gene has been invalidated by homologous recombination, we explored the role of this re-expression and showed that vimentin expression affects selectively the activity of Na-glucose cotransporters. In the absence of vimentin, ischemia-induced glycosuria persists for a longer period of time. These data support an important role of vimentin in tubular function after ischemia.
The second example concerns the molecular basis of renal phosphate leak, a defect responsible for hypophosphatemia, nephrolithiasis, and bone demineralization. In numerous patients, this syndrome is not accounted for by endocrine disorders such as hyperparathyroidism. In 20 of these patients with persistent idiopathic hypophosphatemia associated with a decrease in maximal renal phosphate reabsorption, we looked for mutations of type 2a Na-phosphate cotransporter (NPT2a). Two patients, one with recurrent urolithiasis and one with bone demineralization, were heterozygous for two distinct mutations. Phosphate-induced current and sodium-dependent phosphate uptake were impaired in Xenopus oocytes expressing the mutant NPT2a. Coinjection of oocytes with wild-type and mutant RNA indicated that the mutant protein had altered function.
These examples illustrate the interest of combining different approaches in renal pathophysiology.

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S7-2

USE OF KNOCK-OUT MOUSE MODELS FOR THE STUDY OF RENAL ION CHANNELS

Poujeol P., Barrière H., Belfodil R., Rubera I., Poujeol C., Barhanin J., Tauc M.

The knock-out (KO) mice represent powerful tools to investigate the physiological role of membrane transport proteins. Transcripts encoding for CFTR, TASK2 and KCNE1 are highly expressed in kidney although the role of the translated products was not demonstrated in this organ. To elucidate this role, Cl^- and K^+ currents were studied in primary cultures of proximal (PCT), distal (DCT) and cortical collecting tubules (CCT) from wild type and CFTR-/-, TASK2-/- and KCNE1-/- mice.
In wild type mice, transcripts encoding CFTR were found in PCT, DCT and CCT cells but concomitant cAMP-activated K^+ and Cl^- currents were recorded in DCT and CCT cells only. As expected, these cAMP dependent currents were abolished in CFTR-/- mice. The regulatory volume decrease (RVD) process was also investigated. Surprisingly RVD was impaired in the three KO mice. From the data obtained in PCT, DCT and CCT cells of CFTR-/- mice, it could be concluded that CFTR modulates the swelling-activated Cl^- currents by controlling a cascade that involves apical ATP release, adenosine production and Ca^{2+} entry. The activation of swelling K^+ currents exhibited an identical regulation in DCT and CCT cells only suggesting that these currents could belong to maxi K^+ channels. TASK2 transcripts were localized mainly in PCT and the results obtained from TASK2-/- mice indicated that TASK2 was the swelling activated K^+ channel responsible for cell volume regulation process during osmolyte absorption in the proximal tubules. Finally, in PCT cells from KCNE1-/- the impairment of RVD was due to the loss of both swelling-activated Cl^- and K^+ currents. These observations suggest a possible link between KCNE1 and TASK2.
In conclusion, the present studies reveal that CFTR, TASK2 and KCNE1 participate in the control of the renal cell volume regulation. The consequence of the invalidation of these genes on the overall renal function in vivo is now under investigation.

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OC07-1

FUNCTIONS OF THE TWIK-1 K^+ CHANNEL IN MURINE RENAL CORTICAL COLLECTING DUCT PRINCIPAL CELLS

Millar I.D., Taylor H., Arrighi I., Barhanin J., Kibble J.D., Robson L.

TWIK-1 is expressed in the kidney and is thus a candidate K^+-channel participating in setting the resting membrane potential of renal tubule epithelial cells, including the cortical collecting duct (CCD). The aim of the present study, using CCD isolated from wildtype (WT) and TWIK-1 knockout (KO) mice, was to test the hypothesis that loss of TWIK-1 expression results in depolarization of the resting membrane potential and altered cell volume of CCD principal cells.
WT and KO mice were humanely killed by cervical dislocation and collecting ducts were isolated by an enzymatic technique from renal cortical slices. CCDs and their component principal and intercalated cells were identified by light microscopy. Zero-current membrane potential was determined by whole cell patch clamp using standard techniques. The bath solution was a high NaCl Ringer and the pipette solution was a high KCl Ringer. Zero current potential was taken as the steady state potential determined in current clamp mode. CCD diameter was determined using a digital video camera and real–time analysis software. Data are expressed as means ± SEM and statistical significance was tested using Students' t-test and assumed at the 5% level.
Zero current potential of WT principal cells was –62.2 ± 1.5 mV (n=26). TWIK-1 KO principal cells were significantly hyperpolarised at –67.0 ± 1.6 mV (n=16). KCl-induced depolarisation was increased in KO principal cells compared to WT cells. The steady-state diameter of CCD isolated from WT mice was less than the diameter of tubules isolated from KO mice (26.8 ± 0.8 and 32.2 ± 1.8 μm; n = 20 and 12 respectively).
These data show that cell function is altered in renal principal cells isolated from TWIK-1 KO mice. There is a paradoxical increase in K^+ conductance with an increase in tubule diameter in KO cells. The data are consistent with increased K^+ conductance and K^+ fluxes in the absence of TWIK-1 expression.

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OC07-2

REGULATION OF ROMK TRAFFICKING BY PROTEIN TYROSINE KINASE AND PHOSPHATASE

Wang W.H., Lin D.H., Sterling H.

We used the immunocytochemistry to study the role of PTK in the regulation of ROMK membrane location in the cortical collecting duct (CCD) of rat kidney. The expression of c-Src in the renal cortex and outer medulla has been confirmed in kidneys obtained from rats on normal rat chow or on K-deficient (KD) diet. Moreover, the expression of c-Src is always positive in the renal tubules demonstrating a positive staining with antibody of ROMK. To study the role of PTK and PTP in the regulation of ROMK membrane expression, we have carried out the immunocytochemical staining with ROMK antibody in the CCD or cortical thick ascending limb (cTAL) from rats on high K (HK) or on KD diet. A clear membrane staining of ROMK was observed in the cTAL from rats on both HK and KD diet. However, a clear membrane surface staining could be found only in the CCD from rats on HK diet but not from those on KD diet. Treatment of the CCDs from rats on a KD diet with herbimycin A to inhibit PTK increases the ROMK staining in the cell surface. In contrast, treatment of the CCDs from rats on a HK diet with phenylarsine oxide (PAO) to block PTP decreases the positive staining in the cell surface. However, neither herbimycin A nor PAO treatment has significantly changed the staining pattern of ROMK in the cTAL. Two-electrode voltage clamp technique demonstrated that inhibition of PTK or PTP has no significant effect on K current in oocytes injected with ROMK2 and c-Src. Moreover, biotinylation technique has also confirmed that neither herbimycin A not PAO has significantly changed the surface labeled ROMK2 in HEK293 cells transfected with ROMK2 and c-Src. In contrast, herbimycin A significantly increases whereas PAO decreases the surface biotin-labeled ROMK1 in HEK cells transfected with ROMK1 and c-Src. We conclude that c-Src is colocalized with ROMK and that PTK and PTP play an important role in the regulation of ROMK1 surface expression in the CCD.

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OC07-3

CLAUDINS DEPICT RENAL SEGMENTAL DISTRIBUTION THAT CHANGES WITH DEVELOPMENT

Reyes J.L., Lamas M., Martin D., Namorado M.C., Islas S., Luna J., Tauc M., González-Mariscal L.

Objectives. The transepithelial electrical resistance (TER) and complexity of the tight junction (TJ) vary along the different tubular segments suggesting that the molecules that constitute this structure may change in the different segments of the nephron. We studied the differential expression of occludin and several claudins in isolated renal tubules from newborn and adult rabbits. Methods. Isolated renal tubules from newborn and adult rabbits were processed for occludin, claudin-1 and claudin-2 immunofluorescence and Western blot detection of claudin-1 and –2. RT-PCR from isolated tubules was performed for claudins 1 to 8. Results. Immunofluorescence revealed that occludin, claudin-1 and –2 are present at the cell boundaries since the neonatal stage. Claudin-1 is detected in the tighter segments of the nephron (distal and collecting duct), while claudin-2 is found in the leaky portions (proximal). PCR amplification of claudins revealed the presence of claudins 1 to 4 in newborn tubules. In adults, claudin 1, 2 and 4 are present in proximal, Henle's and collecting segments, claudin 3 in proximal and collecting tubules, while claudins 5 and 6 are absent from all tubular portions. Claudin 7 is restricted to proximal tubules, while claudin 8 is present in proximal and Henle's segment. Conclusions. The pattern of occludin distribution is present from the neonatal age. Claudins 4, 7 and 8 are upregulated after birth. Each tubular segment expresses a peculiar set of claudins that might be responsible for the permeability properties of their TJs.

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OC07-4

A NOVEL RENAL SPLICE VARIANT OF CFTR: FUNCTIONAL CHARACTERIZATION IN PROXIMAL TUBULE CELLS.

Barriere H., Belfodil R., Rubera I., Tauc M., Poujeol C., Poujeol P.

The cystic fibrosis transmembrane conductance regulator (CFTR) is known to be both a cAMP-activated Cl^- channel and a regulator of other membrane proteins. In the kidney, we have previously shown that CFTR is expressed as a Cl^- channel in primary cultures of distal and cortical collecting tubule cells, whereas in proximal convoluted tubule cells, no cAMP-activated Cl^- currents have been detected using whole-cell patch-clamp recording. Moreover, it was also demonstrated that CFTR plays an important role in the control of cell volume regulation in the three nephron segments. Therefore, in proximal tubule, CFTR is not expressed as a cAMP dependent Cl^- channel but participates in the stimulation of swelling activated Cl^- currents.
By RT-PCR technique, CFTR mRNA expression was examined in immortalized cells from either proximal or distal tubules. Using primer pair in exons 9 and 13, a 650 bp fragment was amplified in distal cells. This product represents the fragment expected from the published mouse CFTR cDNA sequence. In proximal cells, a 440 bp fragment was detected. The sequence analysis of this amplified segment showed an exon 9-exon 11 splice junction, indicating that the entire exon 10 sequence was eliminated. Using the same strategy, two CFTR-specific products were detected in the whole kidney tissue (i.e. the expected 650 bp PCR product and a smaller 440 bp fragment).
These findings suggest that distal cells express wild-type CFTR and exhibit cAMP-dependent Cl^- currents. In proximal cells, an alternatively spliced CFTR mRNA missing exon 10 is translated and fails to produce cAMP-dependent Cl^- channels. However, this isoform is able to modulate cell volume by controlling the ATP release during hypotonic shock. Experiments are now performed to determine the physiological relevance of such a splice variant of CFTR in the proximal tubule.

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S7-3

REGULATION OF SODIUM REABSORPTION : EARLY STEPS IN ALDOSTERONE AND VASOPRESSIN ACTION

Courtois-Coutry N., Boulkroun S., Le Moellic C., Blot Chabaud M., Farman N

Two hormones play a cooperative role to regulate sodium handling in the distal parts of the nephron : aldosterone (aldo) and vasopressin (AVP). Aldo binds to the mineralocorticoid receptor and promotes transcription of early response genes, as part of the early response preceeding the delayed increase in sodium reabsorption. Beside its well established rapid effects on water and sodium permeability, AVP can also modify transcriptional events, and both hormones converge to regulate the activity of the epithelial sodium channel ENaC. By differential display PCR, we have identify NDRG2 (for N-myc Downstream Regulated Gene 2) as an early aldosterone-induced gene, and calcyclin as an AVP-induced gene in renal collecting duct (CD) cells. Hormonal induction was confirmed and characterized in differenciated CD cell lines (RCCD1 and 2) and in vivo in the native rat epithelium. NDRG2 has four isoforms ; it belongs to a family of genes of unknown functions which are conserved through evolution. It has a wide pattern of expression, in epithelial cells with high transepithelial resistance and also in other tissues including cardiac and squeletal muscle. We have shown that NDRG2 is specifically increased in CD cells after 15-30 min exposure to aldosterone, while glucocorticoid hormones or AVP are uneffective. NDRG2 may activate the Ras-MAPK cascades to enhance ENaC activity. Calcyclin belongs to the family of calcium-binding proteins which may participate to regulation of exocytosis. Calcyclin is expressed all along the renal CD; AVP increases its abundance within 1-7hrs depending on the cell model tested. It appears as necessary to the AVP-induced delayed increase in transepithelial sodium transport, since anti-calcyclin antibodies can block this response. These two early-responsive genes represent new elements of the regulatory pathways controlling sodium reabsorption and putatively blood pressure levels.

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S7-4

THE SERUM AND GLUCOCORTICOID INDUCIBLE KINASE SGK1 IN THE REGULATION OF TRANSPORT

Lang F., Vallon V., Busjahn A., Wulff P., Palmada M., Henke G., Böhmer C., Kuhl D.

The Serum and Glucocorticoid inducible Kinase SGK1 is transcriptionally regulated by a variety of stimuli including cell shrinkage, cytosolic Ca^{2+}, glucocorticoids, mineralocorticoids and calcitriol, and activated by several stimuli including oxidative stress, IGF1 and insulin. SGK1 inactivates the ubiquitinligase Nedd4-2 and thus delays degradation of several cell membrane proteins. Moreover, SGK1 cooperates with NHE regulating factor NHERF2 to enhance the abundance of certain transport proteins in the cell membrane. SGK1-regulated transport proteins include Na^+/K^+ ATPase, Ca^{2+} channels (TRPV5), Na^+ channels (ENaC, SCN5A), K^+ channels (ROMK1, KCNE1/KCNQ1, Kv1.3), and several transporters of organic solutes. The functional significance of SGK1 is illustrated by observations in SGK1 knockout mice (sgk1-/-). At normal diet, renal salt excretion is seemingly normal but requires enhanced plasma aldosterone levels in sgk1-/- mice as compared to wildtype littermates (sgk+/+). Moreover, a salt deficient diet discloses the impaired ability of sgk1-/- mice to adequately decrease renal NaCl excretion despite excessive plasma aldosterone levels, decrease of glomerular filtration rate, and decline of blood pressure. Similarly, the renal excretion of an acute K^+ load is delayed and the plasma K^+ concentration during a chronic K^+ load is enhanced in sgk1-/- mice as compared to sgk1+/+ mice. Certain gain of function modifications of the SGK1 gene have been found in as many as 5 % of an unselected Caucasian population. Significantly enhanced blood pressure in those individuals points to the functional significance of enhanced SGK1 activity in humans. In vitro studies, observations in sgk1-/- mice and in human tissues reveal that the functional significance of SGK1 is not restricted to the regulation of renal and intestinal transport, but that SGK1 affects a wide variety of further functions including neuronal and cardiac excitability, cell proliferation and fibrosis.

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S7-5

STRUCTURE/FUNCTION RELATIONSHIP OF RENAL BRUSH BORDER MEMBRANE Na/Pi COTRANSPORTERS

Murer H.

Renal proximal tubular (PT) brush border membrane (BBM) phosphate (Pi) transport is sodium (Na) dependent and involves Na/Pi-cotransport. In adult animals (rats/mice) the type IIa Na/Pi-cotransporter determines rates of BBM

Pi-flux, in young animals a type IIc transporter contributes to transport activity. In small intestine (SI) BBM Pi-flux is via the type IIb transporter. Physiological regulation occurs via altered BBM expression of type II transporters.
The type IIa Na/Pi-cotransporter mediates an electrogenic cotransport of 3 Na and 1 Pi; a Na-leak (1 Na ion) occurs in the absence of Pi. Type II transporters most likely contain 8 transmembrane segments, with intracellular NH2- and COOH-termini. Parts of predicted intracellular loop (ICL) 1 and extracellular loop (ECL) 3 are homologous and may contribute to a permeation pore. The transporter operates as a monomer.
Internalization of the type IIa cotransporter is in response to a variety of agonists activating PK-A, PK-C and/or PK-G; these kinases converge in the MAP/ERK-kinase pathway, leading by unknown mechanisms to internalization (megalin dependent).
Specificity for internalization depends on two basic amino acid residues in predicted ICL3. (Re-)insertion depends on sequences located in the COOH-terminus: three terminal (TRL) and two internal residues. The terminal amino acids are required for apical scaffolding via PDZ interactions involving PDZK-1 (4 modules) and NHE-RF1 (2 modules). Interaction with the transporter is via one module allowing further interactions either with other brush border constituents (e.g.: transporters, receptors) or with elements of the cellular regulatory machinery (e.g. kinase anchoring proteins, cytoskeletal elements).

For reference: Murer H. et al: Annu. Rev. Physiol. (2003) 65:531-542

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POSTER SESSION

P07-01

VITAMIN E TREATMENT PREVENTS DIABETES-INDUCED DECREASE IN RENAL TISSUE OXYGEN TENSION AND BLOOD FLOW

Palm F., Hansell H., Fasching A., Liss P., Carlsson P.-O.

We have previously recorded decreased renal oxygen tension and local renal blood flow, with associated metabolic disturbances, in rats with streptozotocin-induced diabetes. This study aimed to investigate if a diet enriched with 5% (wt/wt) of the antioxidant vitamin E, yielding an approximate daily dosage of 5 g/kg of vitamin E, can prevent these hemodynamic changes. Oxygen tension was recorded with Clark-type microelectrodes (o.d. 3-6 µm), whereas laser-Doppler flowmetry was used to measure local blood flow. The oxygen tension profile, with values recorded each mm from cortex to papilla, was 48±1, 25±2, 46±1, 34±1 and 24±1 mm Hg in non-diabetic animals (n=8). In comparison, animals diabetic for 4 weeks (n=7) had ~35 % lower oxygen tension values at all corresponding depths. The decrease in oxygen tension was more pronounced in the medullary region and totally preventable by daily administration of vitamin E (n=9) throughout the course of diabetes. A marked blood flow gradient existed between the cortical and medullary region in all animals. The untreated diabetic animals displayed ~20 % decreased blood flow in the medullary region compared to control animals. This decrease could be prevented by daily administration of vitamin E.
In conclusion, daily administration of the antioxidant vitamin E can prevent diabetes-induced disturbances in renal tissue oxygen tension and blood flow.

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P07-02

SIMULTANEOUS IN VIVO MEASUREMENTS OF NITRIC OXIDE, BLOOD FLOW AND OXYGEN TENSION IN THE RENAL CORTEX

Palm F., Hansell P., Fasching A., Carlsson P.-O., Liss P.

This study aimed to investigate the effects of the nitric oxide substrate L-arginine and the unspecific nitric oxide synthase inhibitor L-Nw-nitro-L-arginine metyl ester (L-NAME) on nitric oxide (NO) formation, blood flow and oxygen tension in the renal cortex of control and diabetic rats. NO was measured using Whalen-type recessed microelectrodes (o.d. 10-15 µm) coated with a nafion membrane. Laser-Doppler flowmetry was used to measure local blood flow, whereas oxygen tension was recorded with Clark-type microelectrodes (3-6 µm o.d.). The diabetic animals had compared to control animals a larger transient increase in renal cortical NO concentration [Δ 40 nM (diabetic, n=8) vs Δ5 nM (control, n=7)] and a concomitantly larger blood flow increase after infusion of L-arginine (50 mg/kg BW). Mean arterial blood pressure and cortical oxygen tension were not affected by L-arginine in either control or diabetic animals. In control animals, injection of L-NAME induced a progressive decrease in renal cortical NO concentration throughout the study period (20 min, Δ90 nM). Concomitantly, renal cortical blood flow decreased by ~5% and cortical oxygen tension by ~50%. The renal cortical NO concentration in diabetic animals was not significantly affected by infusion of L-NAME. Despite this, the blood flow decrease was more pronounced (22%) and the oxygen tension decrease similar to that in control animals.
In conclusion, the diabetic animals responded with a larger increase in NO formation after infusion of L-arginine, but did not respond at all to infusion of L-NAME. This suggests a limitation of substrate for the formation of NO. Furthermore, the exaggerated cortical blood flow responses to L-arginine and L-NAME administration in diabetic animals indicate NO hypersensitivity in the renal cortex.

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P07-03

EFFECT OF FUROSEMIDE AND HYDROCHLOROTHIAZIDE ON SODIUM AND POTASSIUM EXCRETION IN ROMK KNOCKOUT MICE

Wang T., Yang, X., Yan, Q., Hebert, S., Giebisch G.

We recently reported (JBC 277:37881, 2002) the absence of the small-conductance K^+ channel in KCNJ1 (Kir1.1) null mice, ROMK(-/-). This channel mediates K^+ secretion in cortical collecting duct (CCD) and K^+ recycling in the thick ascending limb (TAL). ROMK (-/-) mice show significant natriuresis and kaluresis with volume depletion. The reduction of Na^+ and water absorption is compensated by increased water and food intake. To investigate the effects of ROMK deletion on salt transport in the TAL and on enhanced salt delivery to the distal convoluted tubule (DCT) thiazide-sensitive NaCl-cotransporter, we compared the effects of furosemide (F) and hydrochlorothiazide (HCTZ) on urinary Na^+ and K^+ excretion in ROMK wild-type (+/+) and null (-/-) mice. Two types of the experiments were performed: (1) 6 hour metabolic studies with urine collections before and after injection of F or HCTZ, and (2) renal clearances in anesthetized animals. Both F and HCTZ produced significant diuretic, natriuretic and kaluretic effects in +/+ mice. However, F did not change either urine volume or Na^+ excretion in ROMK (-/-) mice. In contrast, HCTZ produced larger natriuretic effects in ROMK (-/-) than (+/+) mice. ENa increased 51% in (+/+) and increased 156% in (-/-) mice. Renal clearance data show that the baseline of GFR was significantly reduced in ROMK (-/-) mice (0.38 vs. 0.82 ml/min/100gBW). The increments of FENa and FEK by iv F were diminished by 70% and 73%, yet the effects of HCTZ on FENa increased 124% times in (-/-) mice. In contrast, FEK values were similar in (+/+) and (-/-) mice given HCTZ despite the larger FENa in (-/-) mice. Conclusions: (1) ROMK channels are important in K^+ recycling supporting Na^+ absorption in the TAL via Na-K-2Cl cotransport. (2) HCTZ-sensitive NaCl-cotransporter activities in the DCT are upregulated in ROMK null mice likely due to increased salt delivery from the loop, and (3), K^+ secretion in the CCD is compromised in ROMK (-/-) mice.

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P07-04

ARACHIDONIC ACID STIMULATES CALCIUM ENTRY IN THE THICK ASCENDING LIMB OF MOUSE NEPHRON
Paulais M., Teixeira M., Butlen D., Teulon J.

Arachidonic acid (AA), a cis poly-unsaturated fatty acid, is an ubiquitous component of membrane phospholipids. In the thick ascending limb (TAL) of the nephron, AA modulates NaCl reabsorption using different pathways: (i) it modulates the activity of ionic channels either directly or after its degradation by cyclooxygenase or cytochrome P450 pathways and (ii) also affects the cAMP-dependent transduction pathway. Our aim was to determine whether, as in many other cell types, AA could also affect the Ca^{2+}-dependent transduction pathway in the TAL.
Intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in mouse cortical TAL (CTAL) tubules was measured with the Ca^{2+}-sensitive fluorescent probe Fura2. Exposure of CTAL tubular fragments to AA caused a monophasic increase in $[Ca^{2+}]i$. This effect was quite specific among other fatty acids. AA acted intracellularly but not through one of its known degradation products. Thus, ETYA, a non-specific inhibitor of known AA degradation pathways, had no effect on the $[Ca^{2+}]i$ increase. The response to AA was abolished upon removal of external Ca^{2+}, indicating that it may have stimulated Ca^{2+} entry. Indeed, experiments monitoring Fura2 quenching by Mn^{2+} revealed a profound and rapid stimulation of Mn^{2+} entry rate upon exposure to AA.
We conclude that, in addition to its known effects through its degradation products, AA stimulates a Ca^{2+} entry pathway in the TAL either directly or by a yet undefined metabolite. This may provide an additional pathway for the modulation of NaCl reabsorption by this nephron segment.

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P07-05

NITRIC OXIDE AND PROSTAGLANDINS INFLUENCE RENOMEDULLARY HYALURONAN CONTENT
Rügheimer L., Hansell P.

The interstitial glucoseaminoglycan hyaluronan (HA) forms a gel-like substance with water, which influence the transport properties primarily of water. There is a large amount of HA in the renal papillary interstitium while in the renal cortex the HA level is only about 1% of that in the papilla during normal physiological conditions. Increased HA levels in the renal papilla are found during water loading and a decrease is found during dehydration. An HA-elevation favors excretion of excessive water by changing the physico-chemical characteristics of the papillary interstitium. The hormonal regulation of papillary HA turnover is not completely understood. The interest of the present investigation was to study the influence of NO and prostaglandins on kidney HA content. Anaesthetized male Sprague-Dawley rats were given either isotonic saline (control), hypotonic saline (water loading for 2h), Indomethacin or L-NAME. The influence of Indomethacin or L-NAME was also tested in combination with water loading. The regional intrarenal HA content was determined using a radioimmunoassay. Baseline papillary HA levels were not affected by Indomethacin treatment (2h) while it was slightly reduced by L-NAME. In the water loaded animals an increased urine flow rate was accompanied by increased papillary HA thereby confirming earlier observations. The water loaded animals given L-NAME or Indomethacin did not respond with the normal elevation of papillary HA. No changes in cortical HA levels occurred during any treatment modality. We suggest that NO and prostaglandins are involved in the process whereby papillary HA is elevated in response to water loading. Furthermore, NO but not prostaglandins influence the baseline renal papillary HA content.

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P07-06

PROPERTIES OF A P2X RECEPTOR IN FROG ISOLATED PROXIMAL TUBULE CELLS
Davis J.P., Robson L.

P2X receptors are a subfamily of purinoceptors that are activated by extracellular ATP, forming Ca^{2+} permeable channels. A number of P2X receptors have been cloned. These cloned receptors form homomeric P2X receptors that have specific pharmacological profiles. In addition, some may also exist as heteromeric receptors with altered properties. The aim of this study was to determine the properties of an ATP activated current in frog proximal tubule cells and compare these with those of the cloned P2X receptors.
Single proximal tubule cells were isolated from frog kidneys by enzyme digestion and whole cell patches obtained via the basolateral membrane. The pipette and bath contained symmetrical NaCl amphibian Ringers, with low pipette Ca^{2+} and 0.5 mM Ca^{2+} in the bath. Whole cell potential was held at –100 mV and was then ramped between –100 to +20 mV. Agonist potency was examined by exposing patches to 500μM ATP and 500μM αβmethylene-ATP (αβmeATP) with the order of agonist exposure altered with each patch. Antagonist potency was examined by exposing patches to 500μM ATP, in the absence or presence of 100μM suramin. Data are expressed as mean ± S.E.M.
In paired patches, addition of ATP to the extracellular surface of patches increased outward conductance (Gout) by 13.03 ± 1.60 μS/cm2. αβmeATP increased Gout by 14.15 ± 2.68 μS/cm2 (n=13). The response to ATP was inhibited by suramin. In paired patches ATP increased Gout by 10.10 ± 2.76 μS/cm2 and 4.58 ± 0.86 μS/cm2 (n=16), in the absence and presence of suramin, respectively.
These data show that proximal tubule cells isolated from the frog contain a conductance equipotently activated by ATP and αβmeATP. The conductance is also sensitive to the P2X inhibitor suramin. Of the cloned P2X receptors these properties most closely match those of P2X2/P2X3 heteromeric channels.

University of Sheffield – UNITED KINGDOM

P07-07

PREVENTION OF CASPASE-DEPENDENT APOPTOSIS, RENAL DYSFUNCTION BY MELATONIN AFTER ISCHEMIA/ REPERFUSION
Kunduzova O., Escourrou G., Seguelas M., Delagrange P.*, De La Farge F., Cambon C., Parini A.

The pineal hormone melatonin has been reported to protect the tissue from oxidative damage. This study was designed to determine whether melatonin could prevent cell events leading to tissue injury and renal dysfunction after ischemia/reperfusion. Using an in vivo rat model of ischemia-reperfusion, we show a significant increases in kidney malondialdehyde concentrations, reflecting lipid peroxidation, and cell apoptosis measured by TUNEL staining. This apoptotic cell death was associated with an increase in the activity of the pro-apoptotic factor caspase-3, determined by fluorometric protease activity assay. Histomorphological analysis of ischemic kidneys revealed that most extensive tubular necrosis occurred at 24 and 48 h after reperfusion, which correlated with peak elevations in blood urea nitrogen and creatinine. Rat pretreatment with melatonin prevented lipid peroxidation,

cell apoptosis and necrosis and blocked caspase-3 activity. The prevention of tissue injury was associated with the improvement of renal function as shown by the decrease in blood urea nitrogen and creatinine concentrations. The demonstration that melatonin prevents post-reperfusion apoptotic and necrotic cell death and improves renal function suggests that melatonin may represent a novel therapeutic approach for prevention of ischemia/reperfusion injury.

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P07-08

RENAL RESISTANCE TO CARDIAC NATRIURETIC PEPTIDES IN PATIENTS WITH PULMONARY HYPERTENSION

Charloux A., Chaouat A., Piquard F., Doutreleau S., Brandenberger G., Weitzenblum E., Geny B.

Patients with pulmonary hypertension (PHP) demonstrate increased cardiac atrial and brain natriuretic peptides (ANP, BNP) that correlate with parameters of right ventricular function. However, PHP develops peripheral edema as the disease progresses. We evaluated, for the first time in PHP without left heart dysfunction, the ANP and BNP renal responsiveness in response to an acute saline load.
Seven PHP (primary PH or thrombembolic disease), NYHA II/III, free of edema, and 7 controls (CTL) were included. After a standardized breakfast, the subjects remained supine. Three hours later ten ml/kg isotonic saline solution were infused over 30 minutes. Blood and urine samples were obtained before infusion, and at 60, 120 and 180 min after the beginning of infusion.
ANP and BNP were elevated in PHP (140±32 vs 27±4 pg/mL, $p<0.001$ and 97±24 vs 7.5±2 pg/mL, $p<0.001$) but did not increase after infusion. Cyclic guanosine monophosphate (cGMP), ANP's second messenger was 60% higher in PHP. Plasma renin activity and aldosterone were in the range of normal values and decreased after infusion.
PHP excreted 22 ±8% of the sodium load over 3 hours vs 37±3% in CTL ($p=0.007$). Natriuresis was 0.08±0.03 in PH vs 0.23±0.05 mmol/L in CTL ($p<0.01$) over the first hour. PHP had lower glomerular filtration rates (GFR) than CTL (71±8 vs 91±6 mL/min/1.73m², $p<0.05$). Fractional Na^+ excretion was lower in PHP than in CTL after infusion (0.65±0.24 vs 1.18±0.15, $p<0.05$).
PHP without evidence of fluid retention have an impaired capacity to excrete sodium that results from decreased GFR and enhanced Na^+ tubular reabsorption. Patients's ANP, BNP and cGMP levels were 20, 13 and 1.6 fold those observed in CTL, with a 3 fold lower natriuresis, supporting evidence of renal hyporesponsiveness to ANP and BNP. Such impaired response appears thus to be located both at the cGMP production level and beyond cGMP formation. The circulating renin-angiotensin system is unlikely to play a role

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P07-09

POLYETHYLENE GLYCOL ADDED TO A HIGH-Na UW SOLUTION PROTECTS KIDNEY GRAFTS AFTER TRANSPLANTATION

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The gold standard liquid for organs cold preservation is the University of Wisconsin (UW) solution. Its efficiency seems linked to the presence of colloids as well as agents able to decrease oxidative stress. Although that it is largely used and incontestably efficient, performances of this liquid are limited by the presence of hydroxyethyl starch (HES). HES benefit has never been clearly demonstrated and high-K^+ concentration in UW could damage endothelial cells.
In the present study, we report how a high-Na UW solution containing polyethylene glycol (PEG) as oncotic supply (Na-PEG-UW solution) could improve kidney function after auto transplantation in pigs.
The study report the results obtained in 16 pigs randomly divided between a control group (n=4) subjected to sham surgery and 2 preserved groups: kidneys were harvested, washed-out and cold-stored for 24h in UW (group1, n=6) or Na-PEG-UW (group2, n=6) solutions and then transplanted after 1h of warm ischemia. Renal function studies included daily plasma creatinine (CrP) and urea (UrP) levels. Renal biopsies were performed at postoperative day (POD) 1, before and after revascularization and before sacrifice at POD7.
The results showed that CrP and UrP peaked at POD3 in both preserved groups. At POD6 and 7, the group2 (Na-PEG-UW) animals decreased dramatically their CrP and UrP to merge the levels in shown in sham group whereas group1 (UW) pigs still kept very high levels of creatinine and urea ($p<0.05$). Moreover, histological studies showed less tubular necrosis, less inflammatory infiltration and almost no fibrosis in group2 compared to group1.
In conclusion, Na-PEG-UW solution protects more efficiently renal grafts against ischemia-reperfusion injuries than conventional UW solution. PEG has been shown to bind phospholipids and be an effective antioxidant. In addition, high-Na level in our solution may decrease intrarenal micro-vascular injuries.

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P07-10

EFFECT OF CHROMANOL 293B ON RENAL Na^+ AND GLUCOSE EXCRETION IN THE ANESTHETIZED MOUSE

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The KCNQ1 and KCNE1 proteins interact to form a voltage-regulated K^+ channel, and are coexpressed in the renal proximal tubule. KCNE1 knockout mice have increased urinary flow rate and increased urinary excretion of Na^+ and glucose, suggesting that the KCNQ1/E1 complex may play an important role in maintaining renal proximal tubule Na^+ coupled transport. The aim of this study was to examine the role of KCNQ1 in normal mice by studying the renal effects of the KCNQ1 inhibitor chromanol 293B. Adult male SV129 mice were anaesthetised and surgically prepared for renal clearance measurements. Animals received an intravenous infusion of BSA in isotonic saline. After surgery, chromanol 293B was administered via the infusate as a bolus for 15 minutes followed by a maintenance infusion for a further 90 minutes. 45 minutes equilibration was allowed and then urine collected over a 60-minute period. 3H-inulin was also infused to allow estimation of GFR. Two doses of chromanol 293B were used: 4/2 mg/kg and 8/4 mg/kg (bolus/maintenance). Controls were given an equivalent dose of DMSO vehicle in the infusate. A terminal blood sample was taken to obtain plasma. 3H-inulin was assayed by liquid scintillation counting, Na^+ by flame photometry and glucose by microfluorometry. Statistical significance was assessed using one-way ANOVA.
In control mice urine flow was 7.6 ± 0.7 µl/min, Na^+ clearance (CNa) was 9.8 ± 0.5 µl/min and glucose clearance (CGluc) was 0.7 ± 0.1 µl/min, n=6. These were unaltered in mice infused with either 4/2 mg/kg or 8/4 mg/kg chromanol 293B. Urine flow was 8.5 ± 0.4 µl/min (n=6) and 7.6 ± 0.9 µl/min (n=5), respectively. CNa was 9.3 ± 0.4 µl/min and 6.9 ± 0.9 µl/min, respectively. CGluc was 0.6 ± 0.0 µl/min and 0.9 ± 0.2 µl/min, respectively. This lack of effect of chromanol 293B suggests that KCNQ1 does not play a role in maintaining renal proximal tubule Na^+ coupled transport.

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P07-11

EARLY CHANGES IN GLOMERULAR CHARGE SELECTIVITY IN STREPTOZOTOCIN DIABETIC RATS

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The objective of this study was to evaluate possible alterations in glomerular charge selectivity in early diabetes by assessing the ratio of the glomerular sieving coefficient of neutral (charge modified) albumin (n-alb) to that of native (negatively charged) albumin (alb) in diabetic Wistar rats made diabetic (at the age of 7-8 w) using streptozotocin (90 mg/kg BW). The blood glucose levels were kept between 15 and 25 mmol/L by daily i.p. insulin administration. The rats were investigated at 3 w and 9 w of diabetes duration, respectively, and compared to non-diabetic (10-12 w old) control rats.
The n-alb/alb sieving coefficient ratio was determined from the 7-8 min protein clearance to the kidney cortex and urine of 131-I-n-alb and 125-I-alb, simultaneously infused i.v., using a tissue uptake technique and urinary sampling (Tenstad et al, Scand J Clin Invest 56:409-414, 1996). The glomerular filtration rate (GFR), the renal plasma flow (RPF) and filtration fraction (FF) were assessed using 51-Cr-EDTA and 125-I-Hippuran clearances from plasma to urine, respectively.
GFR was significantly increased in diabetic rats at both 3 w and 9 w of diabetes duration (2.7+/-0.2 (SE) ml/min (n=8) and 2.6+/-0.1 ml/min (n=10))

compared to control (2.1+/-0.1 ml/min (n=10); p<0.01), as was also the FF in the diabetic groups (0.37+/-0.01 vs. 0.31+/-0.01) The n-alb/alb sieving coefficient ratio was slightly, but significantly, increased after 3 w of diabetes duration (12.5+/-0.3 vs. 8.9+/-0.6 in control; p<0.05), but significantly fell at 9 w of diabetes duration to 5.4+/-0.2 (p<0.001 vs. 3 w).
The present study indicates that glomerular charge selectivity is maintained intact during the earliest phase of experimental diabetes (at 3 w), but that loss of anionic glomerular membrane charge is an important feature of the further development of diabetic glomerular disease.

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P07-12

ACE INHIBITION REDUCES HEART FAILURE-INDUCED RENAL INCREASE IN PHOSPHODIESTERASE ACTIVITY
Clauss F., Charloux A., Zoll J., Monassier L., Doutreleau S., N'Guessan B., Piquard F., Lugnier C., Geny B.

Objectives: Emergence of renal resistance to atrial natriuretic peptide (ANP) is considered as a turning point in chronic heart failure (HF) evolution, indicating the progression from a compensated to a decompensated state. Its mechanisms remain to elucidated. We hypothetized that increased renal phosphodiesterase (PDE) activity might reduce ANP's second messenger availability (cGMP), blunting thus its natriuretic effect during HF and resulting in abnormal fluid homeostasis.
Methods : HF was induced by left descending coronary artery occlusion in rats not treated (placebo) or treated with the angiotensin-converting inhibitor (ACEi, Perindopril). After 4 months, kidneys were quickly removed and the cortex total and PDE 2 specific cGMP-PDE activities were determined by radioenzymatic assay later and compared to that of sham-operated rats.
Results: ACEi reduced rats'mortality and improved their echocardiographic ejection fractions. Both total and specific cGMP-PDE 2 activities increased in HF rats untreated as compared to shams (165 ± 20 vs 106 ± 16 pmol/min/mg (P = 0.05) and 96 ± 10 vs 76 ± 18 pmol/min/mg, respectively). Interestingly, ACEi reduced significantly such total and specific activities in treated rats (from 165 ± 20 to 80 ± 6 pmol/min/mg (P < 0.01) and from 96 ± 10 to 43 ± 4 pmol/min/mg (P < 0.05); for total and cGMP-PDE 2 activities in placebo and treated HF rats, respectively).
Conclusions: Renal hyporesponsiveness to ANP during HF is likely to occur beyond cGMP production. Such intracellular alteration appears reversible under ACEi. These results support the use of ACEi in diseases characterized by elevated ANP and reduced natriuresis such as HF, renal failure and cirrhosis.

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P07-13

LOCALIZATION OF SWELLING-ACTIVATED Cl^- AND cAMP-SENSITIVE Cl^- CURRENTS IN IMMORTALIZED DISTAL CELLS
Belfodil R., Barrière H., Tauc M., Poujeol C., Poujeol P.

CFTR is not only a chloride channel activated by cAMP but it is a modulator of other ion channels as ENac, ROMK, ORCC...
The aim of this study was to localize the Cl^- currents implicated in the RVD process and regulated by CFTR in apical or basolateral cell membranes of mouse kidney.
In a previous study performed in distal convoluted (DCT) and cortical collecting tubules (CCT) we demonstrated the existence of CFTR Cl^- currents activated by cAMP and of swelling-activated Cl^- currents controlled by CFTR. To obtain further informations on the membrane localization of these currents, iodide ($125I^-$) efflux experiments were carried out in immortalized DCT cells from CFTR wild-type and CFTR-/- mice. Growing on collagen coated permeable filters, the cells developed a high transepithelial resistance (700 to 1800 Ohm.cm2) within 4-6 days.
In CFTR+/+ DCT cells, hypotonic shock on the basolateral membrane did not induce $125I^-$efflux either across the basolateral or the apical membrane. By contrast, a hypotonic shock on apical membrane induced a $125I^-$ loss through the apical membrane only. Moreover the addition of forskolin (10µM) strongly increased an apical $125I^-$ efflux. These effluxes were completely blocked by 100 µM of NPPB. In CFTR-/- DCT cells hypotonic shock or FK application were completely inefficient in increasing apical or basolateral 125 I^-efflux.
In conclusion, these results indicate that the swelling-activated Cl^- and the cAMP sensitive Cl^- channels depend from the presence of CFTR and are colocalized on the apical membrane of DCT cells. This colocalization strengthens the observation that CFTR could control the swelling-activated Cl^- conductance by controlling a cascade which involved apical ATP release, adenosine production and Ca^{2+} entry. Further experiments are now undertaken to localize the K^+ currents involved in RVD process.

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P07-14

THE URINARY BLADDER SMOOTH MUSCLE REACTIVITY IN VITRO – HUMAN VERSUS GUINEA PIG
Mokry J., Svihra J., Nosalova G., Kliment J., Urdzik J.

Introduction: Various pathological models of urinary bladder smooth muscle hyper- or hyporeactivity and different laboratory animals instead of human in various experiments are used. Mechanisms of bladder smooth muscle contraction and relaxation and interspecies differences in various pathologic states are of incremental interest of clinicians because of relative high rate of overactive bladder patients in population.
Objectives: The aim of our study was to assess the in vitro reactivity of urinary bladder smooth muscle in human and in guinea pigs to two different pharmacological agents – muscarinic agonists acetylcholine (ACH) and carbachol (CAR).
Methods: Both kinds of strips were aerated under tension 4g (30 min) and consecutively 2g (another 30 min) in Krebs-Henseleit´s solution in organ-bath. Thereafter CAR and ACH were added to organ-baths in cumulative manner (concentrations 10-8 – 10-5 mol/l) and concentration-response curves were constructed.
Results: We observed significantly higher reactivity of smooth muscle strips to CAR, comparing to ACH at the same concentrations both in strips from guinea pigs (10-5 mol/l and 10-6 mol/l p<0,001; 10-7 mol/l p<0,01) and in human tissue (10-5 mol/l and 10-6 mol/l p<0,01). The reactivity differences between human and guinea pigs strips to adding of ACH and CAR were significant (p<0,001) at all observed concentrations.
Conclusions: In our experiments we confirmed that CAR was more potent constrictor than acetylcholine in detrusor smooth muscle strips of guinea pigs and in human. The interspecies difference is significantly shifted to the guinea pig tissue comparing to human urinary bladder smooth muscle.
Acknowledgement: This work was supported by APVT Grant No. 20-013102.

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P07-15

THE DROSOPHILA RENAL TUBULE AS A GENETIC MODEL EPITHELIUM
Dow J.A.T., Kerr M., Davies S.A.

Every physiologist's goal is to be able to study function non-invasively under 'physiological' conditions. However, this is rarely straightforward, and it is usually necessary to simplify the problem until it becomes tractable. Transgenic technology, in an appropriate genetic model organism, can provide a valuable platform for truly integrative physiology.
The Drosophila Malpighian (renal) tubule is an ideal such model. Although among the smallest epithelia ever studied (150 cells), it is amenable to both physiological study and genetic intervention. It is also capable of pumping fluid at a prodigious rate, transporting its own cell volume every 10 s. There are two major cell types that are defined by the genetic 'enhancer trapping' technique: one specialised for electrogenic ion transport energised by an apical plasma-membrane V-ATPase, and the other for shunt chloride conductance and water flow. (This spatial segregation of V-ATPase is reminiscent of CCD intercalated cells). Cation transport in principal cells is stimulated by cyclic nucleotides or autocrine nitric oxide, whereas chloride flux is regulated by calcium in stellate cells.
To analyse the control of these transport processes further, we have developed a series of transgenes that can be targeted to any cell domain of our choice. These include apoaequorin, allowing real-time monitoring of calcium; two serotonin receptors, allowing manipulation of cAMP and calcium: and a rat ANP receptor, which directly elevates cGMP levels. In this way, it was possible to demonstrate a new signalling modality for the stellate cell in the tubule, implying the existence of novel hormones and receptors.
These technologies allow us to perform new, cleaner experiments in integrative physiology of renal function, or indeed in any tissue of our

choice, in fly. In principle, there is no reason why they cannot be extended to other models, such as mouse.

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P07-16

INVOLVEMENT OF DMT-1 IN RENAL HANDLING OF IRON
Gburek J., Thevenod F., Nielsen S., Christensen E.I., Smith C.P.

We have recently demonstrated that substantial amounts of transferrin are filtered by glomeruli and that the protein is reabsorbed in the kidney proximal tubule [Kozyraki R et al. PNAS, 98:12491-6, 2001]. While the protein part of transferrin is degraded in lysosomes the intracellular routes of released iron are not characterized. It is also possible that some amounts of iron may dissociate from transferrin due to tubular fluid acidification and reach distal parts of nephron. Our studies have shown that Divalent Metal Transporter 1 (DMT-1) is broadly expressed in the kidney [Ferguson CJ et al. Am. J. Physiol., 280: F803 - F814, 2001]. Thus, herein we examined a possible involvement of DMT-1 in renal iron handling. Measurements of iron concentration in tubular fluid collected from PCT yielded an iron concentration profile confirming significant iron reabsorption along the length of the PCT. DMT1 expression in the kidney was sensitive to dietary iron intake and the expression level of DMT1 was inversely related to the dietary iron content. Changes in DMT1 expression occurred intracellularly in the proximal tubule and in the apical and subapical regions of the distal convoluted tubule. This was consistent with the subcellular distribution of the transporter as revealed by immuno-gold/electron microscopy. In the proximal tubule and distal convoluted tubule DMT-1 was localized in the lysosomal membrane. Increased DMT1 expression was accompanied by a decrease in urinary iron excretion rate, and vice versa when DMT1 expression is reduced. Together these findings suggest that modulation of renal DMT1 expression may influence renal iron handling.

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P07-17

Na-COUPLED ANION TRANSPORT IN TOAD COLLECTING DUCT SYSTEM MAY INVOLVE A NaPi COTRANSPORTER
Moebjerg N., Amstrup J., Werner A., Novak I.

In freshwater, amphibians produce very dilute urine and the collecting tubules and ducts of the kidney - the collecting duct system - contribute to dilution. In terrestrial amphibians one major task of the collecting duct system may be K secretion [1]. We studied mechanisms of ion transport in isolated and perfused collecting tubules and ducts from toads, Bufo bufo, kept under terrestrial conditions. Cells were impaled with glass microelectrodes across the basal cell membrane. A rapid depolarization of the basolateral membrane potential (Vbl) occurred upon lowering bath [Na] from 102 to 7 mM. In collecting tubules a Vbl of -67±2 mV depolarized by 16±2 mV (n = 21) and in ducts a Vbl of -73±3 mV depolarized by 18±3 mV (n = 14). No significant changes in Vbl were observed in response to basal application of amiloride (up to 1 mM), demonstrating that secondary pH effects following Na-H exchange were not responsible for the depolarization. This would indicate the presence of a Na-coupled cotransporter carrying an excess of negative charge. This transporter does not seem be a Na-bicarbonate (bic) cotransporter as the depolarization did not correlate with change in bath [bic], or addition of DIDS (0,1 mM) to the bath. In another set of experiments Vbl hyperpolarized upon an increase in bath inorganic phosphate (Pi) concentration, indicating that an electrogenic Pi transporter is present in the basolateral membrane. This transporter could be the Na-coupled anion transporter. Therefore, collecting tubules and ducts were dissected from kidney tissue and using RT-PCR and degenerate primers we were able to detect NaPi-II-related transcripts in the tubules. In conclusion, the toad collecting duct system expresses a NaPi cotransporter type and our functional investigations indicate basolateral Na-coupled anion transport. The two transporters may be related and involved in Pi secretion. [1] Møbjerg, Larsen, Novak. 2002. J.Exp.Biol. 205.

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P07-18

CHRONIC CADMIUM INTOXICATION BY INTRAPERITONEAL AND ORAL ADMINISTRATION: A RENAL STUDY IN THE RAT.
Barbier O., Jacquillet J., Tauc M., Poujeol C., Martin D., Namorado MC., Sierra G., Reyes J.L., Poujeol P.

It is well known that exposure to cadmium (Cd^{2+}) may induce severe renal and bone pathologies. The effects and the time course of the renal alterations induced by cadmium intoxication are not well understood. The aim of our study was to evaluate the impact and to determine the kinetics of a chronic intoxication with cadmium (500 µg/kg/day) on the renal function. Wistar rats were intoxicated daily for 5 days, either intraperitoneally or through an oral administration (gavage). At the end of the intoxication , a recovery period of 5 days was followed. The mainly indicators of renal function (urinary flow, glomerular filtration GFR, fractional excretions of Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cl^{-} and PO_4^{3-}, free water and osmolar clearances) were estimated in the following groups:controls and on days 1, 3, 5, 7 and 10 using the clearance technique. During chronic intraperitoneal intoxication, renal failure appeared after the end of intoxication indicating a "delayed" toxic effect of Cd^{2+}: dramatic decrease of GFR and increase of fractionnal excretions of overall ions at day 10. In the same time, oral intoxication was performed with the same schedule followed for intraperitoneal treatment. For collection of urine samples, rats were in metabolic cages and collections lasted 24 hours. Animals were deprived of water for 24 hours, in order to study its renal capacity to concentrate. Urine and plasma creatinine and osmolality-were measured and clearances for creatinine, osmolar and free-water were estimated. Creatinine clearance continuously decreased from day 1 to day 10. Free-water clearance showed a tendency to become less negative than in the-control group, suggesting that Cd induced a lower capacity to concentrate. The results showed that intoxication with Cd affects renal function in both conditions but during oral intoxication the alteration of GFR occurs quickly while during intraperitoneal intoxication this toxic effect appears more than 5 days after the end of intoxication.

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P07-19

PODOCALYXIN INTERACTS DIRECTLY WITH EZRIN AND ACTIVATES RHOA THROUGH RECRUITMENT OF RHOGDI
Schmieder S., Nagai M., Orlando R. A., Takeda T., Farquhar M. G.

Podocalyxin (PC) is the major sialoglycoprotein at the apical plasma membrane (PM) of podocytes, where it serves as an anti-adhesin to maintain the glomerular filtration slits between foot processes open. PC is connected to actin filaments through a PC/NHERF2/ezrin complex that is disrupted in experimental models of nephrosis in which cell shape is altered and foot processes are lost. To assess whether expression of PC affects the organization of the actin cytoskeleton, we expressed PC in MDCK cells and examined its effects on the actin cytoskeleton by immunofluorescence (IF) and confocal microscopy. We found that expression of PC increases actin staining near the apical and lateral PM and reduces staining near the basal PM. The PC mutant, PC-DTHL, lacking the C-terminal PDZ binding motif fails to bind the PDZ protein NHERF and does not induce actin redistribution. We further examined the effects of PC expression on RhoA, which is known to activate the actin-binding protein ezrin and to regulate actin organization and changes in cell shape. By IF, expression of PC and PC-DTHL resulted in increased RhoA staining near the lateral PM. Furthermore, RhoGDI, a negative regulator of RhoA, was concentrated at the apical PM where PC and PC-DTHL are found. However, we found that PC, but not PC-DTHL, activated RhoA. Using pull-down assays we found that ezrin interacts directly with PC tail. By mutational analysis, we established that basic amino acid residues in the juxtamembrane region of the cytoplasmic tail of PC are crucial for ezrin-binding. We conclude that 1) expression of PC induces reorganization of the actin cytoskeleton through activation of RhoA, and 2) PC binds to ezrin directly (juxtamembrane domain) and indirectly (via NHERF). We propose a model in which ezrin recruits RhoGDI into the PC/NHERF/ezrin complex, initiating the activation of RhoA. In turn, activated RhoA provides a positive feedback to activate ezrin, allowing the connection of PC to actin filaments.

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P07-20

RENAL VASCULAR AND GLOMERULAR FIBROSIS: ROLE OF EPIDERMAL GROWTH FACTOR RECEPTOR TRANSACTIVATION

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Study objectives: Vasoactive peptides, participate in hypertension-associated vascular fibrosis. We have recently observed that EGF receptor (EGFR) mediates the endothelin 1-induced collagen I activation and contraction in the aortic wall ex vivo. In the present in vivo study, we tested the hypothesis that EGFR transactivation mediated these events in pathophysiological conditions. To this end, we studied the effects of chronic inhibition of EGFR phosphorylation on the renal vascular lesions in NO-deficient rats (L-NAME model).
Methods: 20 mg/kg of L-NAME was orally administered alone or associated with Iressa®, a specific inhibitor of the phosphorylated form of EGFR. The histological and biochemical indexes of renal fibrosis were compared in each group. Collagen I mRNA expression and Mitogen Activated Kinases (MAPK) activity were assessed by real-time PCR and Western Blot, respectively.
Results : After 4 wk of treatment, animals receiving L-NAME demonstrated hypertension associated to proteinuria (1.58± 0.37 mg/µmolCreat) and histological damages(glomerulosclerosis, glomerular ischemia and microvascular lesions). EGFR phosphorylation (determined by western blot) was activated in the renal cortex. Silmutaneously, the MAPK pathway vas activated (144% ±14 of the control) and the Collagen I mRNA expression was increased (1.35 ± 0.21 vs 0.87 ± 0.10).
Iressa® normalized the L-NAME-induced MAPK activation (95 ± 12% of the control, $p<0.05$ vs L-NAME), partially prevented the development of glomerular fibrosis, blunted the increase of collagen I gene expression (0.64 ± 0.17, $p<0.05$ vs L-NAME), and decreased the L-NAME-induced proteinuria (0.74 ± 0.23 mg/µmolCreat, $p<0.05$ vs L-NAME).
Conclusion: In the model of NO deficiency, EGFR activation participates in the fibrogenic process leading to arteriolosclerosis and glomerulosclerosis. EGFR might be thus a novel target in the treatment of hypertension-induced vascular fibrosis.

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P07-21

A NOVEL HUMAN CATALYTICALLY-INACTIVE SECRETED PHOSPHOLIPASE A2: CLONING AND RECOMBINANT EXPRESSION

Rouault M., Gelb M., Lazdunski M., Lambeau G.

Mammalian secreted phospholipases A2 (sPLA2s, 14 kDa) form a growing family of enzymes catalyzing the hydrolysis of phospholipid to release free fatty acid and lysophospholipid. They have been implicated in various physiological and pathological conditions including cell proliferation, cell contraction, hormone release, inflammation, cancer and antibacterial defense. sPLA2s are also known to bind to specific membrane receptors, suggesting that they may exert their functions not only as enzymes but also as ligands.
Here, we report the cloning and recombinant expression of a novel sPLA2 isolated from human liver. The mature protein has a molecular mass of 19.7 kDa and the typical structural features of group XII sPLA2s. This novel sPLA2 thus appears to be a new member of group XII sPLA2s, and has been called human group XIIB (hGXIIB). However, this novel sPLA2 has a mutation in the active site replacing the canonical histidine by a leucine, suggesting that this sPLA2 is catalytically-inactive.
Recombinant expression of human (hGXIIB) and mouse (mGXIIB) group XIIB sPLA2s in E.coli indicates that proteins are indeed inactive enzymes that furthermore display altered binding properties to phospholipid vesicles. Initial binding experiments indicate that these proteins are unable to bind to the well known M-type sPLA2 receptor. The RNA tissue distribution of group XIIB sPLA2 is distinct from all other sPLA2s including hGXIIA. A strong expression was observed in liver, small intestine and kidney from mouse and human species. Finally, we found that the expression of hGXIIB is downregulated in human tumors from small intestine, kidney and liver.
The absence of enzymatic activity suggests that these novel sPLA2-like proteins probably exert their biological roles by acting as ligands for as yet unidentified receptors.

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P07-22

PROXIMAL TUBULAR AND COLLECTING DUCT ECTONUCLEOTIDASES IN THE RAT KIDNEY

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Evidence is accumulating that extracellular nucleotides (e.g., ATP, ADP), and the nucleoside adenosine, act as autocrine/paracrine agents in most tissues, including the kidneys, via P2 and P1 receptors, respectively. In this context, intraluminal ATP has been shown to modulate tubular ion transport. Nucleotides can be degraded by surface-located enzymes collectively known as ecto-nucleotidases, of which several families exist, with differing affinities and rates of hydrolysis for ATP and ADP, which can ultimately produce adenosine. In the present study we have examined the distribution within the kidney of individual members of three of the four families of ectonucleotidases.
Kidneys from anaesthetised male Sprague-Dawley rats were perfusion-fixed with 8% paraformaldehyde solution. Cryostat sections displaying cortical and medullary regions of the kidney were incubated with antibodies specific for rat ecto-nucleoside 5' triphosphate diphosphohydrolyase-3 (E-NTPDase3), ecto-nucleotide pyrophosphatase/phosphodiesterase-3 (NPP3) or ecto-5-nucleotidase and were subsequently fluorescently labelled. Co-staining of the sections with a biotinylated fluorescent marker, phaseolus vulgaris erythroagglutinin (PVE) and fluorescently labelled antibody aquaporin 2 (AQP2) revealed proximal tubule and collecting duct segments, respectively.
These studies showed intense staining for NPP3 and ecto-5-nucleotidase in the apical membrane of some, but not all, of proximal tubular segments with little or no staining for these enzymes in the collecting duct. In contrast, expression of E-NTPDase3 was observed only in the collecting ducts.
We conclude that renal ecto-nucleotidases are likely to play an important role in controlling the duration, nature and extent of activation of apical P2 and P1 receptors in the kidney.

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S8 CONTROL OF CALCIUM TRANSPORT IN THE HEART : PHYSIOLOGY AND PATHOPHYSIOLOGY

ORAL SESSION

S8-1

CALCIUM SIGNALING AND EXCITATION-CONTRACTION COUPLING IN CARDIAC MUSCLE

Niggli E., Lindegger N., Egger M., Szentesi P.

In cardiac muscle, depolarization of the cell membrane leads to the opening of voltage-dependent L-type Ca channels, giving rise to an initial influx of Ca that is further amplified by "Ca-induced Ca release" (CICR) from the sarcoplasmic reticulum (SR). CICR occurs via elementary Ca release units termed "Ca sparks", presumably arising from the concerted opening of a few SR Ca release channels (termed ryanodine receptors; RyRs). Interestingly, in cardiac myocytes the probability of a single L-type Ca channel opening to trigger a Ca spark was found to be variable. This recent notion of a variable Ca signal trigger-probability has important implications, not only for the regulation of cardiac force (e.g. via mechanisms changing the Ca sensitivity of the RyRs, such as SR Ca-load or phosphorylation of the RyRs), but also for new concepts of impaired cardiac EC-coupling. Indeed, a reduced efficacy of cardiac EC-coupling has been observed on this molecular level in models of cardiac disease, such as cardiac hypertrophy and failure. In addition, the Ca concentration inside the SR may modulate the Ca sensitivity of the RyRs. Related to this modulatory effect, a partial functional depletion of the SR Ca content may underlie the refractoriness of CICR and the termination of Ca release during a Ca spark. We used laser-scanning confocal microscopy in combination with UV-laser flash and two-photon excitation photolysis of caged Ca to examine the mechanisms responsible for CICR refractoriness in isolated cardiac myocytes. Using a variety of pharmacological tools and transgenic animal approaches we found that recovery from refractoriness was mainly dependent on the rate of SR Ca refilling via the Ca-pump. These observations suggest a pivotal role for the intra-SR Ca concentration as a modulator for the Ca trigger sensitivity of the RyRs. Conversely, SR Ca depletion during release may lead to a "desensitization" of the RyRs and subsequenty allow for termination of Ca release.

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S8-2

INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS IN CARDIAC MYOCYTES- WHERE ARE THEY AND WHAT DO THEY DO?

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The role of inositol 1,4,5-trisphosphate (InsP3) in cardiac myocyte function is unclear and controversial, although agonists activating InsP3 generation are positive inotropic agents in the heart and have been implicated in various cardiac pathologies. We investigated the expression and subcellular localisation of InsP3 receptors (InsP3Rs) in rat ventricular and atrial myocytes. In addition, the consequences of activating InsP3Rs on spontaneous Ca^{2+} release were monitored using laser-scanning confocal microscopy. PCR, Western blotting and InsP3-binding analyses indicated that atrial and ventricular myocytes expressed InsP3Rs. Both cell types mainly expressed type II InsP3Rs, with atrial myocytes displaying 5-fold higher levels of InsP3Rs than ventricular cells. We observed that stimulation of atrial myocytes with InsP3-generating hormones increased the likelihood of pro-arrhythmogenic events such as Ca^{2+} sparks, Ca^{2+} waves and action potentials. Direct activation of InsP3Rs by application of a membrane-permeant InsP3 ester to the cells evoked similar responses, indicating that InsP3R activity alone can underlie some of the established effects of hormonal stimulation. In atrial myocytes, the predominant form of Ca^{2+} release during stimulation with hormones or InsP3 esters was an increase in Ca^{2+} spark frequency. Such increases in Ca^{2+} spark activity were most commonly observed in the cellular regions where InsP3Rs and ryanodine receptors (RyRs) were co-localised. The activation of Ca^{2+} sparks by hormones and the InsP3 ester suggest that cross talk between InsP3Rs and RyRs was responsible for the enhancement of Ca^{2+} release by InsP3. Our data indicate that InsP3Rs are abundantly expressed in atrial and ventricular myocytes, and that their activation can modulate cardiac function.

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OC08-1

HSP70 OVEREXPRESSED IN H9C2 MYOCYTES IMPAIRS FREE CALCIUM BURST PROVOKED BY SIMULATED ISCHEMIA

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A sharp increase in the concentration of free cytosolic Ca^{2+} occurring in cardiomyocytes upon ischemia/reperfusion is associated with the physiological dysfunction and cell death. It is known that the 70 kD heat shock protein (Hsp70), when overexpressed in cardiac cells, can minimize their death resulting from ischemia/reperfusion. However, mechanisms of the Hsp70-mediated cardioprotection remain to be determined. We have supposed that excess Hsp70 in cardiomyocytes is able to attenuate the detrimental burst of free cytosolic Ca^{2+} arising in the course of ischemia/reperfusion. Our aim was to examine this supposition in an in vitro model.

Rat embryonic heart-derived myocytes (H9c2 line) were exposed to anoxia for 10 h followed by reoxygenation under normoxic conditions. The cells were infected (12 h before anoxia) with a herpes simplex virus-based vector expressing human Hsp70; herein, a 5-fold increase in the intracellular Hsp70 content was confirmed by immunoblotting. The vector expressing green fluorescent protein (GFP) was used for control. Concentrations of free cytosolic Ca^{2+} were determined using fura 2-acetoxymethyl ester. Cell death/survival was evaluated by staining with propidium iodide and the MTT assay.

Our data show that in the uninfected or GFP-overexpressing cells the concentrations of free cytosolic Ca^{2+} increased 10-fold within 4 h of post-hypoxic reoxygenation; this calcium burst preceded and probably promoted massive cell death (necrosis). In contrast, in myocytes overexpressing Hsp70 the stress-provoked increase in free cytosolic Ca^{2+} was only 4-fold and these cells exhibited the significantly improved survival. We conclude that up-regulation of the intracellular Hsp70 level can confer better buffering of free cytosolic Ca^{2+} during ischemia/reperfusion, thereby attenuating death of the involved cells. This study was supported by the Wellcome Trust grant 062891.

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OC08-2

THE Ca^{2+}-ACTIVATED K^+ CHANNELS OF THE CORONARY ARTERY IN LEFT VENTRICULAR HYPERTROPHY

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Ca^{2+}-activated K^+ (KCa) channels are very abundant in smooth muscle cells (SMCs), where they play an important role in the regulation of arterial tone and vascular resistance. It has been suggested that the impairment of SMC function by alterations in the KCa channels accounts for the reduction in coronary reserve during left ventricular hypertrophy (LVH). However, this hypothesis has not been fully investigated. The goal of this study was to combine patch-clamp and Western blot methods with isometric contraction experiments to compare the levels of KCa channel current, protein expression, and the contractility of the coronary arteries in control and LVH specimens. In patch-clamp experiments, the unitary current amplitude and open probability for the KCa channels were significantly reduced in LVH patches compared with control patches. The concentration-response curve of the KCa channel to $[Ca^{2+}]i$ was shifted to the right. Inhibition of the KCa channels with TEA was more pronounced in LVH cells than in the control cells. Western blot analysis indicated no differences in KCa channel expression between the control and LVH coronary SM membranes. In contraction experiments, the effect of a high K^+ concentration on the resting tension of the LVH coronary artery was greater than on that of the control. The effect of TEA on the resting tension of the LVH coronary artery was reduced as compared with the effect on the control. Our findings imply a novel mechanism for reduced coronary reserve in LVH.

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OC08-3

EFFECTS OF THYMOL ON THE ACTION POTENTIAL AND THE CALCIUM CURRENT IN CANINE CARDIAC CELLS

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Thymol is a well-known natural antioxidant having bactericidal and antifungal activity in millimolar concentration range. It is widely used therefore, as cosmetic vehicles, in dental care and as natural food

preservatives. Since thymol is a lipophil substance it can accumulate in various membranes, and may alter this way the function of the cells. The aim of this study was to characterize the effects of thymol on the electrophysiological properties of canine myocardium.
Dogs were euthanized then cardiac cells were enzymatically dispersed. Action potentials were recorded by conventional glass microelectrode technique. L-type calcium current (ICa) was measured at 37 C, using the whole-cell version of the patch clamp technique. Data represent mean±S.E.M.
Thymol (10 micromol/l) reduced the notch of the action potentials. 100 micromol/l thymol fully abolished the notch, depressed the plateau and shortened the duration of action potentials.
Thymol reduced the peak amplitude of ICa in a concentration dependent manner. The blocking effect of thymol on ICa was partly reversible. The half-maximal block of ICa was obtained at 158.2±6.8 micromol/l while the Hill coefficient was 2.97±0.43. Thymol shifted the steady-state inactivation curve of ICa toward negative direction, while it had no effect on the slope of this curve.
The observed plateau depression of action potential may be well explained by the inhibition of ICa induced by thymol. The observed abolition of notch and the shortening effect of thymol on the action potential duration indicates that this drug may influence transient outward currents and ion currents involved in repolarization of action potential.

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OC08-4

IMPACT OF T-TUBULES ON ELECTRICAL ACTIVITY OF CARDIAC CELLS EVALUATED IN A QUANTITATIVE MODEL
Pasek M., Christe G., Simurda J.

We report here the first quantitative evaluation of the role of the transverse-axial tubular (TAT) system in the electrical activity of cardiac cells. Our approach uses a biophysically-based representation of the TAT-system incorporated into a model of cardiac ventricular cell (Jafri et al., Biophys. J. 1998, 74:1149-68). The model was modified to agree more closely with recent published data. The differential distribution of ion transfer mechanisms in peripheral and tubular membranes was included (e.g. K channels; Christe, J. Mol. Cell. Cardiol. 1999, 31:2207-13). Changes of ion concentrations in the TAT-lumen were computed from the total transmembrane ion fluxes and ion exchanges with the pericellular medium. Long term stability of the model was verified at rest and under regular stimulation, the charge conservation principle being respected. The tubular membrane voltage during an action potential was nearly identical with the peripheral membrane voltage, indicating that propagation of excitation along the TAT-system was quasi-instantaneous. Depletion of Ca by 12.8 % and accumulation of K by 4.7 % occurred in the TAT-lumen during the course of an action potential at 1 Hz. However, the course of action potential was only slightly altered when the TAT-system was included into the model (shortening by less than 2 % at 90 % of repolarization). Under conditions of progressive hypokalaemia, the TAT-system retarded the occurrence of delayed after-depolarizations owing principally to Ca depletion in the TAT-system and subsequently to suppression of Ca overload in sarcoplasmic reticulum. They occurred at more severe hypokalaemia. These results show that modulation of the excitation-contraction coupling of ventricular cardiac tissue formerly attributed rather to narrow extracellular spaces is also an intrinsic property of the ventricular cardiac myocyte TAT-system, where the preferential localization of ion transfer mechanisms plays a key role.

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S8-3

CALCIUM SIGNALING IN THE REMODELING HEART
Kerfant BG., Pereira L., Perrier E., Vassort G., Richard S., Benitah JP., Gomez A.M.

Contraction in cardiac myocytes arises when an increase in $[Ca^{2+}]i$ is activated by the Ca^{2+}-induced Ca^{2+} release (CICR) mechanism. The cellular depolarization during an action potential activates sarcolemmal L-type Ca^{2+} channels. The brief opening of the Ca^{2+} channels induces a local increase in $[Ca^{2+}]i$, which is not sufficient to trigger contraction. Neighbouring Ca^{2+} -release channels (ryanodine receptors or RyRs) in the sarcoplasmic reticulum (SR) are activated by this increase in local $[Ca^{2+}]i$ that open to release Ca^{2+} from the SR. The coordinated activation of RyRs results in a global $[Ca^{2+}]i$ transient that activates contraction. The opening of a cluster of RyRs can be visualized in situ, as Ca^{2+} sparks, using a confocal microscope and appropriate Ca^{2+} dyes.
When the heart is chronically submitted to an imposed load, it remodels to provide enough blood supply to the body. This remodeling often implies hypertrophy and can be compensated, when the ejection fraction is maintained. However it can also be decompensated as during heart failure, when the heart function is compromised. In the remodeled heart, alterations in the Ca^{2+} signaling have been reported, some times contradictory. Some of the discrepancies could arise form the different animal models used and /or the different stage of the disease progression.
Confocal microscopy and patch-clamp methods were used to examine CICR in several animal models of cardiac hypertrophy and heart failure. We used a rat model of abdominal aortic constriction, post-myocardial infarction in the rat and diabetic obese mice. While Ca^{2+} handling was altered in all analyzed pathologic models, the characteristics and timing differed with the etiology.

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S8-4

CARDIAC SARCOPLASMIC RETICULUM CALCIUM CYCLING IN HEALTH AND DISEASE
Prestle J.

Calcium (Ca^{2+}) ions are the currency of heart muscle activity. During excitation-contraction coupling Ca^{2+} is rapidly cycled between the cytosol and the sarcoplasmic reticulum (SR), the Ca^{2+} store. These fluxes occur by the transient activity of Ca^{2+}-pumps and -channels. In the failing human heart, changes in activity and expression profile of Ca^{2+}-handling proteins, in particular the SR Ca^{2+}-ATPase (SERCA2a), are thought to cause an overall reduction in the amount of SR-Ca^{2+} available for contraction. In the steady state, the Ca^{2+}-content of the SR is essentially a balance between Ca^{2+}-uptake via the SERCA2a pump and Ca^{2+}-release via the cardiac SR Ca^{2+}-release channel complex (Ryanodine receptor, RyR2). Different molecular approaches to improve cardiac SR Ca^{2+} cycling and the implications of these approaches for novel inotropic therapies to human heart failure will be discussed. Two options are considered: (i) activation of SR Ca^{2+}-uptake via SERCA, and (ii) reduction of SR Ca^{2+}-leakage through RyR2. RyR2 forms a macromolecular complex with a number of regulatory proteins that either remain permanently bound or that interact in a time- and/or Ca^{2+}-dependant manner. These regulatory proteins can dramatically affect RyR2 function, e.g. over-expression of the accessory protein FK 506-binding protein 12.6 (FKBP12.6) has recently been shown to reduce SR Ca^{2+}-leak. Furthermore, FKBP12.6 appears to play a crucial role in synchronising SR Ca^{2+}-release.
If gene therapy will ever turn out to be feasible to treat heart failure, the SERCA pump will be a prime candidate. However, too much of a goog thing is sometimes bad. Under certain conditions, overexpression of SERCA may also lead to a loss in contractility due to extensive Ca^{2+}-uptake activity of the SR and Ca^{2+}-buffering.

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S8-5

CONTROL OF IONIC CURRENTS BY ALDOSTERONE DURING THE EARLY PHASE OF CARDIAC HYPERTROPHY
Vassort G., Perrier E., Gomez A., Benitah J.P.
The mineralocorticoisteroid aldosterone is associated with the pathogenesis and progression of left ventricular hypertrophy and heart failure, independent of its relation with arterial blood pressure. However, little information exists about the possible influence of aldosterone on cardiomyocyte electrical activity. Using the whole-cell patch-clamp technique, we investigated whether aldosterone affects the whole-cell Ca^{2+} current, ICaL and the 4-aminopyridine-sensitive transient outward K^+ current, Ito1 in isolated adult rat ventricular myocytes. Bath application, or short-term exposure of 10-nM to1-µM aldosterone had no demonstrable influence on both ionic currents. However, incubation of cells for 24 hours increases significantly ICaL density without altering its kinetics and voltage dependence. The intracellular receptor antagonist spironolactone (250-fold excess) blunted the aldosterone-induced increase in ICaL density as well as did inhibitors of transcription and protein synthesis. Cardiomyocytes incubation for 24 h at 37 °C with aldosterone concentrations up to 1 µM did not change Ito1 density while exposure to 100 nM aldosterone for 48 h produced a 1.6-fold decrease in the Ito1 density. The later effect was prevented by RU28318, a specific mineralocorticoid receptor antagonist. After 24 h of aldosterone pretreatment, further co-incubation for 24 h either with nifedipine or with BAPTA-AM prevented the decrease in Ito1 density. After 48 h of

aldosterone treatment, there was a 2.5-fold increase in the occurrence of spontaneous Ca^{2+} sparks, which was blunted by co-treatment with nifedipine. Thus, aldosterone-dependent decrease in Ito1 density is secondary to the modulation of intracellular Ca^{2+} signaling. Besides, ICaL stimulation by aldosterone would result from an increased channel expression. This genomic action contributes to the increased ICaL observed during cardiac remodeling and might control the decrease in Ito1.

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POSTER SESSION

P08-01

SIMULTANEOUS RECORDING OF MEMBRANE POTENTIAL AND CONTRACTION IN THE RAT AORTIC SMOOTH MUSCLE

Serban D.N., Serban I.L., Petrescu G.

Membrane potential is an essential factor in smooth muscle contractility, mainly by its effect upon the opening probability of the L-type calcium channels. To this moment there are relatively few electrophysiological studies upon the rat aortic smooth muscle, although the isolated rat aorta is one of the most widely used models for smooth muscle investigations. The equipment we used is based on an isometric horizontal myograph (JP-Trading), a microelectrode amplifier (Axon Instruments) and an analog-digital converter (WPI). We used glass microelectrodes: Ag/AgCl, KCl 3M, 20-30 MW. The resting membrane potential was 49±3 mV (n=12). We studied the temporal relation between the membrane potential and the active force in de-endothelised aorta rings (from male adult Wistar rats), within the contraction induced by 40 mM extracellular potassium or by 0.01 mM phenylephrine, as well as during relaxation induced by 0.01 mM methoxy-verapamil or by 0.1 mM sodium nitrite. As quantitative reference levels for statistical analysis we used the potential threshold of contraction or relaxation and the potential value the moment of maximum mechanical effect was reached. In the case of potassium-induced contraction these values were -41±2 mV and -16±4 mV, while for phenylephrine they were -48±3 mV and -27±4 mV respectively (n=6 în all cases). It appear that no similar study on the rat aorta has been done so far, thus the results are discussed with reference to data obtained using other methods: aortic smooth muscle cell cultures, closely related vascular preparations (e.g. tail artery), different microelectrophysiology techniques.

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P08-02

EFFECTS OF DENDRANTHEMA MORIFOLIUM ON ISCHEMIA/REPERFUSION INJURY OF ISOLATED RAT HEART

Jiang HD., Xia Q., Zheng M., Xu W.H.

Objectives:The flower of Dendranthema morifolium (Ramat.) tzvel (DM) has been demonstrated to have protective and curative effect on coronary disease in China. This study was to investigate whether DM could protect the isolated rat heart from ischemia/reperfusion injury and weather that protective effect was related to its antioxidation. Methods:Ischemia/reperfusion was induced by ligation the left anterior descending artery for 30min followed by 30min reperfusion in isolated rat heart, the left ventricle functional signals were recorded, SOD activity and MDA content of heart were measured. In vitro and vivo antioxidation were valuated by measuring MDA content. Results:Recovery rate of cardiac function, including LVDP, +/-dp/dt max and coronary flow in DM group (contain DM 0.5g/L in KH solution) after reperfusion 1,5,10,15,30 mins were markedly higher than that of the ischemia/reperfusion (I/R) group ($p<0.01$), these recovery were accompanied by decreased the level of MDA and increased SOD activity as compared to I/R heart ($p<0.05$). In vitro antioxidation experiment,DM inhibited lipid peroxidation in normal rat myocardial mitochondria in the presence or absence of ferrous sulfate and hydrogen peroxide with dose-dependent relationship. In vivo experiment, Mice were pretreated (ig) with DM 2g/kg or 4g/kg for 15 days, the level of MDA in heart decreased to 98.4+/-31.5 nmol/g and 86.6+/-25.7nmol/g tissue, significantly lower than that of the control group (192.0+/-68.0 nmol/g, $p<0.01$). Conclusion: DM could attenuate the ischemia/reperfusion induced alteration in the isolated rat heart by inhibiting lipid peroxidation and improving the activity of antioxidative enzymes.

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P08-03

ROLE OF NITRIC OXIDE IN TUMOR NECROSIS FACTOR-ALPHA INDUCED CARDIOPROTECTION

Fu C., Xia Q., Cao C.M., Lu Y.

Background and Objective: Previous studies have shown that tumor necrosis factor-alpha (TNF-alpha) could elicit tolerance to ischemia/reperfusion (I/R) by activating manganese superoxide dismutase (MnSOD) in cardiac myocytes. TNF-alpha can induce nitric oxide (NO) synthesis in isolated myocytes. We hypothesize that NO may play a role in cardioprotection

against I/R injury by soluble guanylate cyclase (sGC) or protein kinase C (PKC) pathway. Therefore, the objective of the present study is to investigate the role of NO synthase (NOS), sGC and PKC signaling in TNF-alpha-induced cardioprotection against simulated hypoxia/reoxygenation (H/R) injury.
Methods: Neonatal rat ventricular myocytes were pretreated with TNF-alpha or sodium nitroferricyanide (SNP) or L-arginine (L-Arg), respectively, for 12 hr and then subjected to continuous hypoxia for 12 hr, followed by reoxygenation for 6 hr. The MnSOD activity of the cell was measured after H/R. Myocyte injury was determined by the release of lactic dehydrogenase (LDH). To evaluate the effects of TNF-alpha, SNP and L-Arg on cell signaling the effects of NOS inhibitor N-nitro-L-arginine methyl ester (L-NAME), the specific sGC inhibitor ODQ and the specific PKC inhibitor chelerythrine (CHE) were examined.
Results: TNF-alpha (10, 50, 100, or 500 U/ml) significantly increased the MnSOD activity and decreased release of LDH from ventricular myocytes. The cardioprotection against H/R injury was induced by the pretreatment with SNP (5 microM) or L-Arg (5 mM), which was blocked by ODQ (10 microM) and CHE (5 microM). Pretreatment with L-NAME (100 microM) or ODQ (10 microM) or CHE (5 microM), respectively, attenuated the increased MnSOD activity and reduced LDH level induced by TNF-alpha.
Conclusion: The results suggest that NO may play a role in TNF-alpha-induced cardioprotection, which is mediated by sGC and PKC mediated.

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P08-04

THE ROLE OF AKT KINASE IN ISCHEMIC PRECONDITIONING MEDIATED CARDIOPROTECTION IN PIG MYOCARDIUM
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Ischemia and ischemia/reperfusion (I/R) induce cell damage that involves apoptosis. In the heart, ischemic preconditioning (IP) has been shown to prevent it. Akt kinase (Akt) mediates many functions initiated by growth factor receptors through phosphatidylinositol-3-kinase (PI3K)-cascade. It has been implicated also in the mechanisms of cell survival and apoptosis. In our study we looked for the changes in the expression and phosphorylation (activation) of Akt during I/R. We investigated also a possible role and participation of Akt in the IP-mediated cardioprotection in pig.
German landrace-type domestic pigs were used as an experimental model. The hearts were subjected to 2 cycles of 10 min. of LAD coronary artery occlusion and 10 min. reperfusion in open-chest model. We infused also specific inhibitor of extracellular signal regulated kinase (ERK) pathway (UO126) and transcription inhibitor Actinomycin-D (Act-D) before and during IP. Contents of total and phosphorylated Akt were determined by Western blot analysis using anti-Akt and anti-phospho-Akt primary antibodies.
Infusions of UO126 or Act-D significantly reduced the cardioprotective effect of IP. Western blot analysis with anti-Akt antibody showed no differences in the protein levels of Akt. However, there were changes in the phosphorylation of Akt during I/R. After 10 min. of ischemia Akt activity increased and the phosphorylation of kinase returned to the control level after single reperfusion. Important is the fact, that we observed enhanced activation of Akt after complete IP protocol. Interestingly, application of UO126 but not Act-D inhibited also IP-mediated activation of Akt. Our results suggest that the activation of Akt could play positive role in the cardioprotective mechanisms of IP.

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P08-05

ENDOCARDIAL VERSUS EPICARDIAL DIFFERENCES IN THE KINETIC PROPERTIES OF ICa,L IN CANINE MYOCARDIUM
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Canine endocardial and midmyocardial action potential exhibit a prominent plateau, while spike-and-dome appearance is characteristic to the action potential of epicardial cells. Earlier studies on the ionic basis for transmural electrophysiological heterogeneity have focused on contributions of the Ito1 and ICl(Ca). The present work identifies and characterizes the differences in kinetic properties of L-type calcium current (ICa.L) in epicardial versus endocardial cells.
Single cardiac myocytes were enzymatically dissociated from canine ventricular myocardium. Action potentials (AP) and membrane currents were recorded in normal Tyrode solution, at 37°C. Action potential voltage clamp (APVC) was used to measure the time course of ICa.L. The ICa.L was determined as difference current using 1 µmol/l nisoldipine for blocking ICa.L.
The time course of the ICa.L recorded in canine myocardial cells showed rapid activation followed by complete inactivation during the early phase of plateau. Following a rapid inactivation of ICa.L a second activation was observed in epicardial but not in endocardial cells. The timing of this second activation was found to be determined by the depth and the duration of the notch of the AP. The maximum current of this second activation strictly coincided with the raising phase of the dome. The amplitude of the second ICa.L activation was higher in those epicardial cells in where the AP displayed prominent notch. When epicardial AP was applied as voltage command in endocardial cells, the ICa.L displayed biphasic configuration, similar to those observed in epicardial cells.
Inactivation and reactivation kinetics of the ICa.L was determined by square pulse voltage clamp method under our experimental conditions. These data suggest that the reactivation of ICa.L is likely to be present during an action potential in epicardial cells.

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P08-06

LOW MAGNESIUM INCREASES THE SENSITIVITY OF THE CONTRACTION TO ENDOTHELIN-1 IN CORONARY ARTERY
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We have investigated the effects of the external Mg^{2+} concentration ($[Mg^{2+}]o$) on contractile responses induced by endothelin-1 (ET-1) in rabbit coronary arteries. The left anterior descending branch of the coronary artery was mounted in a perfusion chamber, which was perfused with physiological salt saline (PSS) saturated with 5% CO_2 + 95% O_2. From the video images of the pressurized arteries, the diameter changes were recorded using the edge detector. Endothelium was removed by air bolus prior to the experiment.
The contractile responses of coronary arteries to ET-1 were examined by increasing the concentration of ET-1 from 10-10 M to 10-7 M in different $[Mg^{2+}]o$. With physiological $[Mg^{2+}]o$ (1.2 mM), the vasoconstriction started at 5x10-10 M ET-1. When $[Mg^{2+}]o$ was reduced to 0.3 mM ~ 0 mM, the threshold for ET-1 induced vasoconstriction was decreased. This shifts the dose-response relationship for ET-1 to the left. The concentration of ET-1 for the half maximal effect (ED50) were 3.5X10-10, 5.8X10-10, 1.1X10-9, 7.9X10-9 M at 0, 0.3, 1.2, 8 mM $[Mg^{2+}]o$, respectively. The decreases in diameter were 52 + 2.8 % (n=4), 54 + 2.0 % (n=4) and 53 + 3.2 % (n=4), in the presence of 1.2, 0.3 and zero mM $[Mg^{2+}]o$, respectively. These results indicate that the sensitivity to ET-1 is accentuated in low $[Mg^{2+}]o$.
In the presence of nicardipine, ED50 was increased to 2.3X10-9 M, and the fall in $[Mg^{2+}]o$ did not affect the dose-response relationship for ET-1. The effect of Ca^{2+}-removal from the external solution was similar to the one with nicardipine.
These results suggest that the contractile response to ET-1 depend on Ca^{2+} influx through L-type Ca^{2+} channels which are affected by changes in $[Mg^{2+}]o$. In addition, the maximal contractile response induced by ET-1 was independent to changes in external Ca^{2+} and Mg^{2+}. In summary, ET-1 increases vascular tone more significantly in low $[Mg^{2+}]o$, and hypomagnesemia could participate in inducing arterial hypertension.

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P08-07

DETECTION OF ATP-SENSITIVE POTASSIUM CHANNEL IN THE INNER MITOCHONDRIAL MEMBRANE OF THE RAT HEART
Cuong D.V., Kim N., Kim E., Chung JY., Seog D.H., Han J.

Mitochondrial ATP-sensitive potassium (mitoKATP) channels play a pivotal role in early and late ischemic preconditioning. Although this channel is similar to properties of sarcolemmal KATP channels, subunit composition remains unclear. In this study, we investigated the subunit composition of the rat heart mitoKATP channels. Mitochondria were isolated by differential centrifugation and visualized by confocal microscopy. Mitochondrial protein was estimated using the Biuret reaction. We estimated mitoKATP channels by means of green fluorescence probe BODIPY-glibenclamide labeling. Western blotting of mitochondrial proteins was performed with antibodies against the known KATP channel subunits (the sulfonylurea receptor SUR 1 or 2 and the inwardly rectifying potassium channel Kir6.1 or 6.2).

Immunogold electron microscopy was performed with the same primary antibodies. Western blotting showed that a specific 40 kDa Kir6.2 protein was enriched in the mitochondria. We found that the Kir6.2 antibody labeled the mitochondrial inner membrane as shown by electron microscopy. We also observed that heart mitochondria appeared to be significantly enriched in SUR2-specific bands found at 140 kDa and the signal was located in the inner membrane. Our results indicate that mitoKATP channels compose of Kir6.2 subunits and a SUR2-related sulfonylurea binding protein in rat heart mitochondria.

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P08-08

NITRIC OXIDE ATTENUATES DNA DAMAGE IN ISCHEMIA AND REPERFUSION MODEL UTILIZING ISOLATED RAT MYOCYTES
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ATP-sensitive potassium (KATP) channels are thought to play a role in the phenomenon of ischemic preconditioning in the heart and the activation of these channels may improve recovery of regional contractile function of stunned myocardium by shortening action potential duration and attenuating membrane depolarization, thus decreasing contractility and preserving energy during ischemia. The release of myocardial nitric oxide (NO) during ischemia has been suggested to play roles in ischemic preconditioning. A common action mechanism of NO is a direct or indirect increase in tissue cGMP content. Furthermore, cGMP has also been shown to contribute to the cardioprotective effect against ischemia/reperfusion injury. The present investigation tested the hypothesis that KATP channels attenuate DNA strand breaks and oxidative damage in an in vitro model of ischemia/reperfusion utilizing rat ventricular myocytes. We estimated DNA strand breaks and oxidative damage by means of single cell gel electrophoresis with endonuclease III (comet assay). In the ischemia/reperfusion model, the level of DNA damage increased during ischemia/reperfusion period. Preconditioning with a single 5-min anoxia, pinacidil (50 mM), diazoxide (100 mM), SNAP (300 mM) and 8-(4-Chlorophenylthio)-guanosine-3',5'-cyclic monophosphate (8-pCPT-cGMP, 100 mM) followed by 15 min reoxygenation reduced DNA damage level against subsequent 30 min anoxia and 1 h reoxygenation. These protective effects were blocked by the concomitant presence of glibenclamide (50 mM), 5-hydroxydecanoate (100 mM) and 8-(4-Chlorophenylthio)-guanosine-3',5'-cyclic monophosphate, Rp-isomer (Rp-CPT-cGMP, 100 mM). These results suggest that NO/cGMP/PKG-pathway contributes to cardioprotective effect of KATP channels in rat ventricular myocytes.

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P08-09

IDENTIFICATION OF CARDIAC MARKER PROTEIN DURING ISCHEMIA IN RABBIT HEART TISSUES BY 2DE AND MALDI-MS
Lee Y., Kim N., Kim E., Chung J.Y., Seog D.H., Han J.

Myoglobin is released earlier in acute cardiac infraction. In this study we focused on myoglobin, the cardiac marker protein during ischemia, which might provide the earliest identification of cardiac injury. To investigate a molecular basis for cardiac marker protein during ischemia, 3 types of rabbit heart tissues (control, ischemia preconditioning and ischemia) were analyzed by two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS). We have compared rabbit control heart 2DE gel with ischemia preconditioning and ischemia 2DE gels, respectively. More than 400 protein spots were detected on the 2DE gels and localized in the range of bioelectric point from 3 to 10 and relative molecular weight from 10 to 200 kDa. The pattern of proteins was essentially the same for all three gels. However, one spot, 17 kDa with pI of 6.7, was only appeared in ischemia 2DE gel, which was further supported by western blot analysis. For further examination of molecular characteristics, the spot in 2DE was isolated and subjected to trypsin digestion followed by MALDI-MS analysis. The spot was validated by mass fingerprinting of the selected peaks of peptides by applying low tolerance (<20ppm) with recalibration. We were able to identify the spot is myoglobin, 8 peaks of total were detected. This study shows that proteomic techniques are a powerful and sensitive means of myoglobin protein may provide the identification of cardiac injury marker during ischemia-reperfusion.

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P08-10

AUTONOMIC BACKGROUND OF SLEEP IN CARDIAC TRANSPLANT PATIENTS
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Objectives : The aim of this study was to investigate autonomic activity in cardiac transplant patients (CTP) using heart rate (HR) and HR variability (HRV) analysis during sleep, in particular during the phases of transitory activation (PAT) associated with the emergence from slow wave sleep. In normal subjects, PAT is characterized by a pronounced HR surge.
Methods : Polygraphic sleep, cardiac, and respiratory recordings were determined in 14 CTP (male n = 11, female n = 3, 62 ± 2 years). The time elapsed since transplantation was 4 – 14 years. The control group included 10 healthy subjects (male n = 7, female n = 3, 61 ± 2 years). HR was measured during the PAT, and HRV was estimated from the R-R intervals in stationary 5-min segments preceding and following PAT, i.e. during slow wave sleep and subsequent lighter sleep.
Results : In control subjects, HR increased during PAT from 60.0 ± 2.6 to 76.4 ± 3.4 bpm; $p<0.001$. A delayed increase was observed in 5 CTP (from 83.4 ± 2.8 to 92.2 ±3.9 bpm; $p<0.05$) whereas 9 other CTP had no HR variation. This distinction between the two groups of CTP was confirmed by HRV analysis. The same 5 CTP presented, like the control group, a variation in HRV before and after PAT. In contrast, the 9 other CTP did not demonstrate any significative HRV variation in the 5-min segments surrounding PAT.
Conclusion : HR reactivity during the phases of transitory activation associated with the emergence from slow wave sleep, corroborated by HRV variations before and after the phases of transitory activation, demonstrates an improvement of the autonomic drive to the heart in some cardiac transplant patients. Therefore, sleep stage alternation could be proposed as a tool for evaluation of cardiac reinnervation after heart transplantation.

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P08-11

COMBINED METFORMIN - GLIBENCLAMIDE TREATMENT DOES NOT HINDER POST-ISCHEMIC RECOVERY OF ZDF RAT HEART
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In UKPDS, statistical evaluation of data from diabetic patients receiving a combination of the antidiabetic agents Metformin and Glibenclamide has pointed towards concerns about possible interference of this treatment with cardiac function during post-ischemic recovery. Therefore, we tested the effects of chronic exposure to a mixture of both drugs in a sequence of ischemia-reperfusion on isolated perfused hearts from diabetic rats.
Adult male Zucker Diabetic Fatty rats were either untreated (C) or treated for 1 month with either Metformin (M) (10 mg/100 g bw/d) or Glibenclamide (G) (0.2 mg/100 g bw/d) or with both drugs simultaneously (M+G) at the same dosages. Hearts were perfused in a Langendorff model with modified Krebs-Henseleit medium under 100 cm hydrostatic pressure for 30 min, followed by 25 min global low flow (residual flow at 1.5% of preischemic flow). Reperfusion was monitored for 30 min thereafter. The following parameters were measured: coronary flow (CF), heart rate (HR) and left ventricular developed pressure (LVDP). In the normoxic preischemic period, none of the treatments changed CF, LVDP or RPP (HR*LVDP). In contrast, HR was slightly elevated by M and reduced by M+G. Following reperfusion, CF was rapidly normalized to preischemic values in all groups. Heart rate recovery was impaired to a similar extent in all groups. Postischemic LVDP at the end of reperfusion was reduced, except for the M+G group. As compared to controls, M or G alone had no effect on RPP while M+G induced a non-significant improvement in RPP (mean 55% versus 29%). However, there was no significant difference between the treatment groups. In the conditions of the present study, chronic exposure to the combination of Metformin and Glibenclamide did not have any deleterious effect on post-ischemic functional recovery of isolated hearts of Zucker Diabetic Fatty rats. Furthermore, the contractile function was even slightly improved in M+G versus G or M monotherapy.

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P08-12

Ca^{2+}-ACTIVATED Cl^- CURRENT AND SPONTANEOUS ACTION POTENTIAL OF CARDIOCYTES IN RABBIT PULMONARY VEIN

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Atrial fibrillation is the most prevalent arrhythmia but the mechanism of development is not yet clear. Since the most prevalent focus of paroxymal atrial fibrillation is located inside pulmonary veinfocus, we tried to isolated cardiocyte enzymatically in pulmonary vein of rabbit and could record spontaneous action potentials (Nam et al, 2000). These cardiac myocytes may be a source of ectopic focus of atrial fibrillation but still we don't know how these cells can generate such rapid firing action potentials in paroxysmal atrial fibrillation. We tested the effect of the change of intracellular $[Na^+]$ on action potential development. When we increased $[Na^+]i$ from 0 mM to 30 mM, the action potential frequency was increased from 1-2 Hz to over 5Hz. The increase of intracellular Ca^{2+} concentration from 40 nM to 300 nM, the manner of the action potential change is similar. Therefore the effect of $[Na^+]i$ increase is linked to the increase of $[Ca^{2+}]i$. After a short depolarizing pulse of 10 msec to 40 mV from the holding potential of -40 mV, a transient outward tail current was recorded. the transient outward current was increased as $[Na^+]i$ was increased. The transient outward current was abolished by the removal of extracellular Cl^- with glucuronic acid or methansulfonic acid and was blocked by Cl^- channel blocker, 9-AC. This current was not blocked at all by 40 mM tetraethylammonium application. High concentration of EGTA in pipette solution or the removal of extracellular Ca^{2+}, the transient outward current was abolished. From these results, this transient outward current was Ca^{2+}-activated Cl^--dependent current. This transient outward current may participate in the increase of frequency change in Ca^{2+} loaded conditions by speeding up the repolarization.

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S9 EXTRACELLULAR (VOLUME) TRANSMISSION : ITS MECHANISMS AND FUNCTION

ORAL SESSION

S9-1

DIFFUSION IN EXTRACELLULAR SPACE: MECHANISM OF NEURON-GLIA COMMUNICATION

Sykova E.

Extrasynaptic “volume” transmission plays an important role in communication between neurones and glia. It is mediated by the diffusion of neuroactive substances in the extracellular space (ECS). In this way, transmitters can reach high-affinity receptors located outside synapses and on glia. Cell volume changes, the structure of cellular aggregates and the extracellular matrix (ECM) affect the migration of molecules in the ECS. Diffusion in the CNS is inhomogeneous, facilitated or slowed down, or in certain regions facilitated in one direction rather than in another. This diffusion anisotropy, found in white matter, cerebellum and hippocampus,is of importance for neurone-glia communication and 'cross-talk’ between synapses. ECS diffusion parameters (volume and geometry) can be determined by diffusion analysis using ion-selective microelectrodes or diffusion-weighted NMR. Changes in ECS volume and geometry accompany neuronal activity, development and aging, as well as pathological states, e.g. ischemia, seizures, injury and demyelination. Ionic changes and amino acid release result in cellular swelling, compensated by ECS shrinkage and a decrease in the apparent diffusion coefficients of neuroactive substances or water. The structural changes, e.g. astrogliosis and ECM changes, increase diffusion barriers. Demyelination and gray matter pathologies often result in the loss of anisotropy. It is evident that the movement of substances is hindered by the size of the pores between the cells as well as by the cellular structure and ECM. Moreover, the swelling and movement of glial processes towards active synapses result in changes in synaptic efficacy. Changes in diffusion parameters affect neuron-glia communication, ionic homeostasis, movement and accumulation of neuroactive substances and, therefore, play an important role in volume transmission and in functions such as vigilance, depression, chronic pain, LTP, memory formation and other plastic changes in the CNS.

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S9-2

NONSYNAPTIC TRANSPORTERS OF HIGH AFFINITY: SITE OF ACTION OF ANTIDEPRESSANTS

Vizi E.S., Zsilla G., Caron M.G., Kiss J.P.

Monoamines (norepinephrine (NE), serotonin (5-HT)) play a central role in the pathophysiology of depression. The extracellular concentration of these neurotransmitter depends on release from monoaminergic varicosities and neuronal reuptake. The monoamine transporters are located extrasynaptically (nonsynaptically) and represent the primary targets of antidepressant medication since the majority of antidperessants enhances the monoaminergic neurotransmission via inhibition of reuptake into neurons (Vizi, E.S. Pharm. Rev. 52:63-89, 2000).
Our aim was to investigate the functional properties of the monoaminergic systems in genetically modified mice lacking the norepinephrine transporter (NET) therefore we measured the neuronal uptake and release of [3H]NE from hippocampal and cortical slices of NET(-/-) knock-out (KO) and NET(+/+) wild-type (WT) mice. The [3H]NE uptake reduced to 12% (hippocampus) and 34% (cortex) of the wild-type (WT) control in NET(-/-) mice, but this residual uptake further decreased by 50 and 70%, respectively in the presence of the SSRI citalopram (1 μM). The more preserved neuronal release of [3H]NE (hippocampus: 29%, cortex: 76%; compared to WT) almost completely disappeared in both regions in the presence of citalopram.
Our data show that serotonergic varicosities can accumulate and release NE due to the heterologous uptake of transmitters, therefore a functional cooperation exists between nordarenergic and serotonergic systems in the brain. This finding might have an important implication for the action of SSRIs since these drugs, in spite of their exceptional selectivity for 5-HT transporters, might enhance not only serotonergic but also noradrenergic neurotransmission.

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OC09-1

EFFECT OF CHARGE ON INTERSTITIAL DISTRIBUTION OF ALBUMIN IN RAT DERMIS IN VITRO

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Interstitial collagen and glycosaminoglycans limit the space available for plasma proteins and other macromolecules simply due to the fact that two materials cannot occupy the same space at the same time. This phenomenon is called volume exclusion, and is of importance for interstitial fluid balance and fluid volume regulation. Furthermore, information gathered on interstitial exclusion of probes will be useful for clinical adjuvant therapies with macromolecules, e.g. monoclonal antibodies. At physiological pH, negatively charged disaccharide groups in the extracellular matrix may influence distribution volume of a probe. We hypothesized that by varying the probe (here albumin) charge we would be able to observe a graded response of available and thereby excluded volume fraction. Human serum albumin (HSA) (pI 5.0) was made more positive by cationization. Using reaction times of 10, 45 and 60 min, cationized HSA (cHSA) with respective pIs of 6.5, 7.3 and 8.0 were made. After eight days of equilibration in a buffer containing labelled native HSA and cHSA, the distribution volumes were calculated relative to that of 51-Cr-EDTA, an extracellular tracer. The available volume in fully swollen dermis for native albumin relative to that of the extracellular tracer averaged 0.485 ±0.008 (n=49), with corresponding volumes for cHSA-10, cHSA-45 and cHSA-60 min of 0.554 ±0.012 (n=19), 0.647 ± 0.026 (n=19) and 0.718 ± 0.021 (n=12), respectively. Increasing the ionic strength of the bathing solution to 1 M NaCl, thereby screening the fixed charges of tissue elements and probes alike, resulted in similar available and thereby excluded volumes of native HSA and neutral cHSA-45 min. Whereas previous studies have suggested that the weakly positive collagen is the dominating excluding agent, calculations based on the present experiments suggest that collagen and glycosaminoglycans contribute to about 60 and 40 %, respectively, of the exclusion of albumin in fully swollen dermis.

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OC09-2

MECHANISMS OF SENSING LOW EXTERNAL Ca^{2+} BY RAT HIPPOCAMPAL NEURONES

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Activity of neuronal cells is strongly dependent on Ca^{2+} fluxes through plasma membrane Ca^{2+} channels. Extracellular Ca^{2+} reductions have been hypothesised to occur at the peri-synaptic zone, however, only recently they have also been measured by Ca^{2+} dyes. By confocal Ca^{2+} imaging of hippocampal slices we here show that acute reductions of the $[Ca^{2+}]o$ induce prompt intracellular Ca^{2+} rises in neuronal, but not glial cells, when slices are contemporarily activated by agonists of group I metabotropic-glutamate or muscarinic receptors. Conversely, the intracellular Ca^{2+} level of non stimulated neurones is insensitive to low external Ca^{2+}. Evidence is provided demonstrating that this paradoxical response is not simply due to a decrease in divalent cations concentration but it is specifically activated by a reduction in $[Ca^{2+}]o$, being maximal with $[Ca^{2+}]o$ between 0.25,0.5 mM. Among cortical neurones, this response is first and foremost expressed by CA1-CA3 pyramidal neurones (70 to 90 % of responding cells upon maximal stimulation). Neuronal Ca^{2+} rises depend primarily on Ca^{2+} influx through L-type voltage-operated Ca^{2+} channels and lesser on release from intracellular Ca^{2+} stores. Contemporary reduction of external Ca^{2+} and metabotropic receptor stimulation also causes depolarization and increase the firing rate of hippocampal and cortical neurones (from 1.2±0.7 to 4.2±1 spikes/sec). The here described phenomenon is an intrinsic cell property since it can be reproduced in primary cultures of cortical neurones. Inhibition of phospholipase C or protein kinase C failed to suppress the neuronal response, whereas PP2, a selective inhibitor of the Src-family of tyrosine kinases, abolishes the paradoxical neuronal Ca^{2+} rise. A model is presented to explain how this response might be accounted for by charge shielding effects and metabotropic receptor stimulation.

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OC09-3

EXTRACELLULAR SPACE DIFFUSION PARAMETERS IN HUMAN ASTROGLIOMAS - CORRELATION WITH TUMOR MALIGNANCY

Vargova L., Zamecnik J., Homola A., Sykova E.

Tumor cell migration through the extracellular space (ECS) might be affected by its pore size and extracellular matrix (ECM) content. ECS volume fraction alpha (alpha = ECS volume/total tissue volume) and tortuosity lamda (lamda squared = free/apparent diffusion coefficient) were studied by the real-time tetramethylammonium method in both healthy human cortical tissue obtained from surgically treated epileptic patients and in astrocytic neoplasms of various grades. The ECS diffusion parameter values were correlated with proliferation markers, malignancy grade and changes in ECM composition demonstrated by immunohistochemical detection of ECM glycoproteins. The average values of alpha and lamda in control cortex were 0.24 and 1.55, respectively. In pilocytic astrogliomas (WHO grade I), we found a significantly higher alpha, no change in lamda and moderate GFAP positivity. Higher values of both alpha and lamda were found in low-grade diffuse astrocytomas (WHO grade II), with a dense fibrillary net of GFAP-positive tumor cell processes. In high-grade astrocytomas (WHO grade III and IV), alpha and lamda further increased; GFAP-positive tumor cells processes were shortened with reduced branching. Laminin and collagen IV expression was similar in controls and all tumors grades; tenascin and vitronectin were observed only in high-grade gliomas. Our data indicate that tumor malignancy corresponds to increases in ECS volume and tortuosity. The increase in diffusion barriers in low-grade tumors is mainly due to a net of GFAP-positive fibrillary processes, while in high-grade astrocytic tumors the major contributors to the tortuosity increase are extracellular matrix glycoproteins. Supported by: GACR 309/00/1430, J13/98:111300004, AV0Z5039906 and FNM 00000064203.

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OC09-4

SAMPLING OF INTERSTITIAL FLUID AND MEASUREMENTS OF COLLOID OSMOTIC PRESSURES AFTER PERITONEAL DIALYS

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The aims of this study were to develop a method to isolate interstitial fluid from the peritoneal membrane and to measure the interstitial colloid osmotic pressure (COP) in the normal peritoneum, and after peritoneal dialysis (PD).
Twelve female rats were anesthetized subcutaneously with fentanyl-midazolam (1:1). Interstitial fluid was isolated using a method modified from that described for hindlimb muscle. Nylon wicks were implanted postmortem by means of a plastic catheter in the tissue just underneath the peritoneal membrane. The characteristics of this fluid was compared to that isolated from wicks implanted in intermuscular spaces in hindlimb muscle and back subcutis. All wicks were removed after 20 min and centrifuged in siliconized Eppendorff tubes. The wick fluid was collected and analyzed in a colloid osmometer constructed for submicroliter samples, and interstitial fluid COP was compared to that of plasma. All experiments were done at 100% relative humidity. PD was done by injecting 20 ml of 3.86% Dianeal into the peritoneal cavity, with a dwell time of 4h. Control rats received no PD.
The ratios of each COP to that of plasma during control were 0.65±0.05 in peritoneum, 0.53±0.04 in muscle, and 0.59±0.05 in skin. After PD, the ratios were 0.29±0.03 in peritoneum, 0.54±0.08 in muscle, and 0.41±0.06 in skin. Thus, the COP ratio in the peritoneum decreased by 55% (p=0.014), and in the skin by 30% (p=0.03), while the COP ratio in muscle was unchanged.
The results imply that an acute PD dwell alters the COP in the peritoneal membrane, thereby shifting the Starling equilibrium towards an absorptive state. The effect was restricted to the peritoneal membrane, and to a lesser extent to the skin. We speculate that the increase in peritoneal hydrostatic pressure following PD cause an increase in the interstitial tissue volume, with dilution and/or washout of colloids.
This study was supported by a Marie Curie Host Fellowship, contract no: QLK5-CT-2001-60039.

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S9-3

DYNAMICS OF VOLUME TRANSMISSION IN THE BRAIN

Fuxe K., Rivera A., Hoistad M., Jacobsen K., Tinner-Staines B., Jansson A., Leo G., Tanganelli S., Genedani S., Vergoni V., De La Calle A., Horvath T., Staines W., Agnati L.F.

Volume Transmission (VT) is a widespread mode of intercellular communication that occurs in the extracellular fluid and in the cerebrospinal fluid (CSF) of the brain. VT signals move from the source cells to the target cells as a consequence of energy gradients leading to diffusion and convection. The dynamics of VT is examplified below.
We introduce a "Tide Hypothesis" for VT macro-migration (>100 μm) based on the evidence that when arterial pressure waves reach the cerebral arteries in the sub-arachnoid space they create pressure waves in the CSF. These pressure waves in the subarachnoid CSF produce a "push and pull" movement (as a "tide") of the fluid filling the Virchow-Robin spaces and thus of the extracellular fluid in the pericapillary spaces. Temperature gradients can create macro- and micro-migrations (<100 μm) of VT signals. Macro-migration could be caused by large temperature gradients (> 0.2 °C) demonstrated between blood and brain active regions. The hypothesis is introduced that UCP-2 positive neurons may participate in the generation of temperature gradients for micro-migration. This may lead to a convection based flow of GABA, DA and neuropeptides released from these neurons.
The relationships between D4 IR pyramidal cells and dopaminergic and noradrenergic terminal networks in the frontal and cingulate cortex indicate that DA and NA could activate D4 receptors on cortical neurons via VT. Another mismatch exists between D1 immunoreactive nerve cells and TH positive / DBH negative nerve terminals within large parts of the main intercalated island of the amygdala. A slow dopaminergic VT in the rostromedial and caudal part of this nucleus may have a role in tonic excitatory modulation of the intercalated GABAergic cells.
Locally formed interleukin-1beta in the brain can act as a long distance VT signal influencing distant targets such as the paraventricular nucleus via the extracellular and cerebrospinal fluid.

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S9-4

GLUTAMATE AND GABA SPILLOVER ONTO IONOTROPIC AND METABOTROPIC RECEPTORS

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The amino acid neurotransmitters glutamate and GABA are generally thought to subserve fast precise 'wiring' transmission at ionotropic receptors, and more diffuse spillover or 'volume' transmission at metabotropic receptors. However, within the hippocampal circuitry, glutamate and GABA can signal via spillover onto both metabotropic and ionotropic receptors. Moreover, glutamatergic axons can be affected by presynaptic GABA receptors, and GABAergic axons can be affected by presynaptic glutamate receptors. Finally, GABA tonically activates ionotropic GABA receptors at subsets of neurons, and this phenomenon may play a homeostatic role in regulating GABA release. Although precise wiring signalling is preserved for very fast signals, on a slower timescale diffuse volume transmission has profound implications for the computational properties of the cortical microcircuitry.

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S9-5

MECHANISMS OF HYPOTHALAMIC GLIA-NEURON COMMUNICATION AND IMPLICATION IN NEUROENDOCRINE REGULATION

Hussy N.

Astrocytes have been recently shown to actively participate to the integration of neural message through a non-synaptic type of communication, notably by responding to various neurotransmitters and releasing neuroactive substances that act onto neurones to modulate excitability and synaptic transmission. However, the physiological role of such " volume transmission " between glial cells and neurones is still poorly understood. We are taking advantage of the involvement of the hypothalamo-neurohypophysial complex in well characterized neuroendocrine regulations to assess the importance of these glia-neurones interactions in the physiology of the nervous system. We showed that in this complex, astrocytes located in the supraoptic nucleus (SON) and neurohypophysial pituicytes act as osmosensory cells, participating in the control of the electrical and secretory activities of vasopressin (VP) neurones by external fluid osmolarity. These cells specifically accumulate the amino acid taurine, which they release upon swelling induced by a decrease in extracellular osmotic pressure. Once released, taurine activates glycine receptor Cl^- channels located on the neurones in the SON and the axon terminals in the neurohypophysis, thereby inhibiting the firing of VP neurones and the release of VP in the blood

circulation. Taurine release from glial cells is not a vesicular type of release as it occurs through volume-sensitive anion channels. We also showed that clusters of glycine receptors appear to be closely associated with glial fibers surrounding neuronal structures, confirming the non-synaptic nature of this communication. We are currently investigating the glial implication in other regulatory processes of this system, and showed that SON astrocytes in situ also respond to a variety of neurotransmitters, such as ATP, noradrenaline, or histamine, by transient increases in intracellular Ca^{2+}, further implicating these cells in various aspects of the physiology of the system.

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POSTER SESSION

P09-01

EFFECTS OF EXTRACT OF GREWIA TENAX FRUIT ON SMOOTH MUSCLE CONTRACTION IN RAT DUODENUM AND JEJUNUM

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Grewia tenax is a small tree up to 2m high which belongs to the Tiliaceae family. The fruit is traditionally cooked in boiling water. The decoction is used as a valuable nutriment , espacially in case of fatigue and anemia .
We report the effects of aqueous extracts of its fruits on water mouvements and on contractions induced by agents (acetyl choline, histamine and barium chloride) in isolated rat intestine.
To study water mouvements , we used the everted sac technique described previously (WILSON, 1956).Two segments of intestine were considered: duodenum and jejunum. After 90 minutes incubation in the presence of Ringer, the two segments presented a loss of water. In the duodenum the rate is –0.261± 0.081 g of water / g of fresh tissue. The jejunum presented the higher excretion rate (-0.386 ± 0.107 g of water / g of fresh tissue).
The addition of the aqueous extract at 20mg/ml in the mucous side provoked an inhibition of water excretion in the duodenum and the jejunum.The fluxes were respectively -0.1353 ± 0.029 and -0.2396 ± 0.044 g.
The addition of the aqueous extract in the serosal side produced an absorption of water in the 2 segments considered.
Responses to muscle contractions were recorded isotonically by means of transducers. The addition of fruit extract into the bath at 1, 5 or 10mg/ml caused spasmogenic effects in both segments, the duodenum being more sensitive.
However the extract given at 50-100 - 200mg/ml caused a spasmolytic effect.
The results revealed that the crude extract could have spasmogenic and spasmolytic components.With the aim at elucidating the action mechanisms, we are attempting to separate these components and to isolate the active principles present.

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P09-02

DIACYLGLYCEROLS-CONTAINING N-3 AND N-6 FATTY ACIDS BIND TO RASGRP AND MODULATE MAP KINASE ACTIVITY

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We elucidated the effects of different diacylglycerols (DAGs), i.e., [1-stearoyl-2-arachidonoyl-sn-glycerol (SAG), 1-stearoyl-2-docosahexaenoyl-sn-glycerol (SDG) and 1-stearoyl-2-eicosapentaenoyl-sn-glycerol (SEG)], on [3H]PDBu binding to RasGRP. [3H]PDBu bound to RasGRP (Bmax, 152± 1.66 pmol/mg protein) with a dissociation constant (Kd) of 1.5 ± 0.35 nM. The competition studies with these DAGs on [3H]PDBu binding to RasGRP revealed the following Ki values : 4.49±0.01 µM, 8.37±1.02 µM and 4.97±1.04µM, respectively, for SAG, SDG and SEG. Furthermore, we transfected human Jurkat T-cells by a plasmid containing RasGRP and assessed the implication of endogenous DAGs on activation of MAP kinases-ERK1/ERK2, induced by phorbol-12-myristate-13-acetate (PMA). In control cells, GF109203X, an inhibitor of protein kinase C, inhibited ERK1/ERK2 activation. However, this agent curtailed but failed to completely abolish ERK1/ERK2 phosphorylation in RasGRP-overexpressing cells, though calphostin C, a DAG binding inhibitor, suppressed the phosphorylation of these enzymes. In cells incubated with arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), PMA induced the production of endogenous DAGs containing these fatty acids, i.e., respectively, DAG-AA, DAG-DHA, DAG-EPA. The production of DAG-EPA was negligible whereas the production of DAG-DHA was higher than that of DAG-AA in both types of cells. The inhibition of production of DAG-AA and DAG-DHA by employing U73122 , a PI-PLC inhibitor, and propranolol, a PC-PLD inhibitor, significantly inhibited MAPK activation in RasGRP overexpressing, but not in control, cells. Our study demonstrates that three DAGs molecular species bind to RasGRP, but only DAG-AA and DAG-DHA participate in the modulation of RasGRP-mediated activation of MAP kinases in Jurkat T cells.

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P09-03

DOCOSAHEXAENOIC ACID INDUCES A DECREASE IN PHI IN T CELLS: IMPLICATION OF INTRACELLULAR FREE CALCIUM
Aires V., Hichami A., Moutairou K.($)., Khan N.K.

Docosahexaenoic acid (DHA) induced a rapid (t1/2=33sec) and dose-dependent decreases in pHi in BCECF-loaded human (Jurkat) T- cells. Addition of 5-(N,N-dimethyl)-amiloride, an inhibitor of Na^+/H^+ exchanger, prolonged DHA-induced acidification as a function of time, indicating that the exchanger is implicated in pHi recovery. To assess the role of calcium in the DHA-induced acidification, we conducted experiment in Ca^{2+}-free and Ca^{2+}-containing buffer. We observed that there was no difference in the degree of DHA-induced transient acidification in both the experimental conditions, though pHi recovery was faster in 0 % Ca^{2+} medium than that in 100 % Ca^{2+} medium. In the presence of BAPTA, a calcium chelator, a rapid recovery of DHA-induced acidosis was observed. Furthermore, addition of CaCl2 into 0 % Ca^{2+} medium curtailed DHA-evoked rapid pHi recovery. In 0 % Ca^{2+} medium, containing BAPTA, DHA did not evoke increases in $[Ca^{2+}]$i, though this fatty acid still induced a rapid acidification in these cells. These observations suggest that calcium is implicated in the long-lasting DHA-induced acidosis. However, DHA-induced rapid acidification may be due to its protonation in the plasma membrane (flip-flop model) as suggested by the following observations: 1) DHA with a -COOH group induced intracellular acidification but this fatty acid with a -COOCH3 group failed to do so, and 2) DHA, but not propionic acid, -induced acidification was completely reversed by addition of fatty acid-free bovine serum albumin (BSA) in these cells. These results suggest that DHA induces acidosis via deprotonation and Ca^{2+} mobilization in human T-cells.

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P09-04

EPA AND DHA MODULATE MAPK SIGNALLING: IMPLICATION OF PROTEIN KINASE C-ALPHA AND EPSILON SUBTYPES
Denys A., Hichami A., Khan N.A.

We assessed the mechanism of action of two polyunsaturated fatty acids (PUFA) of n-3 family, i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on the activation of two mitogen-activated protein kinases (MAPK), i.e., extracellularly-regulated kinases 1 and 2 (ERK1/ERK21/ERK1/ERK22), in Jurkat T cells. We observed that both DHA and EPA were essentially incorporated into phosphatidylcholine. Furthermore, two isoforms of phospholipase A2, i.e., calcium dependent (cPLA2) and calcium independent (iPLA2), were implicated in the release of DHA and EPA, respectively, during activation of T-cells. These two fatty acids inhibited the phosphorylation of ERK1/ERK2 without affecting that of Raf-1. Furthermore, DHA and EPA also inhibited the translocation of protein kinase C-alpha and -epsilon isotypes, coupled to MAP/ERK1/2 kinase (MEK1/2). These two n-3 PUFAs also inhibited the nuclear translocation of NF-kB and the transcription of IL-2 gene in activated Jurkat T-cells.

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P09-05

SUBTYPES OF EXCITATORY CHOLINERGIC RECEPTORS ON DOPAMINERGIC CELLS IN THE RAT VENTRAL TEGMENTAL AREA
Chen Y., Phillips K., Sher E.

Cholinergic inputs to the ventral tegmental area excite dopaminergic (DA) cells and stimulate dopamine release. The subtypes of both nicotinic and muscarinic receptors mediating the excitation have been examined by recording the spontaneous firing rates of DA cells in rat (S/D 25-30 days old) midbrain slices using the single cell extracellular recording technique. When bath applied, the nicotinic agonists nicotine and epibatidine increased, dose-dependently, the firing rate of DA cells. The nicotinic α4-preferring antagonist dihydro-β-erythroidine (2μM) prevented the increase. The α4β2 subtype selective agonist, TC-2559, also caused excitation of DA cells. Choline, an α7 selective agonist, produced increase in firing in less than 30% of DA cells, and the increase was blocked by the selective α7 antagonist methyllycaconitine (10nM). We could not observe any effects of α-conotoxin MII, an α3 & 6 selective antagonist, on nicotine-induced excitation. We have, therefore, demonstrated a major involvement of α4β2, and to a less extent α7 subtypes, in mediating DA cell excitation. The muscarinic agonist oxotremorine-M (Oxo-M) also caused excitation of DA cells in a dose-dependent manner, and the excitation was blocked by the muscarinic antagonist atropine. The M1 preferring agonist, pilocarpine, failed to produce any significant increase in firing rates. Xanomeline, a M1, 2 & 4 receptor agonist, and BuTAC, a M2 & 4 preferring agonist, also had no effect on DA cell firing. These data rule out the involvement of M1, 2 & 4 receptors in Oxo-M-induced excitation. BuTAC is also an antagonist on M3 & 5 receptors, and BuTAC can indeed inhibit Oxo-M-induced excitation dose-dependently, with a full block at 10μM. Based on published in situ hybridisation data and studies in M5 knock-out mice, the M5 receptor appears to be the subtype mediating Oxo-M induced DA cell excitation.

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P09-06

MK-801 PREVENTS AMPHETAMINE-INDUCED INCREASE OF AMINO ACID AND ACH RELEASE AS WELL AS NO GENERATION
Bashkatova V., Kraus M., Vanin A., Philippu A., Prast H.

Amphetamine (AMPH), a popular drug of abuse, exerts selective toxic effects on brain monoamine-containing neurons in a variety of experimental animal models. While the effects of psychomotor stimulants on the brain dopamine and serotonin neurons are well characterized, the mechanisms underlying AMPH and metamphetamine neurotoxicity remain still unclear. Several neurotransmitter and neuromodulatory systems have been implicated in the AMPH-induced neurotoxicity, including glutamate and, most recently, nitric oxide (NO). However, findings concerning the effects of different doses of AMPH on neurotransmitter release, are controversial. To study whether non-competitive antagonist dizocilpine (MK-801) influences AMPH-induced neurotoxicity and alterations in neurotransmitter release elicited by this psychomotor stimulant, we determined levels of lipid peroxidation (LPO), as well as the release of acetylcholine (ACh), glutamate, aspartate, and GABA using the push-pull superfusion technique. To clarify the role of NO, which is thought to be a crucial factor in AMPH-induced neurotoxicity, we also studied the NO generation. Experiments were carried out on male Sprague-Dawley rats. NO was directly measured in striatum using an electron paramagnetic resonance technique. Repeated, systemically applied AMPH (2.5 mg/kg x 4 every 2h) elevated LPO products and NO generation in striatum and increased the release of ACh, aspartate and GABA in the in the nucleus accumbens (NAc). Glutamate release was not affected. Dizocilpine abolished the AMPH-induced LPO and NO formation and diminished the elevation of neurotransmitter release. This study provides evidence that acute neurotoxicity of AMPH, expressed as high LPO levels and increased NO formation, is prevented by inhibiting NMDA receptor-mediated effects. The AMPH-induced neurotransmitter release is also mainly caused by NMDA receptor-dependent mechanisms. Supported by grants RFH 03-06-00085a and RFBR 03-06-49050.

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S12 RESPONSES TO OSMOTIC CHALLENGES

ORAL SESSION

S12-1

HOW DO BACTERIA COPE WITH OSMOTIC STRESS ? THE SINORHIZOBIUM MELILOTI MODEL

Dupont L., Boscari A., Mandon K., Alloing G., Poggi M-C., Le Rudulier D.

Osmotic regulation is an essential mechanism for bacteria submitted to desiccation or hypersaline conditions, and is mainly achieved by accumulation of inorganic ions and selected organic compounds known as compatible solutes. These solutes accumulate in the cytoplasm of stressed cells at high concentration without alteration in the cellular functions, thereby allowing water content to be adjusted by osmosis.
To appreciate the physiological mechanisms and the genetic determinism of osmoregulation in bacteria, the symbiotic partner of alfalfa, Sinorhizobium meliloti, was chosen as a model. Upon osmotic fluctuations in the rhizosphere, S. meliloti accumulates, via uptake systems or de novo biosynthesis, a large spectrum of compatible solutes, such as glycine betaine (GB), proline betaine (PB), trehalose, glutamate…S. meliloti can also use ectoine and sucrose for osmoprotection without any accumulation in the cells. Our research is focused on betaines, powerful osmoprotectants widely found in bacteria, plants and animals. In S. meliloti, GB is synthetised from choline or choline-O-sulfate by the betCBA gene products, whose expression will be presented. An ABC transporter (ChoT) involved in choline uptake has also been identified. While ChoT is not regulated by salt but by choline, it might play a role in osmoprotection via choline conversion into GB. GB and PB are also actively taken up by ABC-transporters and symporters. Two systems, Hut and BetS, have been characterized at physiological and genetical levels. Hut is an histidine and betaines transporter involved in catabolism of these molecules, while BetS plays an essential role in immediate osmotic protection. A third mechanism, contributing to betaine accumulation in S. meliloti, is a temporary repression of catabolism at high osmolarity. Indeed, this organism has the specificity to use betaines as carbon and nitrogen sources when osmotic stress is alleviated, while they represent endproducts in other bacteria.

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S12-2

ENGINEERING OSMOTOLERANCE IN HIGHER PLANTS: POSSIBILITIES AND LIMITATIONS

Bartels D., Sunkar R., Kirch H.H., Rahmanzadeh R., Souer E.

A major problem in agriculture is a decrease in productivity through adverse environmental factors like water deficit or salt stress. Different approaches have been taken to improve stress tolerence either through selection of adapted genotypes or by producing transgenic plants. A number of strategies led to the production of transgenic plants, which performed better under stress conditions in the laboratory. Improved stress tolerance was obtained through engineering the production of osmolytes and compatible solutes, overexpressing stress relevant transcription factors, enhanced synthesis of protective proteins or overproduction of enzymes, which are most likely involved in preventing oxidative stress reactions. Despite the fact that plants show improved tolerance, often yield penalties have been observed.
We are using the extreme desiccation tolerant plant Craterostigma plantagineum as a source for genes, which contribute to osmotic stress tolerance and to understand which physiological factors may be coupled to obtain extreme tolerance. Recently a gene encoding aldehyde dehydrogenase was used to engineer osmotolerance plants. Plants transformed with aldehyde dehydrogenase showed improved tolerance to osmotic stress but also to oxidative stress and some heavy metals. Overexpression seems to induce a detoxification mechanism limiting accumulation of aldehydes. Analysis of desiccation tolerance in C. plantagineum shows that detoxification is only one component in acquisition of osmotic stress tolerance. Other factors are being revealed through studying evolution of desiccation tolerance, and they will be discussed.

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S12-3

GENE EXPRESSION AND SIGNAL TRANSDUCTION IN OSMOTIC STRESS RESPONSE

Shinozaki K.

Plants respond and adapt to a variety of environmental stresses including drought, cold and high salinity to survive in severe stress conditions. These stresses induce various physiological and biochemical responses in plants. Moreover, a variety of genes have been described that respond to these stresses at transcriptional level. Their gene products are thought to function in stress tolerance and response. Many stress-inducible genes have been used to improve stress tolerance of plants by gene transfer. It is important to analyze functions of stress-inducible genes not only for further understanding of molecular mechanisms of stress tolerance and response of higher plants but also for improvement of stress tolerance of crops by gene manipulation.
Dehydration triggers the production of abscisic acid (ABA), which, in turn, not only causes stomata closure but also induces various genes. There are at least two ABA-independent as well as two ABA-dependent signal-transduction cascades between the perception of drought-stress signal and the expression of specific genes. Cis- and trans-acting elements that function in ABA-independent and ABA-responsive gene expression by drought stress have been precisely analyzed (Cur Opin Plant Biol. 3: 217-223, 2000). In this conference, we present recent progress on global analysis of expression profiles of stress responsive gene expression using 7,000 full-length cDNA microarray, and functions of stress-inducible genes (Seki et al. Plant J. 31: 279-292, 2002). Cis- and trans-acting factors involved in ABA-independent and ABA–dependent gene expression systems are also described. We also discuss application of stress-inducible genes and their promoters for molecular breeding of drought-stress tolerant crops.

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OC12-1

OSMOTIC ACTIVATION OF BETS, A BETAINE TRANSPORTER FROM SINORHIZOBIUM MELILOTI

BoscariI A., Mandon K., Le Rudulier D.

The most frequent mechanism developed by bacteria to encounter osmotic fluctuations of their habitat is the accumulation of a restricted number of molecules, called compatible solutes. Betaines, mainly glycine betaine and proline betaine, are common osmoprotectant in Gram-negative bacteria and can be accumulated in the microsymbiont of alfalfa, Sinorhizobium meliloti, to restore turgor pressure in response to salt stress. A secondary transporter, BetS, has been characterized and growth experiments have underscored the crucial role of BetS as an emergency system involved in the rapid acquisition of betaines by S. meliloti subjected to osmotic upshock. BetS-mediated betaine uptake is the consequence of immediate activation of preexisting proteins in response to high osmolarity. This Na^+-dependent symport is predicted to show 12 transmembrane segments, with N- and C-terminal extensions in the cytoplasm. We have investigated the role of both hydrophilic ends in the regulatory response to osmotic stress by construction of various deletion mutants in both extensions. One deletion of only 11 amino acids in the C-terminal part, strongly affected BetS transport capacity. Whereas the affinity of this truncated protein towards glycine betaine remained unchanged, a 6-fold reduction in affinity towards Na^+ was measured. This result suggests a role of the C-terminal domain in Na^+-binding activity, either directly or indirectly by affecting the conformation of the Na^+- binding site.

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S12-4

CELL VOLUME REGULATION IN ANIMAL CELLS: OSMOLYTES, OSMOLYTE TRANSPORT, AND SIGNAL TRANSDUCTION

Kinne R.K.H., Olsen H., Tinel H., Kinne-Saffran E., Wehner F., Planck M.

In recent years, it has become evident that the volume of a given cell is an important factor not only in defining its intracellular osmolality and its shape, but also in defining other cellular functions, such as transepithelial transport, cell migration, cell growth, cell death, and the regulation of

intracellular metabolism. In addition, besides inorganic osmolytes, the existence of organic osmolytes in animal cells has been discovered. Osmolyte transport systems-channels and carriers alike-have been identified and characterized at a molecular level and also, to a certain extent, the intracellular signals regulating osmolyte movements across the plasma membrane. The current review reflects some of these developments and focuses on the contribution of organic osmolytes and their transport systems in regulatory volume increase (RVI) and regulatory volume decrease (RVD). Furthermore, the current knowledge on signal transduction in volume regulation is compiled, revealing an astonishing diversity in transport systems, as well as of regulatory signals.

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S12-5

THE CHINESE CRAB: AN OUTSTANDING ARCHETYPE TO UNDERSTAND HOW CRUSTACEA OVERCOME OSMOTIC CHALLENGES.
Péqueux A.

An exhaustive investigation of all aspects of osmotic regulation in crustaceans is a difficult task and would result in an unbelievably large amount of information of limited interest for a clear and good synthetic approach to the question. Crustacean representatives exhibit indeed almost all possible patterns of osmotic regulation. They are abundant and widely distributed in most of known biotopes, being highly adaptable. The major problem these animal species have to face is to maintain, or to restore if disturbed, a cellular volume and a basic pattern of intracellular solutes within some narrow range compatible with the different life-supporting cell activities.
A first and basic way to effect osmotic regulation is to maintain the intracellular fluid isosmotic to the extracellular medium, either the external medium or the body fluid. A second way is frequently evolved aside the basic one; it is to maintain the osmotic concentration of extracellular fluids more or less constant.
This lecture will focus on these processes by considering the various mechanisms at work in boundary epithelia that could possibly be involved or even specialised in the transport of osmotic effectors, essentially inorganic ions. The major part will be devoted to the gills and, more especially to the gills of the fully euryhaline crab Eriocheir sinensis where a clear-cut separation based on structure and physiology exists between “respiratory” and “salt-transporting” gills. Considering the question of the applicability of the gills physiology findings in E.sinensis to other crustaceans, we'll show how that species may actually be considered as an almost unique model to study not only ion-transport processes but also the structure-function relation in NaCl-transporting epithelia of Crustaceans at large.

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OC12-2

THE VOLUME-REGULATED ANION CHANNELS IS A RELEASE PATHWAY FOR ATP FROM AORTIC ENDOTHELIAL CELLS.
Droogmans G., Hisadome K., Koyama T., Kimura C., Ito Y., Oike M.

Mechanical stress induces an auto/paracrine ATP release from endothelial cells, but the mechanism underlying this release is not well understood. Here we show that the release of ATP induced by hypotonic stress (HTS) in bovine aortic endothelial cells (BAEC) occurs through volume-regulated anion channels (VRAC).
Currents through VRAC were measured using whole-cell patch clamp, ATP release was monitored using the luciferin/luciferase bioluminesence assay.
VRAC currents and ATP release share some common features. Both are activated through protein tyrosine phosphorylation, and modulated via the Rho/Rho kinase pathway. Various blockers of VRAC channels also inhibit ATP release with a similar IC50 value.
Extracellular ATP inhibits VRAC currents in a voltage-dependent manner: block was absent at negative potentials, was manifest at positive potentials but decreased at highly depolarized potentials. This phenomenon could be described with a “permeating blocker model” in which ATP binds with an affinity of 1.0±0.5 mM at 0 mV to a site at an electrical distance of 0.41 inside the channel. Bound ATP occludes the channel at moderate positive potentials, but permeates into the cytosol at more depolarized potentials. The triphosphate nucleotides UTP, GTP and CTP, and the adenine nucleotide ADP exerted a similar voltage-dependent inhibition of VRAC currents, which could also be described by binding to a same site at the same electrical distance as ATP.
The current-voltage relationships in the absence and presence of ATP could also be described with a "multi-ion, single site" barrier model with a Kd value for ATP binding similar to that obtained from the previous approach, and a PATP/PCl of 0.0045. Moreover, the flux of ATP calculated from these parameters was compatible with the experimentally observed value.
We conclude that VRAC is an ATP-permeable channel, which may serve as a pathway for HTS-induced ATP release.

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OC12-3

EFFECT OF OSMOTIC ALTERATIONS ON CELL VOLUME AND MEMBRANE PROPERTIES OF LEECH RETZIUS NEURONES
Coulon P., Klees G., Langer J., Dierkes P.W., Schlue W.-R.

To find out whether leech neurones possess volume-sensitive ion channels we investigated the effects of reducing and raising the extracellular osmolarity on the cell volume and on the electrophysiological properties of leech Retzius neurones, using ion-selective microelectrodes as well as the current-clamp and the voltage-clamp technique. A decrease in the extracellular osmolarity of 40 % by omitting NaCl from the bathing solution induced a cell swelling by 40 ± 3 % (n = 4), a hyperpolarisation by -5.5 ± 6.5 mV (n = 15), and a decrease in the input resistance by -37 ± 20 % (n = 11). All these changes were stable and fully reversible. Voltage-clamp experiments revealed that the decrease in input resistance was most prominent near the resting potential. To investigate which of these effects were caused by the reduction of the gradients for Na^+ or Cl^-, or by the reduction of ionic strength, Na^+ and Cl^- were replaced by impermeable ions (NMDG and gluconate) or by saccharose. Under these isosmotic conditions, the hyperpolarisation was similar to that in hyposmotic solution, suggesting that it was due to the reduction of NaCl. In contrast, cell volume and input resistance remained almost unchanged, indicating that these effects were caused by the drop in extracellular osmolarity. The decrease in input resistance indicates an activation of volume-sensitive ion channels. However, these channels seem to have no volume-regulating effect.
An increase in the extracellular osmolarity by 45%, achieved by adding NaCl to the bathing solution, induced a depolarisation by 13.2 ± 4.8 mV (n = 7), and an increase in the input resistance by 11.1 ± 5.2 % (n = 6). A similar depolarisation in voltage-clamp experiments did not lead to an increase in input resistance; hence the resistance increase is likely to have been caused by cell shrinkage. The results suggest the closing of ion channels under hyposmotic conditions, possibly to reduce ion influx.

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POSTER SESSION

P12-01

INTESTINAL PRECONDITIONING INDUCED BY HYPEROSMOTIC SOLUTION

Gál P., Varga S., Kaszaki J., Boros M.

Objectives: Ischemic preconditioning (IPC) effectively protects against intestinal ischemia-reperfusion (I/R)-caused tissue damage, but the method is not feasible in the clinical practice. Nitric oxide (NO) plays important roles in this mechanism, and previously we have shown that circulating NO level might be increased by hyperosmotic solutions. The aims were to examine the possibility of hyperosmotic saline preconditioning (HSPC), and demonstrate the role of NO release in this condition.
Methods: Intestinal I/R was induced in anesthetized dogs (n=6) by clamping the superior mesenteric artery for 60 min; in group 2 (n=6) IPC was performed by means of 3x5 min arterial occlusion 60 min before ischemia. In the HSPC group (n=6) 4 ml/kg of 7.5% hyperosmotic saline was administered 3x5 min i.v. 60 min prior to ischemia. Local hemodynamics, intramucosal pHi were monitored, intestinal biopsies were taken to quantify leukocyte accumulation (with myeloperoxidase assay), constitutive and inducible NO synthase (cNOS and iNOS, respectively) activities.
Results: I/R decreased cNOS (16.4 +/- 3.1 vs 11.2 +/- 3.8 fmol/mg/min), did not influence iNOS activities; significantly decreased pHi (from 7.23 +/- 0.07 to 6.81 +/- 0.075) and increased tissue myeloperoxidase activity (430 +/- 78 vs 848 +/- 117 mU/mg/min). IPC resulted a 2.8-fold increase in cNOS activity (14.9 +/- 2.8 vs 46.6 +/- 9.1 fmol/mg/min), prevented the deterioration of pHi and decreased myeloperoxidase activity. In the HSPC group cNOS activity was 2.2 times higher (17.6 +/- 4.6 vs 40.2 +/- 11 fmol/mg/min) as compared to I/R group and myeloperoxidase activity was significantly decreased.
In conclusion, HSPC is a promising method to diminish I/R-induced inflammatory reaction by elevating NO synthesis in the small intestine. (Supported by research grant OTKA T035275).

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P12-02

OSMOTICALLY-INDUCED ATP RELEASE IN A6 EPITHELIA UNRELATED TO CELL VOLUME

Segal A., Jans D., Lariviere E., Simaels J., Van Driessche W.

ATP release from the apical and basolateral membrane of cultured epithelia from the distal part of the kidney of Xenopus laevis was recorded with luciferin-luciferase luminescence. We developed a pulse protocol to determine in stop flow conditions the rate of ATP release from the rate of rise of the amount of ATP. Gradual lowering of the osmolality of the basolateral bath from 260 to 140 mOsm/(kg H2O) at a rate of 1 mOsm/(kg H2O.min) did not change cell volume over a period of 120 min. The rate of ATP release recorded after reaching an osmolality of 140 mOsm/(kg H2O) amounted 0.78±0.08 pmol/min. In experiments where the osmolality was reduced in a shockwise manner, similar release rates (0.51±0.06 pmol/min) were recorded after the regulatory volume decrease had returned cell volume to control. Apical ATP release was negligible in conditions where the epithelium was bilaterally exposed to isotonic conditions and did not increase when basolateral osmolality was lowered. However, reducing the osmolality of the apical bath markedly increased the apical release. The effect is strikingly dependent on the age of the cultured cells and the degree of the osmotic perturbation. We reduced apical osmolality from 260 to 140 or 20 mOsm/(kg H2O). Such drastic reductions of the osmolality of the apical solutions do not damage the epithelium, because of the negligible water permeability of this barrier. Most experiments were done with epithelia used 7-9 days after being seeded. During an exposure period of 40 min to 20 mOsm/(kg H2O), 1 ml of cells released an amount of 61 pmol ATP. In cells of 15 and 21 days old the amount was 7 and 1.4 pmol, respectively. The apical release induced by apical hyposmotic solutions was markedly inhibited by reducing the tonicity of the basolateral bath. We speculate that apical release is triggered by lowering ionic strength or the reduction of the concentration of one of the involved ions.

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P12-03

MOLECULAR ANALYSIS UNDER DROUGHT STRESS OF DIFFERENTIALLY EXPRESSED GENES IN A LEGUME PLANT MODEL

Clément M., Boncompagni E., Hérouart D.

Legumes are very important as sources of protein and oil for the agro-industry. Abiotic stresses such as osmotic stress induce large decreases in yield and seed quality, especially in soybean which is very sensitive to drought stress. The symbiotic interaction between soybean and the soil bacteria, Bradyrhizobium japonicum, results in the formation of a specific organ, the root nodules, in which the bacteria fix atmospheric nitrogen. During drought stress, nitrogen fixation drastically decreases, leading a large loss in yield, especially when the stress occurs during pod filling. In order to aid the selection of osmotic stress tolerant soybean a clearer understanding of the molecular basis of the inhibition in N2 Fixation Capacity (NFC) by water deficit in root nodules is required. Differential analysis of expressed transcripts between well watered plants (100% of NFC) and stressed plants (50% of NFC) was performed using the Suppression Substractive Hybridization technique, leading to an enrichment of specific drought induced or drought down regulated genes. A first library has been constructed. Using a reverse northern analysis by dot blot hybridization of 200 clones, 40 cDNA clones up regulated during drought stress were selected and are currently been sequenced. The time course expression pattern of revelant clones during drought stress will be presented. Two new libraries are under construction and the validation of 1000 cDNA clones, from each library using a microarray strategy will be shown. A better understanding of the mechanisms involved in the sensing of drought stress and in adaptative response inside root nodules will help us to develop molecular strategies to maintain a high nitrogen fixation even under adverse conditions.

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P12-04

THE Cl DEPENDENCE OF THE HYDROOSMOTIC RESPONSE OF RANA TEMPORARIA BLADDER TO HYPEROSMOLAR SOLUTIONS

Hanna-Mitchell A.T., Gonoud D., Gebruers E.M.

Exposure of the serosal face of anuran urinary bladder to hyperosmotic solutions (SH) results in an increase in water flux (Jw) which is reproducible and is similar in time course and magnitude to that elicited by AVP application. Unlike the response to AVP the response to SH is absent in nominally chloride free solutions. (Hanna-Mitchell, & Gebruers, (2001). To examine the chloride dependence of the water flux, male Rana Temporaria bladders were studied in a series of experiments using a modification of the gravimetric method of Bentley, (1958). Bathing fluid was made hyperosmotic (+100mOsm) and the increase in water flux examined where chloride concentration was reduced, by replacement with gluconate, from 162mM in normal SH Ringer to 110 mM on second exposure of the bladder, series 1, and to 55mM in series 2. Flux results represent cumulative values for the 30 minute period following stimulation with SH and a similar period following hyperosmotic stimulation in low chloride. Results were analysed using Students t test and expressed as Mean ± S.E.M. Mean Jw in SH Ringer (Series 1) was 54ul ± 10.9 µl/30min this decreased to 34.5ul ± 7.57ul /30min on reducing chloride to 110mM ($P < 0.02$, n=7). In series 2 experiments in SH Ringer Jw was 47± 5.6 µl/30min falling to 27.9± 3.62ul/30 min ($P<0.02$) in 55mM chloride solution. Jw was lower in SH solution with 55mM chloride than in 110mM chloride ($P<0.05$, n=7). Control experiments showed no significant difference between exposures 1 and 2 in normal SH medium. At zero Chloride the response was abolished. It could be slowly reversed when bladders were returned to normal SH medium. Bumetadine (100uM) did not abolish the response. NPPB application serosally (50uM) reduced the response. The graded reduction in the response, taken with the absence of the response in zero chloride, supports a physiological role for chloride in hormone-independent water transport.

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P12-05

THE E. COLI POTASSIUM CHANNEL, KCH, IS ACTIVATED BY HYPO-OSMOLARITY

Munsey T.S., Aziz Q., Dondas N.Y, Sivaprasadarao A.

We have previously shown that the activity of the E.coli potassium channel, Kch, can be monitored by examining the change in the phenotype of E.coli conferred by the overexpression of Kch: cells overexpressing Kch fail to survive on nutrient agar plates deficient in K^+ ions (BBRC 297, 10-16, 2002). In this study, we have investigated the hypothesis that the activity of Kch might be regulated by the osmolarity of the environment. For this, we have plated E.coli harbouring the expression vector construct, pKch, on low K^+-nutrient agar plates containing (induced) or lacking (control) the inducer of expression, isopropyl-b-thio-D-galactoside. While growth was normal on control plates, no growth was observed on plates containing the inducer, indicating that overexpression of Kch was toxic to E.coli. However, when the osmolarity of the medium was increased with sorbitol, growth was rescued, reaching a maximum at ~400 mOsm. This suggests that higher osmolarity suppresses the activity of Kch, thereby promoting the cell survival. Unlike sorbitol, however, NaCl failed to rescue the growth. On the contrary, NaCl (³ 30 mM) prevented the growth rescue conferred by sorbitol. This suggests that NaCl might activate Kch. Progressive deletion of the C-terminal region of the channel, including the RCK domain, failed to alter these regulatory properties. This suggests that both osmosensing and Na^+ sensing functions are associated with the transmembrane portion of the channel. What is the physiological significance of this regulatory behaviour? Bacteria jettison K^+ ions in response to a sudden decline in the osmolarity of the surrounding medium, as a protective mechanism against osmotic swelling and cell bursting. We propose that Kch plays a key role in this osmoadaptive mechanism by opening its pore during hypoosmotic shock to allow rapid exit of its cytoplasmic K^+. This in turn leads to the efflux of water, thereby preventing cell swelling and death. Supported by the Wellcome Trust.

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S27 CELL VOLUME: REGULATION AND FUNCTIONAL IMPACT

ORAL SESSION

S27-1

SIGNAL TRANSDUCTION IN CELL VOLUME CONTROL AND APOPTOSIS

Hoffmann E. K.

Most animal cells regulate their volume very precisely, and cell volume homeostasis is crucial for the integrated function of cells. Moreover change in cell volume seems to be a signal used in basic physiological mechanisms. Thus cell swelling plays a primary role in cell proliferation, and cell shrinkage is a hallmark in programmed cell death (apoptosis). The signaling events evoked by changes in cell volume consist of: a signal, a volume sensor, signal transduction pathway(s), and a number of effectors, which are the volume sensitive membrane transport systems. K^+ - and anion channels are important effectors after cell swelling, whereas a Na^+,K^+,$2Cl^-$ co-transporter and a Na^+/H^+ exchanger play major roles in the volume recovery following cell shrinkage. The signal transduction mechanisms involved in the activation of these transport systems by changes in cell volume were studied in Ehrlich ascites tumor cells, NIH3T3 cells and astrocytes, and recent evidence regarding the relation between the signaling cascade, the F-actin cytoskeleton, and volume-regulatory transporters will be presented. MLC phosphorylation by MLCK and translocation of myosin II to the cortical region of the cell appeared to be important for the initial shrinkage-induced activation of the ion transporters. Longer lasting cells shrinkage on the other hand resulted in activation of c-Jun-N-terminal kinase (JNK) and p38 mitogen activated protein kinase (p38MAPK) but not in extra cellular regulated kinases (Erks), that instead were activated by cell swelling. The shrinkage activation of JNK and p38 is followed by p53 phosphorylation at serine 15 and by activation of the apoptotic enzyme caspase-3. The process is dependent on the small G protein Rac. Despite caspase3 activation by cell shrinkage it is not yet certain whether shrinkage per see induces apoptotic cell death or it just sensitized the cells towards other apoptotic inducers.

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S27-2

PROTEIN–PROTEIN INTERACTIONS AND PROTEIN–MEMBRANE INTERACTIONS OF THE ICLN PROTEIN

Ritter M., Fuerst J., Jakab M., Ravasio A., Eichmueller S., Chwatal S., Garavaglia L., Botta G., Meyer G., Paulmichl M.

ICln is a multifunctional protein essential for cell volume regulation. It can be detected in the cytosol and in association with the cell membrane. Beside its role in the splicing process ICln is involved in the generation of the ion currents activated during regulatory volume decrease after cell swelling (RVDC). ICln reconstituted in artificial bilayers can form ion channels with biophysical properties related to RVDC. In cultured fibroblasts fluorescence resonance energy transfer (FRET) between a fusion protein of cyano fluorescent protein and ICln (CFP-ICln) as donor and a membrane tagged YFP (YFP-Mem) as acceptor can be detected, indicating association of ICln with the cell membrane. Upon cell swelling the FRET signals significantly increase, unmasking ICln redistribution from the cytosol to the cell membrane. This redistribution occurs in parallel to altered kinetics of RVDC in MDCK cells and in NIH 3T3 fibroblasts, i.e. the rate of swelling induced depolarization of the cell membrane potential is accelerated and RVDC develops more rapidly if these cells are swollen for a second time. Addition of purified ICln protein to the extracellular solution or overexpression of farnesylated ICln increases the anion permeability, measured by MEQ fluorescence.
In C.elegans the ICln gene is organized in an operon and transcribed with two other proteins termed Nx and Ny. The transcript of the ICln gene is alternatively spliced to yield two protein variants, termed IClnN1 and IClnN2. IClnN1 is highly homologues to all ICln proteins identified so far, whereas IClnN2 bears additional 20 AAs (encoded by exon 2a) close to the inner mouth of the putative channel pore. In contrast to IClnN1, IClnN2 exhibits strong voltage dependent channel inactivation if reconstituted in lipid bilayers. Reconstitution of IClnN2 and protein Nx reveals suppression of this voltage dependent inactivation indicating a functional interaction between these proteins.

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OC27-1

G-PROTEINS, Na^+ AND CYCLIC AMP PARTICIPATE IN SWELLING-ACTIVATED TAURINE EFFLUX IN RAT THYROCYTES.

Fugelli K.

Animal cells must deal with changes in osmotic and hydrostatic pressure between their environment and their interior and counteract volume changes. Swelling activated channels is one group of effectors in the cell membrane that is important in preventing excessive volume increases by releasing inorganic ions and organic solutes that include taurine. Swelling activated channels are associated with several physiological processes, but little is known about their activation mechanisms. We have used rat thyroid cells (FRTL-5) to investigate the activation of a swelling sensitive [3H]taurine efflux pathway. Both hypo-osmotic induced swelling and thyrotropin (TSH) increase transiently the rate coefficient for [3H]taurine efflux and show the same pattern of activation. However, the TSH activation does not involve an initial swelling induced by a Na^+ accumulation as Na^+-free medium, 5-(N,N-dimethyl) amiloride or furosemide reinforced and ouabain inhibited the activation. Cholera toxin stimulated the basal efflux activity but completely blocked the TSH activation. Swelling activation of these treated cells was however, strongly increased. TSH stimulation is known to increase the cellular cAMP pool in FRTL-5 cells. A phosphodiesterase inhibitor increased the swelling activated efflux rate coefficient 6.4 times and a cAMP analogue (db-cAMP) activated this pathway, suggesting that cAMP is involved in both swelling and TSH activated taurine efflux. A concomitant db-cAMP and swelling activation showed a synergistic effect, but the same db-cAMP stimulation at hyper-osmotic conditions strongly inhibited the db-cAMP effect. Based on our and others results we propose that the TSH-receptor-G protein complex is a transducer sensitive for intracellular Na^+ ions that mediates the signal that activates the swelling sensitive taurine efflux pathway in FRTL-5 cells. An element in this transduction pathway downstream from adenylyl cyclase is inhibited depending on the cell volume.

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OC27-2

CERTAIN K^+ CHANNELS ARE SENSORS OF CELL VOLUME.

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Many important physiological processes involve changes in cell volume, e.g. the transport of salt and water in epithelial cells and the contraction of cardiomyocytes. Cell volume changes result in significant changes in the K^+ conductance of the plasma membrane, but neither the molecular identity of the involved K^+ channels nor the mechanism for this regulation have been clear. To examine the effect of cell volume changes on cloned K^+ channels, voltage-regulated K^+ channels of the KCNQ type (KCNQ1-4) or Ca^{2+}-activated K^+ channels (SK, IK and BK channels) were co-expressed with aquaporin 1 water-channels in Xenopus oocytes to ensure adequate cell volume changes in response to altered extracellular osmolarity. The KCNQ1 and KCNQ4 current amplitudes precisely reflected the volume of the oocytes; during cell swelling the currents increased by approx. 70 % and decreased to approx. 50 % during cell shrinkage. In both cases maximal effects were seen after cell volume changes of 5-10 %. In contrast, the related KCNQ2 and KCNQ3 channels, which are prominently expressed in neurons, were insensitive to cell volume changes. Incubation of the oocytes with cytochalasin D and experiments with truncated KCNQ1 channels suggested that these channels sense cell volume changes through interactions between the cytoskeleton and the N-terminus of the channel protein. For Ca^{2+}-activated K^+ channels, IK and SK channels were sensitive to cell volume changes (activated by swelling and inhibited by shrinkage), whereas the BK channels were insensitive. In conclusion, we suggest that certain K^+ channels (e.g. KCNQ1, KCNQ4, IK and SK) are strictly regulated by small changes in cell volume, whereas others (e.g. KCNQ2/3 and BK) are not. The regulation of K^+ channels by cell volume is most likely mediated through interactions between the cytoskeleton and certain parts of the K^+ channel proteins.

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OC27-3

EFFECTS OF ALTERED ANION CONDUCTANCE IN THE CHEMORECEPTOR CELL OF RAT CAROTID BODY

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The carotid bodies are responsible for sensing arterial PO_2, PCO_2 and pH and play a substantial role in the control of ventilation. Anion transporter and channel inhibitors, like DIDS and anthracene-9-carboxylic acid (9-AC), have been described to influence the function of the organ, therefore we tested the effect of altered anion conductance on membrane potential (Em), cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]c$) and pH (pHi). Experiments were performed on primary cultures of chemoreceptor cells isolated from 10-20 day old Wistar rats. Em changes were monitored by patch-clamp technique using the cell-attached method, $[Ca^{2+}]c$ and pHi were detected by single cell microflourimetry with Indo-1 and c-SNARF, respectively. All experiments were performed in CO_2/HCO_3^- buffered medium. Inhibiting the pH-sensitive inwardly rectifying chloride current by 9-AC (1 mM) resulted in intracellular alkalinisation (0.09 ± 0.06 unit in 5 min, $n = 7$, $p < 0.01$) and hyperpolarisation (13.7 ± 4.9 mV in 3 out of 5 tested cell), which suggest that this conductance serves as a background base-extrude pathway and also a background depolarising conductance. Activation of the swelling-activated outwardly rectifying chloride channel by decreasing the osmolality from 300 to 250 mosmol·kg-1 caused elevation of $[Ca^{2+}]c$ from 52 ± 6 to 389 ± 31 nM ($n = 19$, $p < 0.001$). Ca^{2+}-free medium abolished the Ca^{2+} response ($n = 5$) and nifedipine (10 μM), a blocker of the L-type Ca^{2+} channel, reduced the response by 67 ± 7 % ($n = 4$, $p< 0.001$). Niflumic acid (300 μM), an inhibitor of this swelling-activated chloride channel, abolished the Ca^{2+} response ($n = 9$), indicating that increasing chloride conductance results in depolarisation and Ca^{2+} channel activation. Our data suggest that augmented chloride/anion conductance contributes to the activation of rat chemoreceptor cells and may explain the effect of osmotic changes on carotid body function and ventilation.

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OC27-4

INTRACELLULAR ATP STUDIED USING LUCIFERASE MUTANTS WITH REDUCED AFFINITY FOR NUCLEOTIDES.

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Cellular ATP release is essential for purinergic signaling and thus plays an important role in autocrine and paracrine regulation, however the pathways mediating efflux remain poorly understood. The goal of the present work was to develop a method for distinguishing transmembrane and exocytotic ATP release pathways based on the hypothesis that cytoplasmic [ATP] should fall slightly during stimulation of transmembrane efflux, but not during exocytosis. To monitor small changes in intracellular [ATP] during mechanically induced release, the predicted nucleotide binding site of luciferase was mutated to reduce its affinity for ATP (i.e., raise Km into the physiological range for cytoplasmic ATP) and stably transfected into human embryonic kidney (HEK) cells. Immunofluorescence staining of fixed samples confirmed that the mutant was expressed at similar levels in all cells. Intracellular [ATP] was studied by luminometry of cell monolayers, and by imaging single cells using a photon counting camera. In situ calibrations of alpha-toxin permeabilized HEK cells yielded an intracellular Km of 0.63 ± 0.2 mM ATP for wild-type luciferase and 3.7 ± 1.0 mM for the mutant. [ATP]i was 4.8 ± 0.8 mM under control conditions, and this was transiently reduced by mechanostimulation, consistent with activation of some transmembrane ATP release under these conditions. 2-deoxyglucose (10 mM) reduced intracellular [ATP] by 70 % within 30 minutes at 25 oC. Residual ATP was abolished by inhibiting aerobic metabolism with 5 mM sodium azide. The results suggest that luciferase mutants with reduced ATP affinity will be useful for monitoring intracellular [ATP] non-invasively in living single cells. This work was supported by a fellowship from the Canadian Lung Association / CIHR to A.G., a Canadian Cystic Fibrosis Foundation summer-studentship to C.G. and grants from the CIHR and Wellcome Trust to J.W.H and G.A.R., respectively.

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S27-3

MECHANISMS OF REGULATORY VOLUME INCREASE IN RAT HEPATOCYTES

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In hepatocytes, cell volume functions as a second messenger tuning metabolism and (most likely) proliferation and apoptosis. In addition to their contribution to homoeostasis, the mechanisms of cell volume regulation are therefore of considerable (patho) physiological significance.
The initial event in the regulatory volume increase (RVI) of rat hepatocytes is an uptake of extracellular Na; Na is then exchanged for K via activation of Na/K-ATPase. It could be shown recently that, in confluent monolayer culture, the main effect of hypertonic stress (300 to 400 mosmol/l) on membrane transport is the activation of a cation channel. Furthermore, when compared to Na/H-antiport and Na-K-2Cl symport the relative contribution of this channel to overall Na uptake equalled 4:1:1. With respect to selectivity, the channel exhibited a PNa/PK of 1.4.
In order of potency, the hypertonicity-induced channel was blocked by EIPA (ethyl-isopropyl-amiloride) > amiloride > benzamil, with an apparent Ki for amiloride of 5 μmol/l. It was also found that rat hepatocytes express all three subunits of the epithelial Na channel (alpha-,beta-,gamma-ENaC) and by use of antisense-oligo nucleotides it could be shown that alpha-ENaC is a functional component of the cation channel.
With respect to regulation, it was found that the hypertonic activation of the cation channel (as well as that of Na-K-2Cl symport, but not Na/H-antiport) is mediated by PKC, most likely PKC-delta. Further upstream to PKC, tyrosine kinases, G-proteins, PI3 kinase, the GTPase Rho as well as the actin cytoskeleton are part of the signalling machinery, and a contribution of integrins is very likely. This illustrates the pronounced physiological impact of changes in cell volume on the intracellular signalling network, on the one hand, and the high integrative potency of hepatocyte volume regulation, on the other.

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S27-4

CHANGES IN INTRACELLULAR CALCIUM AND ATP RELEASE DURING HYPO-OSMOTIC TREATMENT OF RENAL CELLS.

Van Driessche W., Jans D., Eggermont J., Lariviere E., Simaels J., Segal A.

Polarized renal A6 epithelia respond to hyposmotic shocks with an increase in transepithelial capacitance that is inhibited by extracellular Mg^{2+} and dependent on extracellular calcium. Therefore, we examined intracellular calcium (Cai) dynamics, their sensitivity to magnesium during hyposmotic conditions and relation to ATP release. In the absence of extracellular magnesium, the hyposmotic shock induced a biphasic rise in Cai. The first phase peaked within 40 s and Cai increased from 248±12 to 606±24 nM. The second phase depended on Ca^{2+} in the basolateral perfusate, was largely suppressed by 2 mM basolateral Mg^{2+} and independent of the initial intracellular Ca^{2+} release. It therefore constitutes non-capacitative Ca^{2+} entry. Phase one was unaffected by removal of extracellular calcium, but was abolished by depleting stores through activation of P2Y receptors with basolateral ATP or inhibiting PLC. Suramin severely attenuated phase one, suggesting that the fast intracellular calcium rise followed swelling-induced ATP release. We developed a bioluminescence luciferin-luciferase pulse protocol to determine the rate of ATP release (RATP) in the basolateral compartment. Under isosmotic conditions, 1 ml of A6 cells released ATP at a rate of 66±8 fmol.min-1. A sudden reduction of the basolateral osmolality from 260 to 140 mosmol.(kg H2O)-1 elevated RATP rapidly to a peak value of 1.89 ± 0.11 pmol.min-1 followed by a plateau phase reaching 0.51±0.07 pmol.min-1. Both peak and plateau values increased with the degree of dilution. The steady ATP release is unrelated to cell swelling but the time course of the rapid phase correlates with RVD. Experiments with other cell types (CaCO-2) showed a similar behaviour. Pharmacological data and experiments in which expression level of the volume activated cation channels was verified suggest a possible role of the volume activated anion channel for ATP release.

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S27-5

CELL SWELLING-INDUCED EXOCYTOTIC SECRETION
Strbak V., Benicky J., Greer S,E., Bacova Z., Najvirtova M., Greer M.A.

Cell swelling evokes an immediate secretory burst of material (peptide hormones, enzymes) stored in secretory vesicles from various types of cells (endocrine, neurons, leukocytes, exocrine pancreas). Dynamics of this secretion are indistinguishable from those induced by specific secretagogues. This regulated secretion does not require a rise in intracellular Ca^{2+} through opening L-type Ca^{2+} channels. Using various tissues (pituitary, pancreatic islets, brain structures), hormones (prolactin, insulin, thyrotropin releasing hormone - TRH, oxytocin) and inhibitors we found that hormone secretion induced by cell swelling is not depressed by inhibition of stretch activated channels (GdCl3), mercury-sensitive aquaporins, protein kinase C (bisindolylmaleimide), microtubules and microfilaments (colchicine, cytochalasin) and does not involve arachidonic acid metabolites prostaglandins and leukotriens (indomethacin, NDGA). Blocking Na^+-K^+-dependent ATPase, Na^+ channels or blocking K^+ channels had no effect on hyposmolarity-induced hormone secretion in pituitary cells. Norepinephrine, a physiological inhibitor of insulin secretion, did not inhibit hypotonicity-induced secretion from pancreatic islets. Participation of such a general biophysical phenomenon in physiological reactions rises question of specificity. Cell swelling induced by isosmotic ethanol containing medium evoked release of TRH from hypothalamic paraventricular nucleus and posterior pituitary, oxytocin (known to be engaged in water and salt regulation) release was not stimulated. Cell swelling consistently stimulates peptide secretion exploiting a different transduction pathway than that delineated for other secretagogues. Signaling is likely to act at a distal end of the secretory pathway and may involve osmotic swelling of docked vesicles. Cell swelling-induced exocytosis possesses limited selectivity; cells specifically engaged in water and salt regulation retain their specific response to osmotic stimuli.

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POSTER SESSION

P27-01

EtOH INCREASES CELLULAR Ca^{2+}, CLOSES GAP JUNCTIONS AND DECREASES CELL VOLUME IN RABBIT GASTRIC MUCOSA
Mustonen H., Kiviluoto T., Kivilaakso E.

This study investigates the behaviour of cell calcium and gap junctions as well as cell volume during exposure to low dose ethanol.
Isolated rabbit gastric epithelial cells were cultured on collagen type I coated glass cover slips or membranes (Ham's F12 with 10% FBS,5%CO_2 at 37°C). They formed a complete polarized monolayer in 48h. Gap junctional diffusion was measured with 5-carboxyfluorescein diacetate loaded cells bleaching a small area with a laser and the recovery was measured with a confocal microscope with or without 5% (vol/vol) ethanol in Ham's F12. For the measurement of intracellular calcium the cells were loaded with fura-2. Emission intensity (510 nm) was measured at 340 nm and 380 nm excitation. Intracellular free calcium concentration ([Ca^{2+}]i) was calculated from 340/380 ratio. For cell volume measurements the monolayer was loaded with Calcein and imaged along Z-axis with a confocal microscope. The change in fluorescence intensity was intercepted as a measure of cell volume.
The basal [Ca^{2+}]i was 65±9 mM. After 10 minutes exposure to 5% ethanol it increased to 140±17 mM (p=0.03,N=5). In bleaching experiments the recovery after 10 minutes from the bleaching without ethanol was 52.8±11.1% and with 5% ethanol 9.4±3.2% (p=0.01,N=6), indicating that gap junctions were partially closed. After 10 minutes exposure to 5% ethanol, the cell volume decreased by -16.6±5.4% (p<0.05, N=6). This volume decrease was inhibited with basolateral exposure to quinine (1 mM), a potassium channel blocker (-1.1±2.1%, p=0.02, N=6), indicating involvement of basolateral K^+ channel.
Following exposure to 5% ethanol [Ca^{2+}]i is increased, gap junctions are partially closed and cell volume is decreased. Opening the basolateral potassium channel was probably involved in the volume decrease. The Ca^{2+} induced gap junction closure presumably opposes the spread of dissipation of ion gradients and other cytoplasmic derangements from an injured cell to neighbouring cells.

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P27-02

VOLUME REGULATION IN MOUSE ISOLATED PROXIMAL TUBULE CELLS
Hartley J.A., Kibble J.D., Robson L.

Most cells regulate their volume upon exposure to a hypotonic shock. The typical response is an initial swelling phase due to the movement of water into the cell down an osmotic gradient, followed by a regulatory phase, where cell volume decreases towards the initial level. The regulatory phase, regulatory volume decrease (RVD), occurs as a consequence of the loss of solute from the cell. The aim of the following study was to examine the mechanisms underlying RVD in mouse isolated proximal tubule cells.
Single proximal tubule cells were isolated from mouse renal cortex and cell diameter determined using an optical technique. Cells were initially superfused with a high Na^+ mammalian Ringer, which contained 60 mM mannitol. Mannitol was then dropped to 20 mM to expose the cells to a hypotonic shock. This was repeated in separate experiments in the presence of 5 mM barium (a K^+ channel inhibitor), 100 µM DIDS and 10 µM tamoxifen (Cl^- channel inhibitors), 100 µM gadolinium (an inhibitor of stretch-activated channels), on stimulation of cAMP and in the absence of extracellular Ca^{2+}.
The initial diameter of control cells was 9.59 ± 0.09 µm (n=37). Exposure to a hypotonic shock increased diameter to a peak by 0.32 ± 0.04 µm (n=37). At steady-state, after RVD, cell diameter was 0.16 ± 0.04 µm above the control level. In the presence of barium, DIDS and tamoxifen and in the absence of extracellular Ca^{2+} RVD was inhibited. Cell diameter after RVD was 0.63 ± 0.06 µm (n=28), 0.35 ± 0.07 (n=15), 0.34 ± 0.07 µm (n=14) and 0.43 ± 0.16 (n=6) above the control level, respectively. Activation of cAMP and the presence of gadolinium were without effect on RVD.
In conclusion, removing extracellular Ca^{2+} inhibited RVD, suggesting that Ca^{2+} influx may play a role in RVD in mouse proximal tubule. The inhibitory actions of barium, DIDS and tamoxifen support the hypothesis that K^+ and Cl^- channels mediate solute efflux during RVD in mouse proximal tubule cells.

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P27-03

IONIC MECHANISM OF OUABAIN-INDUCED CELL SWELLING IN LEECH RETZIUS NEURONS

Dierkes P.W., Klees G., Wüsten H.J., Müller A., Schlue W.-R.

It is widely accepted that the Na^+/K^+-pump plays a central role in the maintenance of cell volume. In leech Retzius neurons ouabain, a blocker of the Na^+/K^+-pump, induced a reversible cell swelling. To elucidate the mechanism of this swelling we monitored the effect of ouabain and K^+-free solution on cell volume, Em and on the cytosolic concentrations of Na^+, K^+, Cl^-, and Ca^{2+} by using the fluorescent indicator Fura-2 and ion-selective microelectrodes.
Bath application of 0.5 mM ouabain induced a polyphasic membrane depolarization (peak depolarization: 30 + 13mV; n = 9), an increase in $[Ca^{2+}]i$ (341 + 95 nM; n = 12), $[Na^+]i$ (75 + 9 mM; n = 5), and $[Cl^-]i$ (25 + 7 mM; n = 4) as well as a decrease in $[K^+]i$ (-73 + 12 mM; n = 8). The increases in $[Cl^-]i$ and $[Ca^{2+}]i$ started with a delay of about 5 min that closely corresponded to the onset of the ouabain-induced cell swelling (35 + 11%; n = 12). Correction for the dilution of the cytosolic ion concentrations during cell swelling showed that oubain induced an electroneutral uptake of NaCl and hence an increase in the cytosolic osmolarity. The resulting change in the osmotic gradient between intra- and extracellular medium seems to be responsible for the induction of cell swelling. This interpretation is underlined by experiments in Cl^-- and Na^+-free solutions in which the oubain-induced cell swelling was abolished. The blockade of the Na^+/K^+-pump in K^+-free solution induced a membrane hyperpolarization, an increase in $[K^+]i$ and $[Na^+]i$, but cell volume, $[Ca^{2+}]i$ and $[Cl^-]i$ remained nearly unchanged. This result indicates that the oubain-induced membrane depolarization is crucially involved in the Cl^- influx and hence cell swelling.

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P27-04

VOLUME REGULATION OF GILL CELLS IN PRIMARY CULTURE FROM SEA BASS

Avella M., Ducoudret O., Duranton C., Tauc M., Poujeol P.

Euryhaline fish face important modifications of external salinity, which can range between 0 and 1100 mOsm/l. Cells of the gill epithelium in direct contact with this environment are naturally confronted with these drastic osmotic challenges. They therefore represent a good model to study the mechanisms of cell volume regulation.
To understand the amazing capacity of the gill epithelium to accomodate these osmotic changes, the cells were challenged with hypoosmotic shocks. The usual response of a wide range of cell types in this situation is the swelling-activated release of KCl and organic osmolytes together with osmotically obliged water, a process known as regulatory volume decrease (RVD).
Previous studies on gill cells showed the presence of apical stretch-activated K^+ channels (TASK ?) sensitive to hypotonic shock. The present study focused mainly on electrophysiological and kinetic aspects of RVD. On the one hand, volume-sensitive chloride currents developed 5 to 7 min after application of a 30% hypotonic shock. These currents were outward rectifying, sensitive to chloride channel blockers (DIDS, NPPB), and did not inactivate with time. On the other hand, efflux of the osmolyte taurine was rapidly stimulated (5-15 times above baseline) following the shock, and this effect was reversible and reproducible. To understand the mechanism of taurine transport, application of chloride current inhibitors was used. Our results indicate that taurine leaves osmotically swollen gill cells via DIDS, NPPB, niflumic acid and DPC-sensitive channels. Both role of Ca^{++} and the cellular location (apical versus basolateral) of this secretion are under investigation.
In conclusion, in gill respiratory-like cells in primary culture, a hypoosmotic shock elicited the activation of volume-sensitive Cl^- currents and taurine release. The interdependence of the pathways involved (presence of volume sensitive organic osmolyte and anion channel – VSOAC ?) is being questioned.

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P27-05

DIFFERENT RESISTANCE TO UNCOUPLER OF RESPIRATION IN RAT LIVER MITOCHONDRIA OF MALES AND FEMALES.

Leontiev D.S., Anishchenko T.G., Fedotcheva N.I., Kondrashova M.N.

It is known, that the high level of mitochondrial membrane potential results in increase in the reactive oxygen species (ROS) production. It was also reported that male sex hormones added in vitro restore membrane potential reduced by low concentrations of uncoupler - a phenomenon of "recoupling". The aim of the present work was to study the phenomenon of recoupling in vivo and to compare a display of it in males and females with simultaneous measurement of lipid peroxidation intensity.
Adult female (n=18) and male rats (n=14) were used. The lipid peroxidation in liver mitochondria was determined by the level of malonic dialdehyde (MDA). The rate of respiration in mitochondria was measured with oxygen electrode before and after addition of 1 μM uncoupler – chlorocarbonylcyanide phenylhydrazone (Cl-CCP). For the maximal preservation of mitochondrial properties a liver homogenate was used. As a substrate we used 4mM succinate.
The basal level of MDA in male mitochondria was higher than in female one by 31% ($p<0,05$). The respiration rate in State 4 was approximately the same in both genders. The addition of 1 μM Cl-CCP resulted in decrease in membrane potential and increase in the respiration rate. The degree of activation of respiration rate was different in males and females. The average stimulation was 37% ($p<0,05$) in males against 85% ($p<0,05$) in females.
These data demonstrate the greater stability of membrane potential (which is the essence of recoupling phenomenon) in male organism than in female one. It can be suggested that the increased level of MDA in male mitochondria is explained by the presence of some factors which more actively support the transmembrane potential.
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P27-06

SURFACE DYNAMICS OF LIVING ENDOTHELIAL CELLS OBSERVED BY ATOMIC FORCE MICROSCOPY

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Endothelial cells change their permeability during inflammatory processes. Important stimuli are tissue hormones like histamine or bradykinine, which diminish the barrier function of intact endothelia. It is still a matter of debate whether the increase in permeability rather arises from intracellular fenestration or intercellular gap formation.
A versatile tool for the investigation of surfaces is atomic force microscopy. Because sample fixation is not necessary for this method, it offers the possibility to observe living cells with the advantage of high vertical resolution. Upon addition of histamine, endothelial cells secrete the polymerizing and thereby "sticky" glycoprotein vonWillebrandt factor whereby stable recording of surface topology is often hampered. Using a tapping mode protocol, individual cells of the human umbilical vein (HUVEC) could be imaged over a time course of more than an hour. The influences of histamine and bradykinine on surface structure and cell-cell-contacts were characterised in a time-dependent manner. In conclusion, morphodynamic changes of endothelial cells as a response to inflammatory mediators were visualised in time-lapse mode.

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P27-07

17ß-ESTRADIOL DOES NOT INFLUENCE VWF EXOCYTOSIS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

Hillebrand U., Riethmueller C., Ossig R., Oberleithner R., Schneider S.W.

17ß-estradiol is known to have protective effects on the vascular endothelium. This is indicated by the reduced incidence of cardiovascular diseases in women. Therefore we investigated the effect of 17ß-estradiol exposure to human umbilical vein endothelial cells (HUVEC) on exocytosis of the procoagulatory protein von Willebrand factor (vWF) and on changes in cell volume. Both reactions are known to be involved in the stimulation of endothelial cells. The release of vWF was measured after short time stimulation (1.5 to 30 minutes) by histamine [50 μM], 17ß-estradiol [3.6 nM] or both substances using ELISA techniques. Changes in cell volume were measured using atomic force microscopy (AFM). After 17ß-estradiol

exposure constitutive level of vWF release showed no significant difference (n=32) compared to non-estradiol treated controls. Stimulation of endothelial cells by the physiological mediator histamine led to a 25+/-1.27% (n=8) increase of vWF exocytosis. Co-stimulation with 17ß-estradiol did not influence the histamine-stimulated vWF release (n=44). Furthermore, stimulation by 17ß-estradiol did not lead to alterations in cell volume as measured by AFM (1946+/-86.3 vs. 1885+/-61.9 fl per cell, n=30). In conclusion 17ß-estradiol exposure does not influence constitutive and stimulated vWF exocytosis in endothelial cells.

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P27-08

SGK1 IS INDUCED IN RESPONSE TO ADVANCED GLYCATION ENDPRODUCTS IN MESOTHELIAL CELLS

Waerntges S., Lang F., Reimann S., Peisch C., Pischetsrieder M., Hugo C., Goppelt-Struebe M.

High glucose levels as present in peritoneal dialysis fluids accelerate the formation of advanced glycation endproducts (AGEs). These are primarily formed by non-enzymatic oxidation and glycation of proteins as e. g. human serum albumin. AGEs are thought to participate in peritoneal membrane dysfunction observed in dialysis patients, but the molecular mechanisms of their action have not yet been characterized completely. The serum- and glucocorticoid-regulated protein kinase SGK1, which previously has been characterized as a target of TGFß, was suggested to be one of the possible mediators of AGE-mediated functional changes in mesothelial cells. Western blot analyses of pleural mesothelial cells (MeT-5A) revealed that hypo- as well as hyper-phosphorylated SGK was upregulated by TGFß1 and by dexamethasone after 24 and 48 hours. AGEs were prepared in vitro by incubating HSA with peritoneal dialysis solution to obtain AGE-HSA. Incubation of the mesothelial cells with AGE-HSA (10 ug/ml) markedly enhanced SGK1 protein expression. The induction was detectable already at 1h of exposition and SGK levels remained elevated for at least 48 h. Human serum albumin (HSA, 10 ug/ml) did not significantly enhance SGK expression within 24 hours. Coincubation of the cells with AGE-HSA and dexamethasone led to a further rise of SGK levels. Thus, our studies show that AGEs induce a long-term upregulation of SGK1. The functional implications of SGK1 expression remain to be elucidated but may be related to volume regulation and fibrotic alterations of the mesothelium.

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S13 NEW ASPECTS OF IONIC TRANSPORT (II)

ORAL SESSION

S13-1

KCNQ1 AND KCNE1 CO-ASSEMBLE EARLY IN THE BIOSYNTHETIC PATHWAY OF CARDIAC IKS CHANNELS

Peretz A., Uziyel Y., Schmitt N., Ben Aharon L., Schottelndreier H., Pongs O., Attali B.

The IKS potassium current mediated by the KCNQ1/KCNE1 K^+ channel complex, plays a major role in cardiac excitability. Mutations in KCNQ1 and KCNE1 genes lead to the long QT (LQT) syndrome. Here, we studied the assembly of functional KCNQ1 and KCNQ1/KCNE1 channels using a decoy peptide (CTD) encompassing the C-terminal assembly domain of KCNQ1 subunits. In the presence of CTD, KCNQ1 currents are not expressed due to a marked decrease in protein expression. The data suggest that CTD traps KCNQ1 subunits and thereby inhibits channel assembly. Co-expression of KCNE1 with KCNQ1 prevents the effects of CTD on functional K^+ channel assembly. Co-expression with the LQT5 mutant W87R KCNE1 does not protect KCNQ1 against the inhibitory action of CTD. The scaffolding protein yotiao which interacts with the channel assembly domain also protects KCNQ1 from the subunit trapping effect of CTD. The data suggest that KCNQ1 and KCNE1 co-assemble early in the biosynthetic pathway of cardiac IKS channels.

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S13-2

LOSS OF FUNCTIONAL M-CHANNELS LEADS TO CHANGES IN NEURONAL EXCITABILITY IN TRANSGENIC MICE

Peters H.C., Hua H., Dehnhardt M., Engler G., Engel A.K., Storm J.F., Pongs O., Isbrandt D.

We have investigated the physiological role of M-type potassium channels (M-current) by expressing a dominant-negative KCNQ2 subunit in mouse brain. The resulting suppression of M-channel activity in CA1 hippocampal pyramidal neurons reduced their early spike frequency adaptation and the corresponding afterhyperpolarizations (mAHPs) following action potential trains. The data also indicate that the characteristic theta frequency resonance behavior of the CA1 neurons is caused by M-channels operating in the subthreshold membrane potential range. The altered neuronal oscillatory properties and excitability were mirrored by abnormal rhythmic brain activity, learning deficits, network hyperexcitability, and by pronounced behavioral hyperactivity accompanied by occasional epileptic seizures. Our data provide new insight into the functional roles of M-channels in neuronal response patterns, cerebral network activity, and behavior.

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OC13-1

REVERSE GENETIC STUDY OF EPITHELIAL V-ATPASE FUNCTION IN DROSOPHILA

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V-ATPases, ubiquitous among eukaryotes, play a 'housekeeping' role in acidifying endomembrane compartments. However, they are also present at far higher densities on plasma membranes of many ion-transporting epithelia. To dissect these contrasting roles, we work on V-ATPase function in the genetic model, Drosophila.
The first animal knockout of a V-ATPase subunit was identified in Drosophila, and was shown to be recessive larval lethal with a characteristic renal phenotype. We have identified the molecular lesions in a series of EMS alleles of vha55, the gene encoding the B subunit. They are all non-conservative substitutions in absolutely conserved regions of the protein. The lethal effect of these mutations in flies can be rescued by overexpression of the wild-type cDNA (in fact, fused to GFP, which in turn provides a valuable resource for mapping expression within the fly).
To show that these mutations really disrupt V-ATPase function (rather than acting in some non-specific way), we attempted to rescue yeast deleted for the homologous vma2 gene. Wild-type vha55::GFP fusions rescue the pH-sensitive conditional phenotype of the vma2 mutation. However, none of the 5 EMS mutant cDNAs can rescue the phenotype. This shows that the role of the B subunit is conserved across 400M years of evolution between fly and yeast. Surprisingly, when the same mutations are introduced into the corresponding residues encoded by vma2, one of them is capable of rescuing the mutant phenotype, implying that the very small differences between the two proteins a sufficiently different context for variation in the residue to be tolerated.
Back in the fly, we employed microarrays to classify the 24 V-ATPase subunit genes into plasma membrane or housekeeping roles, and used a variety of GFP fusion constructs to monitor V-ATPase abundance and subcellular distribution throughout the fly's life cycle. This provides a technology for truly integrative physiology of V-ATPase function.

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OC13-2

PROTEIN KINASE C STIMULATES THE ACID-SENSING ION CHANNEL ASIC2A VIA THE PDZ DOMAIN PROTEIN PICK1

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Acid Sensing Ion Channels (ASICs) are cationic channels activated by extracellular protons. They are expressed in central and sensory neurons where they are involved in neuromodulation and in pain perception. Recently, the PDZ domain-containing protein PICK1 (protein interacting with C-kinase) has been shown to interact with ASIC1a and ASIC2a raising the possibility that PKC could regulate ASICs. We now show that the amplitude of the ASIC2a current, which was only modestly increased (~ +30%) by the PKC activator OAG (50 µM) in the absence of PICK1, was strongly potentiated (~ +300%) in the presence of PICK1. This PICK1-dependent regulatory effect was inhibited in the presence of a PKC inhibitory peptide and required the PDZ domain of PICK1 as well as the PDZ-binding domain of ASIC2a. We have also shown the direct PICK1-dependent phosphorylation of ASIC2a by [32P]phosphate labeling and immunoprecipitation, and identified a major phosphorylation site, T39IR, on the N-terminus part of ASIC2a. The OAG-induced increase in ASIC2a current amplitude did not involve any change in the unitary conductance of the ASIC2a channel, whether co-expressed with PICK1 or not. These data provide the first demonstration of a regulation of ASICs by protein kinase phosphorylation, and its potentiation by the partner protein PICK1.

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OC13-3

THE CORTICOSTEROID HORMONE INDUCED FACTOR (CHIF): A NEW MODULATOR OF KCNQ1 CHANNELS ?

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The corticosteroid hormone induced factor (CHIF) is a member of the one-transmembrane segment protein family named FXYD, which also counts phospholemman and the Na,K-pump γ-subunit. Originally it was suggested that CHIF could induce the expression of ISK current when expressed in Xenopus laevis oocytes, but recently CHIF has attracted attention as a modulatory subunit for the Na,K-pump. In renal and intestinal epithelia the expression of CHIF is dramatically upregulated in response to aldosterone stimulation, and it is an attractive hypothesis that CHIF may also regulate epithelial ion channels. In the present study CHIF was co-expressed with KCNQ1 channels in Xenopus oocytes and mammalian CHO-K1 cells. In both expression systems, we find that CHIF drastically modulates the KCNQ1 current; in the presence of CHIF the KCNQ1 channels become open at all membrane potentials. CHIF is thereby the first accessory subunit shown to be capable of modulating both the Na,K-pump and an ion channel. In Ussing chamber experiments approx. 20 % of the absorptive current in colonic epithelia from salt depleted animals could be blocked by XE-991, a selective inhibitor of KCNQ channels, suggesting a possible role of the constitutively open KCNQ1/CHIF complex during aldosterone stimulation. However, by confocal microscopy we did neither in epithelia from control animals nor from salt depleted animals detect an obvious overlap in localization of KCNQ1 and CHIF. In conclusion, if co-expressed with KCNQ1 channels, CHIF modulates the voltage sensitivity of the KCNQ channels, but so far evidence for an actual co-localization of CHIF and KCNQ1 channels is lacking.

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OC13-4

A MECHANISM FOR THE ACTIVATION OF Na/H EXCHANGE BY CYTOPLASMIC ACIDIFICATIONS AND MITOGENS
Lacroix J., Poët M., Maehrel C., Counillon L.

Because of their metabolic activity and the negative value of their membrane potential, all eukaryotic cells constantly have to fight against internal acidification. In mammals, this task is mainly performed by the ubiquitous Na^+/H^+ exchanger NHE-1, which extrudes intracellular protons against extracellular sodium. This transporter activates sharply, only when cells become acidic, thus exhibiting an allosteric kinetic. Despite its biological importance, the mechanism of its activation is still poorly understood, the most commonly-accepted hypothesis being the existence of a proton-sensor site on the internal face of the transporter. By substituting conserved charged residues located in the intracellular loops of the protein and using a method of rapid kinetic of $22Na^+$-uptake, we discovered a mutation which leads to a form of NHE-1 exhibiting a classical michaelian behavior with a low affinity for intracellular H^+. Using this observation, we show that the allosteric activation of the exchanger can be properly described by the Monod-Wyman-Changeux model. In this model, a dimeric NHE-1 oscillates between two conformations wich possess different affinities for protons (3.6 +/- 0.7 x10-6 M and 1.7 +/- 0.14 x10-8 M). Thus, this mechanism does not require a proton sensor site for the conversion from the low to the high H^+-affinity form. The low affinity form is much more abundant (L0 = 5952 +/- 513) making the exchanger inactive at physiological pH. This model also explains the specific activation of the exchanger by growth signals, which shift the equilibrium towards the high affinity form.

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S13-3

FIRST STEPS IN VISUALIZING CFTR USING ATOMIC FORCE MICROSCOPY
Schillers H., Oberleithner H.

Plasma membrane proteins as ion channels, transporters and receptors are not randomly distributed in the cell membrane but supposed to be organized in "intelligent clusters". One of the key proteins of such clusters is CFTR (cystic fibrosis transmembrane conductance regulator). In order to elucidate cluster formation, we looked for a biological model and a surface technique that should allow to visualize single cluster components in a native plasma membrane. In a first step we tried to identify CFTR at the single molecule level in the cell membrane. Our approach was as follows: Membrane trafficking of CFTR is controlled by the intracellular messenger cAMP. In order to visualize protein insertion we applied atomic force microscopy (AFM) to inside-out oriented plasma membrane patches of CFTR-expressing Xenopus Laevis oocytes stimulated by cAMP. First, oocytes injected with CFTR-cRNA and stimulated by cAMP were voltage-clamped verifying successful CFTR expression. Then, plasma membrane patches were excised, placed (inside out) on glass and scanned by AFM. Gold-labeled antibodies were used to search for CFTR. We found between 60 and 100 single gold labels per µm2 of plasma membrane. Due to the softness of the vesicles we could not resolve the gold labels on the surface of the vesicles. However, we could obtain high resolution images (lateral resolution about 5 nm) from the inner surface of the native plasma membrane spread on glass. Specific ring-shaped structures with several subunits were identified in close vicinity to an individual gold label. From the circular arrangement of the subunits we conclude that CFTR forms a homodimer with a central pore entrance. As a future step we will simultaneously express purinergic receptors and CFTR to test the hypothesis of cluster formation between two functionally related plasma membrane proteins.

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S13-4

DEVELOPPING A MICROARRAY PLATFORM DEDICATED TO PHYSIOLOGICAL STUDIES
Barbry P., Moreilhon C., Prieto H., Magnone V., Christen R.

Most biological demonstrations require a combined use of animal models and clinical or cellular data derived from human. So far, no experimental approach allows to compare easily the transcriptome from different animals using one unique experimental set up. This leads to the development and validation of one microarray for each organism, which represents a difficult and expensive task. We propose a cost-effective strategy, with the production of a “mammalian microarray”. This microarray corresponds in fact to a human microarray, where cDNA probes have been selected in order to be specific for one unique human transcript, and to share a minimal identity of 80% with the mouse orthologue (average=88%±13). Since the clade that comprises mouse and human also comprises most of the placental mammalians, the resulting microarray can therefore be used in most animal models. The production of 4992 probes has been checked by sequencing, and the production strategy was successful for 4186 cDNAs (84% of total). Probes have an average length of 229bp±36, and a mean GC content equal to 45%. The production of 2200 additional probes is currently under work, after a selection of genes of interest by end users. The first results obtained with human and non human samples indicate the good sensitivity of the microarray. These results will be described.
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CNRS/UNSA UMR 6097

S13-5

REGULATION OF THE EPITHELIAL SODIUM CHANNEL (ENAC)
Korbmacher C.

The amiloride-sensitive epithelial Na^+ channel (ENaC) is the rate-limiting step for sodium absorption in a variety of epithelia including the renal collecting duct. The appropriate regulation of this channel is essential for the maintenance of renal sodium balance and hence for long-term regulation of arterial blood pressure. ENaC channel activity and its surface expression appear to be controlled and modified by a range of regulatory proteins including the ubiquitin-protein ligases Nedd4 and Nedd4-2, the serum and glucocorticoid-inducible kinase SGK1, and the cystic fibrosis transmembrane conductance regulator (CFTR). However, the complex interdependence and relative importance of these regulatory interactions are not yet fully understood. ENaC consists of alpha, beta, and gamma subunits and the carboxyl terminus of each ENaC subunit contains a PPxY motif which is believed to be important for interaction with the WW domains of the ubiquitin-protein ligases, Nedd4 and Nedd4-2. Disruption of this interaction, as in Liddle’s syndrome where mutations delete or alter the PPxY motif of either the beta or gamma subunits, has been shown to result in increased ENaC activity and arterial hypertension. We have recently reported that N4WBP5A, a novel Nedd4/Nedd4-2 binding protein, is a potential regulator of ENaC (Konstas et al. J. Biol. Chem. 277: 29406-29416, 2002). In Xenopus laevis oocytes N4WBP5A increases surface expression of ENaC by reducing the rate of ENaC retrieval. Furthermore, N4WBP5A prevents sodium feedback inhibition of ENaC possibly by interfering with the xNedd4-2 mediated regulation of ENaC. As N4WBP5A binds Nedd4/Nedd4 2 via PY motif/WW domain interactions and appears to be associated with specific intracellular vesicles, N4WBP5A probably regulates Nedd4/Nedd4-2 availability and trafficking. Since N4WBP5A is highly expressed in native renal collecting duct and other tissues that express ENaC, it is a likely candidate to modulate ENaC function in vivo.

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POSTER SESSION

P13-01

MgATP INDUCED-CONFORMATIONAL CHANGE OF THE C-SUBUNIT OF CYCLIC-AMP DEPENDENT PROTEIN KINASE

Shumei Y., Kestrel M. R.

Cyclic AMP-dependent protein kinase (cAPK) is present in all eukaryotic cells. Multiple studies have suggested that the conformational change of the catalytic (C) subunits of cAPK is critical for the catalytic events of transferring the gamma-phosphate from ATP onto the targeted protein. The present study is focused on investigating the respective roles played by Mg^{2+}, ATP and MgATP complex in the conformational change of the C-subunit. The conformational change of C-subunits was examined by measuring the time-resolved fluorescence anisotropy of the carboxyfluorescein-labeled C subunit (CFC). The observed time-resolved fluorescence anisotropy decays were best fitted by a sum of two exponentials containing a fast and a slow rotational correlation time phiF and phiS. In the absence of MgATP, CFC subunit has the rotational correlation times phiF = 1.8 ± 0.3 ns and phiS = 20.1 ± 0.6 ns, respectively. In the presence of MgATP complex (1 mM Mg^{2+}, 0.4 mM ATP), CFC subunit has the rotational correlation times phiF = 1.0 ± 0.2 ns and phiS = 12.8 ± 0.3 ns. The reductions in the rotational correlation times indicate that CFC subunit has adopted a more compact shape upon the formation of CFC ·ATPMg complex. Neither Mg^{2+} (1-3 mM) nor ATP (0.4 mM) alone induced the same conformational change of the CFC subunit as the MgATP complex did. The effect of MgATP on the CFC subunits can be removed by adding 10 mM EDTA. In addition, cAPK inhibitor peptide IP20 alone did not induce significant conformational change of the CFC subunit. We conclude that the binding of the MgATP complex to the C-subunit plays a key role in inducing the C-subunit to a proper stereochemical alignment for the substrates so that the following phosphorylation can be done. (supported by an award from Research Corporation)

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P13-02

NONLINEAR MULTIFUNCTIONAL LASER MICROSCOPY (LSM,FCS,MCS,SMD) OF SINGLE FLUORESCING BIOMOLECULES

Opitz N., Kahms M., Oeke B., Kuhlmann J.

Characterization of individual molecular features of single biomolecules in biochemical reactions is of growing importance in molecular physiology due to the increasing impact of individual molecular features in cellular signal transduction and other biomolecular interactions. Thanks to the cutting edge sensitivity of optical detectors and photon counting detection schemes single molecule techniques comprise particularly optical methods such as TCSPC (time correlated single photon counting), FCS (fluorescence correlation spectroscopy) as well as imaging techniques like CLSM (confocal laser scanning
microscopy) and nonlinear laser scanning microscopy (MP-LSM) based on multiphoton excitation processes using, for instance, ultrafast mode-locked titanium-saphire-lasers (Ti-Sap-Laser) with short pulse widths (about 150 femtoseconds) and high repetition rates (ca. 100 MHz). Utilizing highly focused laser beams along with either confocally imaged or multiphoton excited subfemtoliter volume elements we demonstrate here the feasibility of a commercial LSM (Biorad MRC 1024 extended for FCS and MCS measurements) to image and autocorrelate single fluorescent biomolecules eGFP-GST) with high spatial resolution and, simultaneously, to monitor photon bursts of single fluorophores (traversing the open, optically confined volume element) with high temporal resolution using a multichannel scaler (MCS,bin width 0.41 ms) in conjunction with the FCS technique. First investigations demonstrate the unique opportunities of multi-modality nonlinear laser microscopy for probing complex biological systems at the single protein level. In our actual approach we utilize our setup to analyze GFP-tagged membrane anchored proteins in cellular plasma membranes.

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P13-03

ENLIGHTENING CELL BIOLOGY : PARTICULAR ADVANTAGES OF NONLINEAR LASER MICROSCOPY WITH QUANTUMDOTS

Opitz N., Kahms M., Oeke B., Kuhlmann J.

Fluorescing semiconductor nanocrystals (quantumdots (QDs)) are of particular importance in both cellular and molecular physiology due to their outstanding optical and biological features such as brightness, photostability, and biocompatibility. However, despite the fascinating results of biological applications recently reported, these investigations are up to now all restricted to the one-photon excitation regime. Hence, we performed first
nonlinear-microscopy based experiments (using an ultrafast mode-locked Ti-Sap-laser with high repetition rate (76MHz) and short pulse width (ca. 150 fs)) in order to explore the potential of QDs in conjunction with different microscopical techniques like fluorescence correlation spectroscopy (FCS), multi-channel scaling (MCS) and spatially-resolved intracellular imaging (LSM). Using an excitation wavelength range from about 760 nm to 840 nm we find exceptionally high two-photon absorption cross-sections and fluorescence quantum yields resulting in FCS count rates of up to 340 kHz per single quantumdot (which is about a factor of 30 higher than with conventional fluorophores). As a consequence, highly diluted solutions of quantumdots can still be analyzed with high temporal
resolution resulting in few photon bursts of single quantumdots traversing the open, optically-confined 2hv-excitation volume. From the corresponding autocorrelation curve an amplitude of about 60 can be derived, which may be interpreted such that a single quantumdot is measured only about 2% of the overall measuring time. These results
and additional optical features suggest a particular 2hv-advantage of quantumdots for dual color fluorescence cross-correlation measurements. Finally, first investigations concerning intracellular nonlinear imaging of QDs inspire for real-time observation of molecular trafficking within single living cells due to their exceptional brightness
and photostability.

Max-Planck-Inst. for Molecular Physiology – Dortmund - GERMANY

P13-04

RFA-NEUROPEPTIDES DISCERN ACID-SENSING CHANNELS OF NOCICEPTIVE VS. MECHANOCEPTIVE SENSORY NEURONS

Ostrovskaya O., Moroz L., Krishtal O.

Several subtypes of acid-sensing ionic channels (ASICs) are expressed in mammalian sensory neurons. These mechanisms are thought to play a prominent role in nociception and mechanoreception. It is known that the diameters of somata of DRG neurons correlate with diameters of the nerve fibers.
Sensory neurons were acutely isolated from rat DRG and studied in conditions of whole-cell patch clamp.
Our results are as follows:
1. The steady-state desensitization was measured in 35 large (35-50 mcm) and small (10-20 mcm) neurons. The data for large and small cells fit curves with different pK values: pK 7.21±0.01 for small cells, 7.11±0.01 for large cells, Hill slope for both curves was 8±2. At pH 7.15, the response of a large cell is 70% of the control, while in the case of a small cell it is only 24%.
2. In the small (primarily nociceptive) but not in large (primarily mechanoceptive) sensory neurons peptide KNFLRFa in concentration of 50 mcM shifts the desensitization curve in 0.06 units to acidic direction (pKa 7.15±0.01). The higher concentration of peptide results in more significant shift of the desensitization. For FIRFa 200mcM IpH7.17/IpH7.4 = 0.79±0.02. The shift is not linked specifically to the diameter of the cell, but to a mechanism expressed.
Our results show that the RFa-related peptides are capable of changing the sensitivity of nociceptors to protons, as well as the temporal pattern of their activity. RFa-peptides have been found in mammals and may play a role in modulating sensory input.

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P13-05

FUNCTIONAL CHARACTERIZATION OF HUMAN RHAG GLYCOPROTEIN AS AN AMMONIUM TRANSPORTER IN HELA CELLS

Benjelloun F., Bakouh N., Hulin P., Thomas S.R., Fritsch J., Edelman A., Planelles G., Cherif-Zahar B.

RhAG glycoprotein is expressed in the red cell membrane and belongs to the Rh protein family that shares sequence similarity with Mep/Amt ammonium transporters. RhAG was found to complement the growth defect of a yeast lacking ammonium transporters and to mediate uptake of methylammonium when expressed in Xenopus laevis oocytes. We tested whether RhAG

transports NH_4^+/NH_3. To this end, HeLa cells were transiently transfected with GFP-RhAG cDNA (HeLaGFP-RhAG) or with GFP cDNA (controls). We investigated the 10mM NH_4Cl-induced change of intracellular pH (pHi), by using the BCECF proton probe. The results were as follows: (i) pHi increased immediately after exposure of cells to the NH_4Cl-containing solution with no significant difference between HeLaGFP-RhAG cells (DpHi = 0.45±0.05) and controls (DpHi = 0.43±0.07). This increase (alkalinization) was due to the entry of NH_3 and its subsequent combination with H^+. (ii) Alkalinization was followed by a ;plateau-phase; decrease (acidification), that was significantly more pronounced in HeLaGFP-RhAG cells than in controls (DpHi = 0.29±0.02* vs 0.12±0.01). The acidification was consistent with a secondary entry of NH_4^+ which partially dissociates to form NH_3 and H^+. (iii) Upon removal of NH_4Cl, there was a pHi undershoot below its initial value, attributed to previous NH_4^+ entry. This undershoot was significantly larger in HeLaGFP-RhAG cells than in controls (DpHi = 0.48±0.02* vs 0.24±0.01). Total removal of extracellular Na^+ ions or addition of bumetanide (250µM), ouabaine (1mM), and BaCl2 (2,5mM) did not abolish the undershoot acidification in HeLaGFP-RhAG cells. The results were successfully simulated by a mathematical model, including only simple membrane diffusion of NH_4^+ and NH_3 (RhAG-dependent) and a parallel unspecified pH regulatory mechanism. Moreover, preliminary results from patch-clamp experiments suggest that the RhAG-dependent increase of NH_4^+ permeability occurs by an electrogenic mechanism.

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P13-06

FONCTIONAL EXPRESSION OF RHCG IN XENOPUS LEAVIS OOCYTE

Bakouh N., Benjelloun F., Hullin P., Edelman A., Cherif-Zahar B., Planelles G.

The human glycosylated protein RhCG (also known as RhGK) is predominantly expressed in the kidney. RhCG shares homologies with AMT and Mep, the ammonium transporters from plants, yeast and bacteria. A functional role for RhCG in ammonium transport is supported by growth of delta Mep yeasts, after being transfected with RhCG. These findings raise the hypothesis that RhCG is involved in ammonium transport in the kidney.
The aim of our study was to functionally express RhCG, and to check whether it is involved in ammonium transport. Methods: Defolliculated Xenopus laevis oocytes (Stage V-VI) were injected with 10 ng of cRNA corresponding to the GFP-RhCG fusion protein, or injected with water. Three to five days after injection, NH_4Cl-induced currents were measured using the 2-electrode voltage-clamp (Vc) technique, and changes in intracellular pH were monitored using pH-sensitive microelectrodes. Results: in RhCG-expressing oocytes (Vc= -50 mV), increasing NH_4Cl concentrations induced increasing inward currents; half-maximal current was reached for [NH_4Cl] = 0.55 mM. The current-voltage relationship (Vc= -90 to 0 mV) showed that NH_4Cl-induced currents were enhanced with voltage negativity, consistent with the net influx of positive charges into the cell. NH_4^+ influx into RhCG-expressing oocytes was further supported by the slight intracellular acidification observed in the presence of 500 µM NH_4Cl. Ammonium-induced currents were unchanged in Na^+- or K^+-free solutions. Currents induced by 500 µM NH_4Cl were not mimicked by 500 µM NaCl, KCl, cholineCl, or methylamineCl (MeACl). Increasing MeACl to 1 mM induced only a slight inward current, suggesting that the ionic transport system related to RhCG expression is more selective to NH_4^+ than to other amines. Conclusion: Our results are consistent with an enhanced transport of ammonium ions in RhCG-expressing oocytes. The physiological role of RhCG in renal physiology needs to be further investigated.

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P13-07

PHARMACOLOGICAL CHARACTERISATION OF RECENTLY DEVELOPED a7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONISTS

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The alpha 7 (a7) nicotinic receptor is the second most abundant nicotinic receptor in the brain and has been implicated in a number of psychiatric and neurological disorders.
We have synthesised several compounds, recently reported as selective a7 agonists by Astra Zeneca including (R)-(+)-5'-phenylspiro[1-azabicyclo [2.2.2] octane-3,3'(3'H)-furo [2,3-b] pyridine] – patent application WO99/03859 and (R)-N-(1-azabicyclo [2.2.2] oct-3-yl)(5-(2-pyridyl)thiopene-2-carboxamide) patent application WO01/60821A1), here referred to as compounds A and B respectively.
Using two-electrode voltage clamp (TEVC) of Xenopus oocytes we profiled the two compounds, for their agonistic activity on human a7 receptors and their selectivity over other nAChRs.
Both A and B were potent and efficacious agonists of ha7 receptors expressed in Xenopus oocytes (EC50 2.2±2.4mM and 0.95±0.85mM, n=3; Emax 83±6 and 70.7±6.6, n=3). All compounds had no activity on other nicotinic receptor subtypes. However, compound A (but not B) was also a potent agonist of the highly homologous human 5HT3R expressed in oocytes (Emax 66.6±6%, EC50 1.4±0.4mM, n=3)
Both A and B were more potent in desensitising a7 receptors than in activating them, as previously reported for other a7 receptor agonists (IC50 1.1±0.2nM and 0.58±0.3nM, n=3) (Briggs et al., 1998).
A and B were tested on rat native a7 receptors. Rapid and focal application of compounds induced fast activating and fast desensitising somatic currents in cultured hippocampal neurones, and modulated GABA and Glu release in hippocampal cultures and cerebellar slices, (patch-clamp experiments). However, both compounds caused a robust and long lasting desensitisation on longer applications.
These results confirm the feasibility of developing highly selective a7 receptor agonists. The desensitisation profile seen, however, could profoundly limit the efficacy of these compounds in vivo.

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P13-08

MUTAGENESIS STUDIES OF TRANSMEMBRANE SEGMENT IV OF THE MAMMALIAN Na^+/H^+ EXCHANGER ISOFORM 1

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The mammalian Na^+/H^+ exchanger isoform 1 (NHE1) is a ubiquitously expressed integral membrane protein that functions to remove one intracellular proton in exchange for one extracellular sodium ion. Although little is known about the overall mechanism of NHE1 function, several residues in transmembrane segment four (TM IV) of NHE1 have been implicated in ion binding and transport. The purpose of our study was to further investigate the importance of TM IV and to characterize the individual residues in this segment that are required for normal NHE1 function. We used site directed mutagenesis to individually mutate 23 residues in TM IV to cysteine, using the fully active cysteineless NHE1 protein (cNHE1) as the background. We subsequently determined the activities of the mutated NHE1 proteins by measuring intracellular pH changes in stably transfected cells that lack an endogenous Na^+/H^+ exchanger. Of the single cysteine mutants, F155C, F161C, L165C, I169C, L171C, A173C, G174C, and L177C had impaired activity (20-60% of cNHE1 activity) while S158C, F162C, F164C, P167C, P168C, D172C, Y175C, and F176C were inactive (< 20% of cNHE1 activity). Proline residues are known to increase the flexibility within alpha helices and to allow for the availability of free backbone carbonyls that can interact with transported cations. We further investigated the importance of P167 and P168 by mutating these residues to either glycine or alanine in wild-type NHE1. Each of these mutants was also inactive (< 20% of NHE1 activity) regardless of whether the alpha helix promoting alanine or the alpha helix breaking glycine was present. Our results further establish the importance of TM IV in NHE1 activity and suggest that TM IV is in close proximity to the ion transport pore in a very specific conformation. Future studies will use sulfhydryl-reactive reagents to determine the specific pore lining residues of NHE1. Supported by the Canadian Institute of Health Research.

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P13-09

PROTEIN PHOSPHATASE-1 PLAYS A KEY ROLE IN THE REGULATION OF THE Na^+/H^+ EXCHANGER ISOFORM-1

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The Na^+/H^+ exchanger isoform-1(NHE1) is a ubiquitous plasma membrane protein essential for regulating intracellular pH in eukaryotic cells. It removes one intracellular proton in exchange for one extracellular sodium

ion. Hormones such as thrombin, stimulate NHE1 leading to phosphorylation. Reversible phosphorylation, mediated by protein phosphatases, is essential in eukaryotic cells. Regulatory proteins, toxins or inhibitors regulate phosphatase activity. The objective of this study is to characterize phosphatases involved in NHE1 regulation. We examined dephosphorylation of the regulatory C-terminal region of the NHE1 using cardiac myocytes, heart cell extracts and purified phosphatase proteins. Treatment of isolated cardiac myocytes with the toxin, okadaic acid (10 mM), did not affect activity of NHE1. This resistance to inhibition by okadaic acid suggested the phosphatases involved are likely protein phosphatase-1(PP1) or 2B (calcineurin). A C-terminal fusion protein of the last 178 amino acids of NHE1 was phosphorylated in vitro using heart cell extracts. PP1 completely dephosphorylated NHE1 while PP2B lacked this ability. We examined the ability of PP1 and 2B to bind to the NHE1 C-terminus. Cells overexpressing NHE1 were treated with crosslinking reagents followed by immunoprecipitation and Western blot analysis. Native PP1 interacted with NHE1 while PP2B did not show any interaction. To investigate the effects of PP1 on NHE1 in vivo we used cells overexpressing Inhibitor-2, a potent PP1 inhibitor. The rate of recovery from an acid load was significantly stimulated in cells expressing Inhibitor-2. Thrombin stimulated NHE1 activity in acid loaded cells, but did not stimulate this activity in cells expressing Inhibitor-2. The results suggest that dephosphorylation of the NHE1 isoform of the Na^+/H^+ exchanger is mediated at least in part, by PP1. *Supported by Heart and Stroke Foundation of Canada and CIHR.

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P13-10

ANGIOTENSIN II STIMULATES Na^+/K^+ATPASE ACTIVITY THROUGH PKC-ZETA IN MCF-7 BREAST CANCER CELLS
Muscella A., Greco S., Elia M.G., Storelli C., Marsigliante S.

The activity of the Na^+/K^+ATPase expressed by human breast cancer cells has a pivotal role in cell functioning and in the regulation of cell growth. In various cell types the Na^+/K^+ATPase activity is modulated by different isoforms of the protein kinase C (PKC). In previous studies, we demonstrated that in breast cancer MCF-7 cells Ang II stimulation of its AT1 receptor subtype increased the Na^+/K^+ATPase activity and activated the atypical PKC-zeta without affecting the intracellular calcium concentration. Here we examined whether the Ang II-activated PKC-zeta is responsible for the activation of Na^+/K^+ATPase activity in MCF-7 cells. Data that support an essential role for PKC-zeta in Ang II-mediated signalling are the following: a) the specific inhibition of PKC-zeta by a synthetic myristoylated peptide with sequences based on the endogenous PKC-zeta pseudosubstrate region blocked the Ang II-stimulation of Na^+/K^+ATPase activity; b) the Ang II-induced Na^+/K^+ATPase activity is blocked by 10 μM staurosporine; c) the Ang II-mediated Na^+/K^+ATPase activation was unaffected by PKC down-regulation after PMA treatment. To identify the mechanism by which Ang II activates PKC-zeta, we focused on interactions with phosphatidylinositol 3-kinase (PI3K), since it is known to regulate PKC-zeta in other cells: wortmannin and LY294002, inhibitors of PI3K, did not block the cytosol-to-membrane translocation of PKC-zeta nor the activation of Na^+/K^+ATPase. We showed previously that Ang II stimulated ERK1/2 via PKC-zeta in MCF-7; thus, in order to determine whether the MAPK pathway is involved in modulation of Na^+/K^+ATPase activity, the MEK1 inhibitor PD098059 was used. PD098059 inhibited the ERK1/2 phosphorylation induced by Ang II, but it failed to block the effects on Na^+/K^+ATPase activity. In conclusion, the results of the present study strongly support a role for PKC-zeta in Ang II stimulation of the Na^+/K^+ATPase activity in MCF-7 without PI3K/ERK1/2 involvement.

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P13-11

ENGINEERING OF A CYSTIC FIBROSIS MOUSE MODEL BY SPECIFIC CFTR GENE TARGETING IN THE LUNG
Bertin G., Rubera I., Poujeol C., Hasseine L., Poujeol P., Tauc M.

Cystic Fibrosis (CF) is the most common lethal disease among Caucasians caused by mutations in CFTR gene (Cystic Fibrosis Transmembrane Conductance Regulator). CFTR is known as a chloride channel localized in the apical membrane of various epithelial tissues including lung. In human beings, mortality is mainly due to severe inflammatory and infectious attacks of the lung. Thus, obtaining a mouse model mimicking this disease could be helpful. Several cftr KO mouse have been obtained but their extreme fragility prevents any physiological studies allowing for a therapeutic improvement of these phenomenon. To overcome this problem, we have decided to create a transgenic mouse with a lung specific invalidation of cftr using CRE/LoxP system. To inactivate CFTR we used the murine promoter CCSP (Clara Cell Secretory Protein) to direct the expression of Cre recombinasein Clara cells that express CFTR in the airway epithelium. Firstly, 2,2 kilobases of the murine promoter was cloned upstream of Cre gene and microinjected in mouse fertilized eggs pronuclei. 3 transgenic mouse lines have been established. One of them which expresses Cre in the lung is under extensive characterization using immunohistochemistry studies and mating with Rosa26-Lox/STOP/lox-lacZ reporter mouse. A second transgenic mouse strain is under creation by homologous recombination in ES cells. 8 kilobases of the CFTR gene have been cloned and two loxP sites were added to flank CFTR gene exon 11 together with Thymidine kinase and NeomycineR selection genes. Two frt sites were also added in order to remove NeomycineR selection gene after selection in ES cells. The inactivation of CFTR in the lung will be obtained by mating this two different mouse lines. Such a model will be useful for the study of infectious and inflammatory processes of CF disease and could help in therapeutic studies. Moreover, floxed cftr mouse strain could be used to invalidate cftr in other tissues such as intestine, pancreas or kidney.

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P13-12

ACTION OF L703,606 ON IONIC CURRENTS IN AIRWAYS
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The effect of C-fiber ending on functions of the airways was extensively studied. The hyperpolarization of the airways wall during mechanical stimulation was characterized in details and involvement of neurokinins: substance P, NKA, NKB and its receptors was evidenced for the reactions. The aim of the study was to define the action of NK-1 antagonist (L703,606) on airways ion currents.
The experimental model was isolated rabbit tracheal wall mounted in Ussing apparatus. The mechanical stimulation of C-fiber endings was by gentle rinsing of mucosal surface of the trachea by jet flux from peristaltic pump. The 21 specimens of isolated tracheal walls from 7 rabbits were investigated. Every significant reaction was repeated at least ten times. In the smallest applied concentraction of NK-1 antagonist (10-8 M) the hyperpolarization was augmented but in higher concentrations (up to 10-6 M) the reaction was diminished.
It was hypothetized that the inhibitory action of L703,606 resulted from blocked epithelial tachykinins receptors, but stimulatory action of the drug resulted from blocked neuronal receptors located on sensory endings and acting as inhibitory autoreceptor.

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P13-13

NADPH-OXIDASE RELATED PROTON AND ELECTRON CURRENTS IN INSIDE-OUT PATCHES FROM HUMAN EOSINOPHILS
Petheö G. L., Maturana A., Demaurex N.

The phagocytic NADPH-oxidase is an enzyme complex which assembles at the plasma membrane to generate superoxide by transferring e- from cytosolic NADPH to external oxygen. Sustained function of the oxidase requires H^+ extrusion through voltage-gated, outwardly rectifying H^+ channels, but it is not clear whether protons flow through the oxidase itself or through a distinct channel protein. The H^+ channel and oxidase functions are closely connected, as activation of the oxidase evokes profound changes in whole-cell proton current (Ip) characteristics, causing a ~ -40 mV shift in activation threshold that leads to the appearance of inward Ip. To further explore the relationship between the two functions, we performed voltage-clamp experiments on inside-out patches from both PMA (phorbol myristate acetate) activated and untreated eosinophils. Proton currents from untreated cells displayed slow voltage-dependent activation, and moderate or no run-down during prolonged recordings. Proton currents from PMA-treated cells activated faster and at much lower voltages, but drastic run-down was observed. After run-down was complete the remaining Ip shared all characteristics of the current from non-activated cells. Bath application of

NADPH to activated patches evoked e- current (Ie) which progressively ran down and was blocked by diphenyleneiodonium (DPI). Run-down of both Ip and Ie was delayed by ATP and GTP-gamma-S, applied from the cytosolic side. A good correlation was found between the amplitude of inward Ip, measured just before NADPH addition, and the amplitude of Ie, measured just after NADPH addition. Furthermore, bath application of NADPH and/or DPI reduced the amplitude of the inward Ip. Our data suggest that rapid modulation of the oxidase has a direct impact of H^+ channel activity, consistent with the oxidase acting as a H^+ channel or with a protein-protein interaction between channel and oxidase proteins with strict stoichiometry.

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P13-14

KIDNEY SPECIFIC GENE TARGETING OF CFTR USING CRE-LOXP STRATEGY
Hasseine L., Rubera I., Poujeol C., Bertin G., Poujeol P., Tauc M.

CFTR forms a Cl^- channel, the defect of which causes abnormal epithelial electrolyte transport in cystic fibrosis. CFTR is expressed in a variety of epithelia including kidney. This protein is known to be implicated in the control of ionic channels or transporters present in renal tubules but up to now, its role in renal function is not well understood. Thus, to elucidate the physiological function of CFTR in the kidney we have decided to engineer a mouse model by creating a tissue-specific knock-out of CFTR in the kidney by using the Cre-loxP strategy. To target gene deletion specifically in the distal part of the nephron (that functionally express CFTR), we have generated a first transgenic mouse line carrying the Cre recombinase gene under the control of the mouse renal type 2 vasopressin receptor (V2R) promoter. Murine promoter was cloned by homology using primers annealing to the rat promoter sequence. Ten founders were obtained after microinjection in mouse fertilized eggs pronuclei of the 1200 bp promoter inserted upstream of the Cre gene sequence. Extensive characterization using immunohistochemistry studies and mating with Rosa26-Lox/STOP/lox-lacZ reporter mouse revealed that one strain line (pVR2-CRE-L5) functionally expressed Cre only in kidney and spleen.
A second mouse strain which contains a conditional floxed allele at the CFTR gene locus (exon 11 flanked by 2 loxP sites together with Thymidine kinase and NeomycineR selection genes) is under creation by homologous recombination in ES cells. Two frt sites were also added in order to remove NeomycineR selection gene after selection in ES cells.
Finally, kidney-specific invalidation of CFTR will be achieved by mating the two mice strains. This will lead to a better understanding of the importance of CFTR in renal physiology and its implication in renal disease emerging in the older cystic fibrosis patients.

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P13-15

GENERATION OF A TRANSGENIC MOUSE EXPRESSING CRE RECOMBINASE UNDER THE CONTROL OF SGLT2 PROMOTER
Rubera I., Hasseine L., Bertin G., Poujeol C., Poujeol P., Tauc M.

The development of conditional gene targeting techniques is of particular importance in the study of the physiology of renal ionic transporting systems expressed along the mammalian nephron. Generation of a kidney cell type- or nephron segment-specific knock out of transporting proteins requires interbreeding of two lines of mice : one strain engineered using gene targeting strategies, that contains a loxP -flanked target gene of interest and a second transgenic line expressing Cre recombinase under the control of a nephron segment-specific promoter. Mating of these mice may result in the invalidation of target proteins only in a specific renal segment.
With the view of directing the expression of Cre recombinase in vivo specifically in the renal proximal tubule, the promoter of the sodium-dependent glucose transporter SGLT2 has been used. By RT-PCR we have confirmed the kidney-specific expression of sglt2 and its expression in primary cultures of proximal tubules. Thus, 1959 bp of the mouse sglt2 5'-flanking region have been cloned upstream the Cre gene sequence and microinjected into pronuclei of fertilized ovocytes. Three transgenic mice lines expressing the sglt2 promoter-Cre transgene were generated. One of them (iL1-sglt2Cre) is under characterization. RT-PCR analysis showed specific expression of Cre in the kidney; no signal was detected in other tissues such as colon, lung, liver, heart and brain. To test for Cre activity, iL1-sglt2Cre mice were bred to ROSA26 lox-stop-lox reporter mice; Xgal staining on kidney slices revealed Cre-mediated recombination in renal tubules. At present, further experiments are carried on to determine the specific pattern of Cre expression in the nephron segments.
Finally, proximal tubule-specific gene targeting may greatly improved our knowledge of the physiological function of different ionic transporters or channels expressed in this specific segment.

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P13-16

COMPOSITION OF AIRWAY-SURFACE LIQUID AND NASAL FLUID DETERMINED BY X-RAY MICROANALYSIS
Vanthanouvong V., Kozlova I., Almgren B., Högman M., Roomans GM.

The composition of airway-surface liquid (ASL) has been a matter of dispute. The elemental composition of the ASL in pig trachea and principal bronchi was determined by X-ray microanalysis, using two different methods. One method was to analyze the ASL in situ in the frozen state in a scanning electron microscope, with the electron beam perpendicular to the surface. Results indicated a near-isotonic composition of the ASL, but with values for P and K much higher than expected for extracellular fluid. The second method was to let Sephadex G-25 ion-exchange beads equilibrate with the ASL in a moisture chamber. The beads were rinsed in silicon oil to remove excess ASL and dried. Results indicated that the concentrations of Na and Cl in ASL are close to those in serum (Na = 135 mM, Cl = 92 mM), but that the K concentration in the ASL is nearly 5 times that in serum (K = 20 mM). It is concluded that ASL in the lower airways is close to isotonic but with higher K than in serum. The first technique samples the mucus layer of the ASL, which may contain cells and debris, the second method samples the watery component of the ASL. Nasal fluid is an easily accessible form of ASL. Nasal fluid was collected from the inferior turbinate with a micropipette after occlusion of a nostril for 5-10 minutes. Ion concentrations in nasal fluid were (in mM): Na: 127, Cl 140, K 27, and Ca 5. This sampling method proved difficult to apply to cystic fibrosis (CF) patients because of their viscous nasal secretion. Sephadex G-25 ion exchange beads were mounted on double-sided tape, stuck on a filter paper as support. The filter paper with beads was applied for 10 min to the occluded nostril of a subject. After removal of the filter paper with the beads from the nostril, the beads were rinsed with silicon oil to remove excess nasal fluid, dried, and analyzed. This method of collection is not cumbersome for the subject and gives results similar to those obtained by the direct collection method.

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P13-17

IMMEDIATE ACTION OF TOLUENE DIISOCYANATE (TDI) ON AIRWAY ELECTRICAL POTENTIAL DIFFERENCE
Soczywko-Ciudzinska J., Jakubaszko J., Paciorek P., Mlodzik N., Kaczorowski P., Lelinska A., Tyrakowski T.

The toluene diisocyanate caused irritant-induced occupational asthma (IIOA) is suggested to be elicited, at least partially, by disturbed function of C-fibers endings. It was also shown in other studies that neuropeptides liberated from C-fibers influenced transepithelial ion transport.
The experimental model was isolated rabbit tracheal wall mounted in Ussing apparatus. The mechanical stimulation by means of gentle rinsing of sensory receptors of mucosal surface of isolated trachea by jet flux from peristaltic pump. The 45 specimens of isolated tracheal walls from 15 rabbits were investigated. Every significant reaction was repeated at last ten times.
TDI in concentration 0.035 mM (and also in other concentrations) influenced this hyperpolarization. Experiments with inhibitors of transepithelial ion transport revealed that amiloride (0.1 mM) and bumetanide (0,1 mM) applied separately or in combination changed the extent and time course of the hyperpolarization and these experiments made possible the evaluation of TDI action on separate ion transport pathway.
Conclusions: The immediate action of TDI on airway walls caused disturbances in these ion transport processes which took part in hyperpolarization after mechanical stimulation. As the transepithelial ion transport is important determinate of airway fluid lining and as such could influence dyspnea so these changes should be taken into consideration in pathophysiological mechanism of IIOA.

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P13-18

EXPRESSION AND PURIFICATION OF THE HUMAN Na^+/I^- SYMPORTEUR (HNIS)

Basquin C., Darrouzet E., Bellanger L., Marcellin D., Leblanc G., Pourcher T.

Iodine is an essential constituent of thyroid hormones. The Na^+/I^- symporter NIS (for Natrium Iodide Symporter) catalyses the active transport of I- into the thyroid follicular cells and therefore plays a key role in thyroid function. The protein is a member of the superfamily of sodium solute symporters which use the favorable electrochemical gradient for Na^+ to drive I- uptake. The human NIS is an integral membrane protein of 643 amino acids. Its currently proposed secondary structure and topology suggest a 13 transmembrane helices model with the amino terminus localized on the extracellular side and the carboxy terminus on the intracellular side. Numerous studies have been undertaken to analyze NIS function and regulation. However, its biochemical and structural properties are still not well characterized. In order to perform biochemical studies to obtain information about NIS functioning and structure we are developing strategies to produce and purify functional hNIS. Tagged protein has been expressed in yeast and mammalian cells. Subsequently a purification protocol based on affinity chromatography allowed the isolation of hNIS protein. Purified hNIS has been reconstituted in liposomes that are currently being used for iodine flux experiments. Expression level and purification yield remain to be optimized.

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P13-19

POST-TRANSLATIONAL REGULATION OF THE SODIUM/IODIDE SYMPORTER BY PKC

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The Na^+/I^- symporter (NIS for Natrium Iodide Symporter) is a key membrane glycoprotein that mediates active iodide transport into the thyroid follicular cells, the first step in thyroid hormone biosynthesis. NIS is localized in the basolateral membrane of thyrocytes. It has been shown that NIS transcription is regulated by TSH (Thyroid Stimulating Hormone). More recently, evidence for post-transcriptional regulation of NIS function by TSH has been provided. In particular, it has been shown that TSH is required for NIS targeting to or retention in the plasma membrane. These regulatory mechanisms may be altered in thyroid cancers in which NIS is predominantly localized in intracellular compartments leading to decreased iodide uptake. It has been shown that TSH modulates NIS phosphorylation. To date, it is not known which of its multiple phosphorylation consensus sites are targetted. Therefore, we examined the possible post-translational regulation of NIS by different kinases, in particular by PKC. The intracellular distribution of expressed NIS carrying amino acid substitutions at the different PKC consensus sites was studied by immunohistochemistry. We observed that NIS mutant proteins T274A and T548A were localized mainly within the cells, in the perinuclear region. In parallel, 125Iodide uptake was measured in cells expressing these mutants. When compared to control cells (i.e. transfected with non mutated NIS), a two fold decrease in iodide uptake was observed. We suggest that phosphorylation of NIS by PKC may lead to proper localization of the protein in the plasma membrane.

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P13-21

LINEAR MYOCARDIAL AP DURATION = F(RR INTERVAL) RELATION IS MAINTAINED DURING BRAIN DEATH IN PIGS

Christe G., Hadour G., Ferrera R.

Whether the heart of brain-dead donors for transplantation has undergone ischaemic insult is debated. To delineate ischemia-induced changes from a pure frequency effect, we investigated the relation of epicardial monophasic action potential (MAP) duration to the RR interval before and after brain death and under transient ischemia followed by reperfusion.

In open-chest pigs, epicardial MAPs were monitored near the apex of the left ventricle by a suction electrode with two concentric AgCl-Ag electrodes, and sampled at 1 KHz using a MacLab interface and the CHART software (AD Instruments). The data from 10 consecutive MAPs acquired during a period of stable rhythm for at least 3 min were analysed under MATLAB for dV/dtmax, MAP duration at 20% (APD20), 50% and 90% repolarization from plateau level. Time zero was taken as time of maximum dV/dt during MAP upstroke. Brain death was induced (water balloon procedure) in 9 pigs (BD group) or a sham procedure applied in 5 other pigs (SHAM group). In 4 pigs of each group, an additional period of 20 min of ligature of the anterior coronary artery was followed by 60 min reperfusion.

In both BD and SHAM pigs, APD20 was stable during 3 hours and was significantly shortened by more than 60% ($p<0.01$) under ischemia. APD20 returned to control after 60 min reperfusion. APD20 values before ischemia plotted versus RR in BD pigs fall along the same regression line as those for SHAM pigs: APD20 = (0.39±0.02) * RR + (36.6±13.0). The squared regression coefficient was 0.86. Under ischemia, APD20 values for SHAM or BD pigs were no longer correlated to RR values.

The duration of the left ventricular action potential of pigs linearily follows the RR interval with a slope of 0.40. Changes in this duration induced by BD are accounted for by shortened RR intervals. Thus, no ischemic episode is likely to have taken place within 3 hours after brain death in pigs.

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P13-22

D-GLUCOSE TRANSPORT IN RAINBOW TROUT AND LAMPREY HEPATOCYTES, AND IN RTH-149 CELL LINE

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In the present study, glucose transport into rainbow trout (Oncorhynchus mykiss) and river lamprey (Lampetra fluviatilis) hepatocytes was studied. Rainbow trout hepatoblastoma cell line, RTH-149 was also used to evaluate whether it could be used as an alternative tool in studying glucose utilization in fish.

The rainbow trout and lamprey hepatocytes were isolated by collagenase treatment. RTH-149 cells were grown in MEM supplied with 10 % FBS in an incubator containing a humidified atmosphere with 5 % CO_2 at 22 ºC. The kinetics of D-glucose and its non-metabolized analog, 3-O-methyl-D-glucose (3-OMG) uptake into the cells was studied using tracer methods. The effects of phloretin (1 mM), cytochalasin B (25 µM), ouabain (1 mM), and the absence of sodium ions on the uptake were evaluated. To further characterize glucose uptake, glucose transporters were stained immunohistochemically in the hepatocyte cultures.

The half-time for D-glucose equilibration was 15 s for rainbow trout. The half-times for 3-OMG equilibration were 8, 37 and 38 s for rainbow trout, lamprey and RTH-149 cells respectively. The 3-OMG uptake by rainbow trout hepatocytes was carrier-mediated, showing saturation kinetics with the Km of 37 mM and Vmax of 62 mmol/kg cells/min. The uptake was sensitive to phloretin and cytochalasin B, but was not affected by ouabain. The 3-OMG uptake by lamprey hepatocytes and RTH-149 cells showed no sign of saturation, and was not affected by phloretin, cytochalasin B and ouabain, which suggests passive diffusion. However, immunohistochemical stainings revealed the existence of mammalian type GLUT1 and GLUT2 transporters in all cell cultures studied. The lack of carrier-mediated glucose uptake in lamprey hepatocytes might be due to its physiological state (prespawning starvation). The minor 3-OMG uptake into RTH-149 cells compared to freshly isolated rainbow trout hepatocytes might reflect the low metabolic activity that is common to cell lines.

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S14 CALCIUM SIGNALLING AND NEURONAL GLIAL INTERACTIONS

ORAL SESSION

S14-1

NEURONES AND GLIA IN CROSS-TALK: ROLE OF CHEMICAL AND ELECTRICAL SYNAPSES

Verkhratski A.

Brain function is executed by continuous interaction of two major cellular circuits, neuronal and glial. Communication within these cellular networks is achieved through two main pathways, by release of chemical transmitters and by direct cell-to-cell coupling through electrical synapses. These two mechanisms are present in both types of cells, although their relative importance varies. Neurones mainly rely upon chemical neurotransmission, whereas glial cells are integrated directly via gap junctions. Yet, more and more evidence suggests that chemical transmission is widespread among astroglial cells, and gap junctions may form neuronal-neuronal and glial-neuronal connections. Recent discoveries marked an important change in our comprehension of the functional basis of chemical transmission in the central nervous system: a rapid neurotransmission always believed to be solely restricted to neurone-neurone contacts, has been extended to embrace glial circuits. At the same time data are gathering demonstrating an important contribution of glial-neuronal electric synapses in functional networking within the CNS.
The existence of gap junctions coupling neurones and astroglial cells have been initially suggested by Nedergaard (1994) who observed propagated Ca^{2+} waves between astrocytes and neurones in mixed cultures. This observation was somehow neglected until very recently, when gap junctional coupling between co-cultured embryonic neurones and astrocytes was confirmed by both dye-transfer assay and direct measurement of junctional currents. Direct coupling between astrocytes and neurones was further substantiated by experiments in situ, in brainstem slices. Recently, electrical coupling between Bergmann glial cells (BG) and Purkinje neurones (PN) in acutely isolated cerebellar slices was also demosntrated. Thus the brain now emerges as a complex of chemical and electrical synapses connecting neuronal and glial networks.

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S14-2

Ca^{2+}-DEPENDENT GLUTAMATE RELEASE FROM ASTROCYTES: PHYSIOLOGICAL AND PATHOLOGICAL RELEVANCE

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Astrocytes often ensheath brain synapses with fine processes expressing receptors for neurotransmitters and other mediators. Such astrocyte receptors are in the position of sensing neuronal activity and translating it into intracellular calcium ($[Ca^{2+}]i$) elevations. These, in turn, start local or long-range glial communication, notably by glutamate release. We find that stimulation of G-protein coupled receptors (GPCR) in astrocytes, namely mGluR5 for glutamate, P2Y1 for ATP and CXCR4 for the chemokine SDF-1a, leads to glutamate release via a Ca^{2+}-dependent process selectively inhibited by tetanus neurotoxin and bafilomycin A1, two blockers of neuronal exocytosis. The Ca^{2+}-dependent mechanism coupling GPCR activation to glutamate secretion has peculiar features, as it involves both intracellular and extracellular signalling, the latter mediated by TNFalpha and prostaglandin E2 (PGE2) in sequence.
In hippocampal slices, the astrocyte pathway may function in coordination with pre- and post-synaptic activities, giving rise to functional "tripartite synapses", where astrocytic inputs influence synaptic outputs. In pathological conditions, when the morphological and functional neuron-glial relations are perturbed, the glutamate-releasing pathway of astrocytes may become a cause of neuronal damage. Thus, in conditions where glial cells become "reactive" and microglia migrates in apposition to astrocytes, we find that CXCR4 stimulation is followed by a significantly higher TNFalpha production and, as a consequence, by potentiated astrocyte glutamate release, which eventually leads to excitotoxic neuronal apoptosis. This CXCR4-dependent death cascade can be activated by the HIV-1 coat glycoprotein, gp120IIIB acting as unnatural CXCR4 agonist, and play a role in the pathogenesis of AIDS dementia. Agents interfering with this cascade provide neuroprotection.
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IBCM, Univ. Lausanne, Switzerland & Center of Excellence on Neurodegenerative Diseases, Univ. Milan, Italy

OC14-1

AMYLOID BETA PEPTIDE CAUSES MITOCHONDRIAL MEMBRANE POTENTIAL CHANGES IN RAT ASTROCYTES

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The deposition of beta-amyloid (bA) in the brain is a key pathogenic event in Alzheimer's disease (AD). bA is a neurotoxic polypeptide of 39-43 amino acids, which we have shown previously to promote fluctuations of intracellular calcium concentration in astrocytes but not in neurons in culture. We used digital fluorescence imaging to examine the action of bA on mitochondrial membrane potential(using Rhodamine123) in mixed cultures of glia and neurons from rat hippocampus or cortex, or in monocultures of cortical astrocytes. We used either the full peptide (bA 1-42) or the 25-35 peptide fragment, non-toxic peptide fragment (35-25) was used as a control. bA did not cause any change in mitochondrial potential in neurons over the period observed (~1h) but induced profound changes in potential in astrocytes (n=510). These changes consisted of a slow modest mitochondrial depolarisation on which were sometimes superimposed sporadic fast and large depolarisations, that could be reversible but were sometimes sustained. Removal of external calcium prevents the bA –induced calcium signal and also prevented the spike like changes in mitochondrial potential, but did not change the slow depolarisation in astrocytes (n=231 cells). The slow depolarisation seen in astrocytes was completely blocked by high concentrations of metabolic substrates for mitochondrial complexes I and II (1-10 mM glutamate (n=194), methyl succinate (10mM, n=99). The response was also prevented by antioxidants (200mkM TEMPO/catalase, n=156 cells). Incubation of the cells with a combination of the inhibitor of the mitochondrial permeability transition pore Cyclosporin A (500nM) and the antioxidants (TEMPO/catalase)(n=107) prevented the bA-induced changes in mitochondrial potential. These data strongly suggest that bA causes changes in mitochondrial metabolism in astrocytes, but not in neurons, promoting lack of substrate supply and by opening the PTP in response to calcium and oxidant stress.

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OC14-2

PHYSIOLOGICAL PROPERTIES OF HYPOTONIC AND HORMONE-INDUCED TAURINE EFFLUX FROM PITUICYTES

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It has been shown previously, in the whole neurohypophysis preparation, that hypotonic conditions evoke an increased release of taurine (Miyata et al., 1997; Hussy et al., 2001), which then acts on secretory terminals to inhibit hormone secretion (Hussy et al., 2001). Using primary cultures of neurohypophysial astrocytes (pituicytes), we first confirmed that these cells constitute the likely source of neurohypophysial taurine. This was based on results of immunocytochemical experiments with anti-taurine antibody, and on the fact that a mild hypotonic shock (270 mOsm) increases [3H]taurine efflux ~2 fold. Secondly, we found that vasopressin (VP) and oxytocin (OT) also release taurine from pituicytes, which may provide a negative feedback mechanism for hormone secretion. VP appeared to be ~50 times more potent than OT, and the effects of both hormones were blocked by SR 49059, a V1a receptor antagonist. This pharmacological profile matches the one we found for VP- and OT-evoked calcium signaling (Rosso et al., 2002), suggesting involvement of calcium in VP-induced taurine efflux. Accordingly, the latter was blocked by BAPTA-AM incubation, which also blocked hypotonic efflux of taurine, further indicating that calcium is necessary in both cases. However, hypertonicity (330 mOsm) blocked VP-activated taurine efflux, indicating that the osmosensor mechanism overrides the calcium sensor. VP-activated taurine efflux was also blocked by DIDS, which is consistent with a passive efflux of taurine through volume-dependent anion channels. We are currently testing several working hypotheses to propose a unifying mechanism that might account for these results.

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S14-3

NEUROGLIAL INTERACTION AND GAP JUNCTIONAL COMMUNICATION IN ASTROCYTES
Giaume C.

A typical feature of astrocytes is their high degree of intercellular communication mediated by gap junction channels (GJC). Biochemical and electrophysiological studies have demonstrated that connexin 43 (Cx43) and 30 (Cx30) are the major GJC-forming proteins in astrocytes. These channels allow direct cytoplasmic exchanges of ions and small molecules and their permeability is controlled by endogenous bio-active molecules, including neurotransmitters. Accordingly, astrocytes might not be considered as individual entities but rather as groups of connected cells constituting astrocytic networks that can be modulated. In cocultures, the presence of neurons up-regulates the expression of Cx43 and Cx30, and increases gap junctional communication in astrocytes. This effect depends on the age and number of neurons, indicating that the state of differentiation and the density constitute two crucial factors in this interaction. The neuronal facilitation of astrocytic coupling is suppressed following prolonged pharmacological treatments that either induce neuronal death or prevent spontaneous activity. Moreover, the propagation of intercellular calcium waves that represents a mode of intercellular communication, in which astrocyte GJC, are involved is also regulated by the presence of neurons. These observations indicate that GJC in astrocytes is a target for neuroglial interaction. Since astrocytes have recently been shown to facilitate synaptic efficacy, these data suggest that neuronal and astrocytic networks may interact through the mutual setting of their respective mode of communication. Finally, the contribution of astrocyte gap junctions could have some physiopathological relevance since pro-inflammatory treatments (IL-1b, TNFa, LPS) alter GJC and Cx43 expression. Altogether these observations reinforce the ongoing concept that astrocytes play a role in brain functions and pathologies.

INSERM U114, Collège de France, Paris
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S14-4

SPONTANEOUS MOTILITY OF ASTROGLIAL PROCESSES IN LIVING BRAIN SLICES
Kirchhoff F.

Within the tripartite structure of vertebrate synapses, enwrapping astroglial processes regulate synaptic transmission by transmitter uptake and by direct glial transmitter release.
Two-photon laser scanning microscopy was applied to acutely isolated brainstem slices obtained from transgenic mice with human glial fibrillary acidic protein (GFAP) promoter-controlled green fluorescent protein (EGFP) expression. Three-dimensional time-lapse recordings with high-spatial (300 to 500 nm) and temporal (30 to 60 s) resolution uncovered spontaneous motility of highly branched astroglial processes. Almost all processes appear as very dynamic structures in situ. On average one to three motile elements were found in a volume of 250 µm3. Two distinct modes of motility could be discerned: (1) gliding of thin lamellipodia-like structures along neuronal surfaces and (2) transient extension of filopodia-like processes. Recording from slices with the styryl dye FM1-43 labelled presynapses revealed that the structural changes were always either in direct contact with active synaptic terminals or directed towards other neuronal compartments
We conclude that cell-cell contacts as another form of neuron-glia interaction play an important role during synaptic transmission in brain function.

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S14-5

COEXISTENCE OF DISTINCT ASTROCYTE CELL TYPES IN THE HIPPOCAMPUS: IMPACT ON NEURON-GLIA SIGNALLING.
Steinhäuser C.

Experimental In contrast to neurons, gray matter astrocytes are commonly considered a functionally uniform cell population. However, recent studies demonstrated that astroglial functioning differs in various brain regions, and changes during development and in response to brain damage and disease. Here, we asked whether this variety reflects different stages of cellular maturation from precursors to more mature cells or rather indicates the presence of distinct astrocyte cell types. Usage of transgenic mice with GFAP promoter-controlled EGFP-expression allowed the identification of astroglial cells after fresh isolation or in brain slices. Combining patch-clamp recordings and single-cell RT-PCR, we distinguished two morphologically distinct types of EGFP-positive cells in the hippocampus, one expressing glutamate transporters (GluT-cells), the other ionotropic glutamate receptors (AMPA subtype; GluR-cells). None of the EGFP-positive cells co-expressed glutamate receptors and transporters. Subpopulations of EGFP-positive GluR-cells expressed AN2, the mouse homologue of the rat NG2 proteoglycan, or neuronal transcripts indicating the existence of intermediate astrocyte-neuron cell types ('astrons'). Biocytin-filling of GluT-cells led to an extensive spread of the tracer to more than 100 neighbouring cells. In contrast, in GluR-cells the tracer was always confined solely to the recorded cell. No cell displayed an intermediate coupling pattern. Our data reveal the coexistence of distinct, independent types of cells with astroglial properties in the hippocampus, which display diverse morphological, molecular and functional profiles and can differently modulate neuronal signalling pathways. The observed heterogeneity of cells with GFAP promoter activity challenges the hitherto accepted definition of astrocytes. These cells can no longer be considered a homogenous cell population but have to be defined according to their specific functional properties.
Supported by DFG (SFB Tr3).

Neurobiology, Neurosurgery, University of Bonn, Bonn, Germany

POSTER SESSION

P14-01

TIME PROFILE OF CELL DEGENERATION IN HIPPOCAMPUS AFTER KAINIC ACID ADMINISTRATION.

Pokorny J., Langmeier M., Maresova D., Trojan S.

Sequence of neuronal degeneration after the kainic acid administration reflects neuroplastic potential of neuronal circuits. It may depend on the presence and density of membrane receptors, on the activity of neuroplastic mechanism of recovery, on conditions of the internal microenvironment, and it may reflect the relation of neurons in the neuronal circuits.
In the first model of a single dose of kainic acid (i.p. injection to adult Wistar male rats), rats were allowed to survive 2, 4, or 6 days. Perefusion fixed brains were processed for DNA staining (Hoechst) in combination with Fluoro-Jade (FJ) to differentiate surviving and dying cells. Two days after the neurotoxic agent administration, many neurons in CA1, CA2-3 and some neurons in the hilus of the gyrus dentatus were degenerating. After four days, majority of the labelled cells were found in the hilus, CA3 and in the distal part of CA1. After six days, only few stained cells were present in CA1, CA3 and in the hilus.
In the second model, kainate was administered repeatedly in three reduced doses. Two days later, brains were processed in the same way. FJ positive cells were present mainly in the CA1 region, partly also in the hilus of the dentate gyrus. CA3 region and both blades of the dentate gyrus were almost intact. The DNA staining revealed significant reduction of cells in CA1 without major changes in CA3 and dentate gyrus. Dilation of the ventricular system indicated more general degeneration of the brain tissue.

The dynamics of nerve cell extinction indicates that the mechanism of cell death is related not only to the direct effect of this excitatory molecule. It may result also from the specific sensitivity of the neuronal circuits and the level of neuroplastic potential of neuronal circuits involved.
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P14-02

IONIC MECHANISM OF AFTERDEPOLARIZATION IN HIPPOCAMPAL DENTATE GRANULE CELL

Park W.S., Son Y.K., Earm K.H., Earm Y.E.

Granule cells in dentate gyrus of hippocampus relay information from entorhinal cortex to pyramidal cells via perforant fibers in CA3 region. Their electrical activities are known to be closely associated with epileptic seizures as well as memory acquisition. The neuronal firing pattern is known to be dependent upon afterpotentials, which follows the stereotypic Na^+ spike. Thus, we investigated the underlying ionic mechanisms of afterdepolarization (ADP) in dentate granule cells of rat hippocampus.
Action potentials of dentate granule cells showed afterdepolarization, which were characterized by a sharp notch followed by a depolarizing hump starting at about –50 mV (49.1 ± 1.69 mV, mean ± SD) (n = 43) and lasts for 3 - 7 ms. The elevation of extracellular Ca^{2+} from 2 mM to 10 mM significantly increased the ADP in amplitude and duration. 4-aminopyridine (4-AP, 2 mM) enhanced the ADP and often induced the burst firings. The effects of 10 mM Ca^{2+} and 4-AP were additive. Furthermore, the ADP was significantly suppressed by the removal of external Ca^{2+} or by 2 mM NiCl2, even in the presence of 4-AP (2 mM). When the high concentration of BAPTA (10 mM) in pipette solutions, or BAPTA-AM (100 μM) was added to bath solution, ADP was also suppressed. Replacement of extracellular Na^+ with Li^+ to block the Na^+/Ca^{2+} exchanger reduced the ADP. When the exchanger inhibitory peptide (XIP), a peptide which is known to block Na^+/Ca^{2+} exchanger, was added to pipette solution, ADP was also reduced. However, Niflumic acid (100 μM), a Ca^{2+}-activated Cl^- channel blocker, and TTX (100 nM), did not affect ADP. From these results, it can be concluded that the Ca^{2+} influx and Na^+/Ca^{2+} exchangers contribute to the generation of ADP in granule cells.

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P14-04

THE INFLUENCE OF THE NEUROTOXIN MPP+ AND DIPEPTIDE TGS-79 ON THE INTRACELLULAR CALCIUM LEVELS

Vukolova M.N., Marsh S., Brown D.A., Lutsenko V.K., Gudasheva T.A.

In the recent series of experiments was obtained preventive effect of a new dipeptide analogue of the active site neurotensin TGS-79 on MPTP-induced parkinsonian syndrome (PS) in mice[1].This compound was synthesized in Institute of Pharmacology of RAMS, Russia [2]. The aim of present study was to investigate the influence of TGS-79 on the intracellular cytosolic calcium levels after treated MPP+ on the cytosolic calcium levels of striatum neurons and elucidation of the possible mechanism it action.The $[Ca^{2+}]i$ was measured by imaging neurons loaded with fluorescent Ca^{2+} indicators. To monitor dynamic changes of Ca^{2+}, striatum cell culture were loaded with 5 μM Fura-2AM for 30 min at 37 ºC. Images were collected using the 340-nm excitation and 520-nm emission wavelengths. Data from 10-15 neurons were recorded. The level $[Ca^{2+}]i$ was measuring before treated with any drugs, then 10μM glu was added to the neurons and measuring levels again. After that striatum neurons were challenged with 100μM MPP+ + 1μMTGS or only 100μM MPP+, or without drugs (control). $[Ca^{2+}]i$ was measurement in the same region of neurons after 30min, 1 hour. After treated the cells only 100μM MPP+ was observed increase the level $[Ca^{2+}]i$ ($P<0,05$). While after 100μM MPP+ + 1μMTGS treated was received the decrease of the intracellular calcium levels ($P<0,05$). It testifies what decrease or maintaining of levels $[Ca^{2+}]i$ is one of TGS-79 protection mechanisms of neurons from loss. Further studies of TGS-79 action mechanism on biochemical models are needed.
The FEBS Fellowship and the RFBR grant 02-04-06617 supported this work.
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2. S.B. Seredenin, T.A. Voronina, T.A. Gudasheva et al. // Russian patent № 2091390 from 28.02.95.

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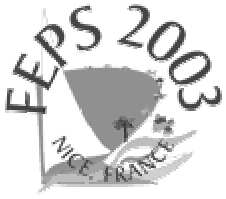

S15 THE NEW INTEGRATIVE BRAIN PHYSIOLOGY

ORAL SESSION

S15-1

PHYSIOLOGICAL MECHANISMS OF MULTISTABLE PERCEPTION

Logothetis N.

Ambiguous or reversible figures are illustrations whose perception changes over time. Although the brain mechanisms underlying this multistable perception have long been a central quest in vision research, they still remain poorly understood and continue to be a topic of intensive research and debate. For the past ten years we recorded the activity of cells in the visual cortex of monkeys trained to report what they perceive when viewing perceptually rivalrous stimuli. In any studied area only a fraction of the neurons were found to respond in a manner that reliably reflects shifts in perception. This small number of neurons is distributed over the entire visual pathway rather than being part of a single area in the brain. Of the areas we have studied the inferior temporal cortex of the temporal lobe was found to have the highest number of perception-related cells. In my talk I'll briefly summarize these results and continue by describing our rivalry experiments, in which both local field potentials and multiple unit activity were measured with multiple electrodes placed over more than one visual areas. Of interest is the study of covariation of activity within and between various occipito-parietal areas under different stimulus and perceptual conditions. Analysis of data collected in such experiments revealed significant coupling between distant sites both in non-stimulated and stimulated conditions. While these patterns were consistent and robust, there were subtle stimulus-specific differences. During rivalry, covariation patterns were significantly diminished, and in some cases completely disappeared. These findings suggest that the coherence in the response of visual neurons during rivalry may be related to a system's stability rather than to the perceptual state of the animal. These results will be discussed in the context of new information obtained from imaging experiments. Finally, psychophysical experiments will be described examing the relationship between the process of stabilizing ambiguous percepts and perceptual memory.

Max-Planck-Institute for Biological Cybernetics – Tuebingen, Germany

S15-2

SPATIO-TEMPORAL DYNAMICS OF RECEPTOR NEURON INPUT TO THE MAMMALIAN OLFACTORY BULB

Spors H.

Odors evoke dynamic glomerular activity patterns in the mammalian olfactory bulb (OB). On the network level voltage sensitive dye imaging revealed odor-specific sequences of glomerular activation and distributed OB activity locked to the nasal respiration cycle. The spatial distribution of its amplitude and phase was heterogeneous and changed by sensory input in an odor-specific manner (Spors and Grinvald, 2002). To analyze the dynamics of these patterns at the level of input to the OB, we selectively loaded olfactory receptor neurons with Calcium Green dextran and imaged afferent glomerular calcium dynamics in freely breathing or artificially sniffing, anesthetized mice (Wachowiak and Cohen, 2001). Glomerular odor responses differed in response latency, rise time, decay time, and modulation by sniffing. In response to esters and hydrocarbons, caudo-lateral glomeruli generally exhibited faster responses and more pronounced respiratory modulation. However, neighboring glomeruli could also exhibit different temporal response characteristics. Temporal response characteristics of individual glomeruli depended on glomerulus identity, odor identity, odor concentration, sniffing frequency, and flow rate. Changing from freely breathing to artificially sniffing altered the degree of respiratory response modulation, while differences in response latency, rise time, and amplitude across glomeruli and odors were preserved. The temporal response properties were consistent for equivalent groups of glomeruli in different preparations. Hence the odor evoked spatial patterns can change significantly over time in a stimulus-specific manner already at the level of the input to the OB. The spatio-temporal dynamics of afferent activity patterns therefore need to be considered in models of olfactory coding.

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OC15-1

IS MK-801 A SPECIFIC NMDA RECEPTOR ANTAGONIST?

Senok S.S., Genever P.*., Cahusac P.M.B.**

Glutamate is the most abundant excitatory amino acid transmitter in the brain. It acts through ionotropic and metabotropic receptors. The ionotropic receptors, consisting of N-methyl-D-Aspartate (NMDA) and non-NMDA types, are responsible for fast synaptic transmission.
MK-801, originally developed as an analgesic and anti-inflammatory agent but later abandoned because of toxic side effects, is widely used in research as an uncompetitive antagonist of the NMDA receptor. MK-801 block is commonly accepted as evidence of signalling through NMDA receptors.
The present study set out to examine the suggestion that glutamate may be the neurotransmitter in the Merkel cell touch receptor. First we showed that the Merkel cell-neurite complexes expressed both NR1 and NR2A/B NMDA receptor subunits, suggestive of the presence of functional NMDA receptors. We subsequently found that MK-801 reduced the number of action potentials evoked in single receptor units to mechanical stimulation in an isolated rat vibrissa preparation. These findings appeared to confirm the involvement of glutamate transmission in this touch receptor.
However, further testing using other classic NMDA and non-NMDA receptor antagonists, including D-AP5, R-CPP, CNQX and DNQX, had no effect on Merkel cell receptor function. Furthermore, the less active enantiomer of MK-801, (-)-MK-801, which is supposed to have 10-fold less activity than MK-801, was equally effective in blocking responses.
From our data, it would appear that MK-801 is either acting on something other glutamate receptors, or that the NMDA receptors in the skin have a qualitatively different pharmacological profile. We speculate that MK-801 is acting on the mechano-gated ion channel and hence directly interfering with mechano-electric transduction. Considering the ubiquity of mechanosensitive channels, results relying solely on MK-801 block need to be interpreted with caution.
This work was supported by the Wellcome Trust.

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OC15-2

A ROLE OF NORADRENALINE IN LATERAL VESTIBULAR NUCLEUS: THE MODULATION OF GABA-EVOKED RESPONSES

Di Mauro M. Li Volsi G. Licata F. Santangelo F.

Neuronal processing of primary vestibular information in the lateral vestibular nucleus (LVN) is controlled by GABAergic cortico-cerebellar fibers. In addition, the firing rate of LVN neurons is depressed by the weak, but diffused action of noradrenergic fibers reaching the whole vestibular complex. The aim of this work was to study the influence of noradrenaline (NA) on the GABA-evoked responses in LVN neurons and to identify the mechanisms involved. Unitary discharges of LVN neurons were recorded extracellularly in deeply anesthetized rats during microiontophoretic injection of GABA, NA, clonidine and yohimbine. Inhibitory responses to repeated GABA applications, recorded during ejection of one or more of the cited drugs, were compared among them to value the entity of NA influence and the type of involved receptors.
NA application (2-10 nA, 3-10 min) modified the GABA-evoked inhibitions in 91% of the studied neurons, enhancing and decreasing them in 26% and 56% respectively. In addition, an inversion of the effect, from an enhancement into a depression, could be evoked in few cases (9%), by increasing the ejection current intensity and therefore the applied doses. The alpha2 receptor antagonist yohimbine was able to block both depressive and enhancing action of NA on the GABA-evoked responses, but in about 50% of cases. The application of clonidine, an alpha2 noradrenergic receptor agonist, enhanced GABA-evoked responses.
These data suggest that the activation of noradrenergic afferents to LVN neurons is able to modify the corticocerebellar control on the nucleus. The sign and intensity of these actions of NA on the neuronal responsiveness to GABA are dose-dependent and are partially mediated by alpha2 noradrenergic receptors.

Department of Physiological Sciences - University of Catania – Italy

OC15-3

THE MODULATION OF SPIKE SYNCHRONIZATION IN RELATION TO IMAGE STRUCTURES

Duret F., Shumikhina S., Molotchnikoff S.

It has been suggested that synchronization of action potentials, between two or more neurons belonging to distant pools of cells within a time-window of 1 to 5 ms, may be an encoding process, allowing the binding of various features of a single visual object. Previous studies have been carried out with multiunit recordings. This method fails to reveal which cells participate in the synchronization process, i.e., do all units of a restricted pool of neurons contribute to the formation of the synchronizing ensemble? We answered this question by investigating the modulation of synchronization between pairs of neurons sorted out from multiunit recordings. The study was performed in areas 17 and 18 of anaesthetized cats. Visual stimuli were composed of a central sine-wave grating patch covering the compound receptive field with two additional, identical patches placed above and below the receptive field. One of the supplementary patches was gradually shifted in small steps. The distance between the central patch and the displaced one is the unique property differentiating the target's structure. Individual cell extraction (3 to 4 neurons) from a pool of recorded cells was performed using software allowing us to discriminate individual action potentials by cluster analysis. Discriminated spikes were individually visualized and monitored along with standard deviations that ensured that the waveform of selected spikes remained within predetermined boundaries, (Z-score >2.5). Results show that the magnitude of synchrony is image dependent. Neural coding assembly is a dynamic process as different cells remain in (or leave) the group as the target form changes. Furthermore, correlating synchrony magnitudes between assembly of larger size and the respective multiunit recordings, reveals that there is a threshold in the number of grouped cells that reliably reproduces the synchronization modulation computed in multiunit recordings.

Université de Montréal – CANADA

OC15-4

OBJECT PROCESSING AND SEMANTIC PRIMING: AN ELECTROPHYSIOLOGICAL INVESTIGATION
Magnie M., N. kahlaoui K., Baccino T.

This study was aimed at investigating the effects of modality and semantic priming in a reality decision task. Event-Related Potentials (ERPs) were recorded from 22 scalp electrodes on 16 participants while performed a mixed reality decision task (object vs. lexical) as a function of the target modality (picture vs. printed word). Target stimuli were presented in isolation (Experiment 1), or preceded by a semantic prime (Experiment 2). In both experiments, half of stimuli were pictures, and the other half were printed words. Target stimuli were meaningful (object picture vs. object name), or meaningless (chimeric object vs. non-object vs. pseudo-word vs. non-word). The prime was always meaningful (object picture vs. name). The Experiment 1 suggests that the reality decision task only requires an access to structural representations when stimuli are presented in isolation. In the Experiment 2, present data are in line with previous ERP studies for intra-modality conditions concerning N300 and N400 patterns and also the P300 component. Moreover, our results show that a semantic priming effect may be obtained with chimeric objects and pseudo-words, independently of the modality prime. Whereas the N400 component was elicited in all conditions requiring an access to the semantic memory, the N300 component was only produced when a picture was presented, whatewer it was the prime or the target. The current study demonstrates that the semantic priming effect may occur in cross-modality conditions even for meaningless stimuli arguing for a semantic mismatching. Finally, our findings provide additional evidence for both similarities and differences in the processing of pictures and words as reflected by ERPs.

PHYSIOLOGY LABORATORY, MEDICAL SCHOOL, NICE, FRANCE

S15-3

NEUROTROPHINS AS RAPID SIGNALLING MOLECULES IN THE MAMMALIAN BRAIN
Konnerth.A.

Brain-derived neurotrophic factor (BDNF) and other neurotrophins are family of structurally related molecules that are essential for the normal function of the mammalian nervous system. These factors are critical for neuronal survival and differentiation. However, there is accumulating evidence that neurotrophins are rapid signalling molecules that act throughout the entire life span, from early embryogenesis through adulthood. They are secreted in an activity-dependent manner and exert a transmitter-like depolarisation of most central neurons. By using whole-cell recordings from neurons in brain slices, we found that the neurotrophins BDNF and Neurotrophin-4/5 (NT-4/5) elicit action potential firing in central neurons through the activation of the receptor tyrosine kinase TrkB (Kafitz et al., Nature, 1999). Neurotrophin-evoked currents resulted from the activation of a TTX-insensitive Na-conductance. By imaging dentate granule cells in mouse hippocampal slices, we established that the BDNF-dependent depolarisation produces large calcium transients through the activation of voltage-gated calcium channels. The BDNF-evoked calcium responses were reliably obtained in the cell's soma and in dendrites, but not in the axon. Particularly large calcium signals were detected in dendritic spines. Pairing a weak burst of synaptic stimulation with a brief dendritic BDNF application caused an immediate and robust induction of long-term potentiation (LTP) (Kovalchuk et al., Science, 2002). By screening candidate genes with an antisense mRNA expression approach and by co-expressing the receptor tyrosine kinase TrkB and various sodium channels, we found that the tetrodotoxin-insensitive sodium channel Nav1.9 underlies the neurotrophin-evoked excitation (Blum et al., Nature, 2002). These results clarified the molecular basis of neurotrophin-evoked depolarisation and revealed a novel mechanism of ligand-mediated sodium channel activation.

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S15-4

DYNAMICS AND PLASTICITY OF CORTICAL COLUMNS
Petersen C.C.H.

This talk will focus on how the neocortex responds to simple sensory stimuli at the level of the synaptic interactions between individual cortical neurons and also how experience can modify both sensory responses and the underlying cortical circuits.
The barrel cortex of rodents is particularly well-suited to this endeavour since the sensory map can be anatomically defined both in vivo and in vitro by the barrel pattern. This allows the physiology of synaptic neuronal networks to be investigated in the context of clearly defined functional cortical regions. The activity of individual neurons can then be monitored in the context of the spatiotemporal dynamics of the ensemble network through a combination of whole-cell recordings and voltage-sensitive dye imaging. The data show highly dynamic views of cortical representations of the sensory periphery.

Dept of Cell Physiology, Max Planck Institut for Medical Research – Heidelberg – Germany

S15-5

OPTICAL IMAGING OF CORTICAL ARCHITECTURE AND DYNAMICS
Grinvald.A., Hildesheim.R., Vanzetta.I., Jancke.D., Chavane.F., Slovin.H.

Objectives: (1) To image the cortical functional architecture at the level of cortical columns, at high resolution of 50 micron. (2) To charagterize the sptio temporal parameters of activity dependent hemodynamic responses, emphasizing the characteristic behavior of the various microvascular compartments and (3) To image cortical dynamics with a millisecond time resolution based on new voltage sensitive dyes. Methods: High resolution optical imaging based on intrinsic signals was used to map the functional architecture of cortex. We measured blood-volume and –oxygenation changes in the anesthetized cat, and awake monkeys using: (1) intrinsic imaging at isosbestic wavelength and others wavelength (2) laser Doppler, (3) imaging spectroscopy, (4) phosphorescence quenching and (5) imaging of activity dependent responses of intramuscularly injected extrinsic-probes. Many of these measurements were done simultaneously. To study cortical dynamics we employed voltage sensitive dyes. Summary: (1) The functional organization of primary sensory areas in cats and monkeys will be reviewed. (2) We found that the onset of the blood-volume increase was delayed (>~200ms) with respect to changes in oxygenation. The peak of the monophasic blood volume response was delayed relative to the peak of the deoxygenation by 1-3s. The initial dip has been confirmed in both the anesthetized cat and the awake monkey. (3) Using voltage sensitive dyes we imaging cortical correlates of illusion in primary visual cortex. Whereas flashing the square or bar alone evoked the expected localized, short latency, high amplitude activity patterns, presenting a square before a bar induced activity patterns resembling that of a moving object. The preceding cue, even though non-moving, creates a propagating gradient of subthreshold neuronal activity that account for the line motion perceptual illusion of motion.

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POSTER SESSION

P15-01

SPATIAL MEMORY TESTING IN RATS TREATED WITH TESTOSTERONE

Okkelová J., Dunčko R.(2), Hodosy J., Ježová D.(2), Ostatníková D.

The nature of the relationships between spatial performance and sex steroids remains controversial. Androgens are supposed to modulate learning and memory in early stages of life. The issue addressed here is to find out whether testosterone exerts any influence in animals during adulthood and whether testosterone affects spatial performance directly or through its active metabolite estradiol. The presented study examined the effects of exogenous testosterone on spatial navigation in maze tasks. Three groups of testosterone treated and control male rats were used. First group was treated with testosterone, the second with testosterone combined with aromatase blocker anastrazol. Rats performed spatial experiments in 8 radial arm maze in three daily sessions. Each arm was baited with food at the start of each day´s trial. The animals placed on central platform were required to learn to find the food. The food was not replenished during the trial so the optimal performance was when animals visited each arm only. Temporal measures with success scoring in three days were recorded. Data analysis revealed that testosterone treated rats had higher testosterone levels than rats treated with testosterone and anastrazol, controls had the lowest levels. Testosterone treated rats outscored control rats in maze performance. Rats treated with testosterone combined with anastrazol reached the lowest scores. The results, which proved testosterone influence on spatial performance were confirmed in Morris water maze tasks. It can be concluded that testosterone treatment resulted in enhanced performance of rats in spatial memory tasks. The study was supported by Grants No. 1/7511/20 and 2007.

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P15-02

ADENOSINE IN SPINAL ANTINOCICEPTION

Kekesi G., Dobos I., Benedek G., Horvath G.

Objectives: Numerous data are in favour of a role for adenosine in nociceptive processes, at the level of peripheral nerve terminals of sensory fibres as well as at central sites. There are a few studies investigating its antinociceptive effect at the spinal level, but most of them determined its influence on the mechanical pain threshold after a bolus injection.
The goal of our study to determine the antinociceptive potency of continuously administered adenosine on thermal hyperalgesia in awake rats. Since the interaction between the endogenous ligands involved in pain processing is not well established, the possible interaction between adenosine and endomorphin-1 was also investigated.
Materials & methods: After obtaining institutional ethical approval, intrathecal catheters were implanted into male Wistar rats. Nociceptive threshold was assessed by using paw withdrawal (PWD) test. The PWD latencies were obtained before unilateral carrageenan injection, 3 h after that and then in every 10-min intervals for 130 min. Dose-dependent effects were determined for adenosine (0.3 - 3 µg/min), endomorphin-1 (0.1-1 µg/min) and for their fixed-dose combination (3:1). Groups were compared by ANOVA with $p<0.05$ considered significant.
Results: Continuous administration of adenosine did not influence the PWD latencies during the infusion, but in the higher doses it resulted in significant increases in PWD latency after the cessation of the infusion. Adenosine dose-dependently potentiated and prolonged the antinociceptive effect of endomorphin-1.
Conclusions: Although adenosine displays low antinociceptive potency during continuous intrathecal administration, it potentiates the effect of endomorphin-1 in thermal hyperalgesia. Our results suggest roles for these endogenous ligands as important mediators of sensory information processing, and combinations similarly to this one might serve as important targets for the therapeutic modulation of pain.

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P15-03

ELECTRICAL SOURCE ANALYSIS OF FAMILIAR FACE RECOGNITION

Mnatsakanian E.V., Tarkka I.M.

The purpose of this study was to model cerebral sources, which could explain the evoked electrical activity during visual processing of familiar faces. This was accomplished by developing multiple dipole source models of the scalp-recorded event-related potential (ERP) data collected from 19 healthy volunteers. Single trial began with one of the two cues (S1) followed by consecutive pictures (S2 and S3). Each picture was a photograph of a familiar face, on which an abstract dot pattern was superimposed. One cue directed attention to compare faces and another to compare patterns. EEG was recorded using 128 channels, with filters of 0.01-100 Hz and sampling rate of 250 Hz. Artifact-free trials (correct performance only) were averaged and analyzed for the FACE task where pairs of photographs of same or different persons were compared. The major components of the waveforms appeared around 120-150 ms, 200-250 ms, 270-300 ms, 350-400 ms, and 500-600 ms. Spatio-temporal multiple dipole source models were created in BESA2000 software for the window of 80-600 ms from S3 onset. The model contained 8 dipoles. Dipole 1 was in anterior cingulate gyrus and dipole 2 was close to caudate nucleus and anterior cingulum. Dipoles 3 and 4 were located in medial temporal gyrus; dipoles 5 and 6 in the visual cortex; and 7 and 8 in the fusiform gyrus. Comparison of different faces elicited larger components than same faces at 400 ms, and these components were mainly explained by frontal sources.

Inst. of HNA and NPh RAS, Moscow, and Brain Res. and Rehab. Center Neuron, Kuopio

P15-05

EVENT RELATED POTENTIAL DIFFERENCES IN PHONOLOGICAL READING OF DYSLEXIC CHILDREN

Georgiewa P., Popatanasov A., Klapp B., Dimitrov B.

Difficulties in phonological processing are currently considered one of the major causes for dyslexia.
Objective is to measure specific Event-Related Potential (ERP)- signs of phonological deficits in children with Dyslexia (ICD-10 diagnosis).
In a sample of 17 dyslexic and 17 control children (aged 9 to 16 years) ERP maps were recorded during four different reading tasks: (1) passive viewing of letter strings (2) passive reading of non-words, (3) passive reading of high frequently used words and (4) a task requiring phonological transformation. Resulting ERPs and performance were tested for differences between intelligence-, age- and sex-matched dyslexic and control group.
Children with Dyslexia had a longer reading time in all tasks and they made more errors in reading of nonwords. The P3a, N4, and Positive Slow Wave (PSW) maps (220-320ms, 400-600ms) revealed reliable group differences, with larger amplitudes for dyslexic children. The P3a- topography indicated left frontal sources. This was only the case in nonword reading and the transformation task.
Brain mapping indicates that children with Dyslexia try to compensate reading difficulties with increased effort mainly in nonword - reading in a period between 220 and 320 ms after reading stimulus (increasing P3a-amplitude). This time window is seen as connected with phonological word processing, and also the left frontal topography extends previous results on difficulties in phonological processing in Dyslexia. The fact that these differences were found specifically for nonword reading provide further evidence for alteration of the phonological system in dyslexic children, and in particular, the system that mediates assembled phonological coding.

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P15-06

AGE DIFFERENCES IN PHONOLOGICAL READING - A STUDY WITH EVENT-RELATED POTENTIALS

Popatanasov A., Dimitrov B., Georgiewa P.

INTRODUCTION: The present study addresses the development of phonological processing in children. Following a model of two different reading strategies (piecemeal versus whole-word- reading) tasks was applied that specifically control for different kinds of phonological coding (assembled versus addressed phonological strategies).

OBJECTIVE: To measure specific Event-Related Potential (ERP) - signs of development of phonological abilities in younger and elder children.
METHOD: In a sample of 15 children (aged 7 to 9 years) and 15 children (aged 10 to 12 years) ERP maps were recorded during four different reading tasks: (1) passive viewing of letter strings (2) passive reading of non-words, (3) passive reading of words and (4) a phonological transformation task.
Resulting ERPs and performance were tested for differences between intelligence-, and sex-matched groups.
RESULTS: Younger children in all tasks had a longer reading time and made more errors than the elder group. The P3, N4, and Positive Slow Wave (PSW) maps (250-350ms, 400-600ms) revealed reliable group differences mainly in word reading, with larger amplitudes for younger children.
CONCLUSIONS: Performance data and brain mapping of ERPs indicate that elder children read with decreased effort mainly the high frequently used words (smaller P3 amplitudes). The time window is seen as connected with semantic and phonological word processing. The fact that these differences are found specifically for word reading provide evidence for a faster development of whole-word reading abilities, requiring particular addressed phonological strategies. The improvement of assembled phonological coding strategies seems smaller.

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P15-07

SEASONAL CHANGES IN STRESS-INDUCED ANALGESIA IN THE LOW-PAIN AND THE HIGH-PAIN THRESHOLD RATS
Rokyta R., Yamamotova A., Pometlova M., Harmatha J.

Changes in stress reactivity generally depend on the previous stress experiences (types, intensity and predictability of stressors), age, gender and seasonal influences. In our experiments we studied several of these factors in Wistar male rats after the treatment with possible anxiolytic drug N-feruloylserotonin (10 mg/kg), which is structurally similar to melatonin and serotonin. This substance was isolated from the root of Leuzea carthamoides, the plant growing mostly in Siberia and used as a roborans. In two series of experiments (in June and December), the stress-induced analgesia after the Porsolt's swimming stress (3 minutes in water of 32°C) was measured using the plantar test and the tail-flick test. Before the stress exposure the animals were tested on their nociceptive reactions and animals were selected into two groups – the low-pain (LPT) and the high-pain threshold (HPT) rats. The rest pain threshold was the same in both seasons in both groups contrary to stress-analgesia threshold, which was considerably higher in summer. Neither in June nor in December N-feruloylserotonin influenced the pain threshold and the magnitude of stress analgesia in both methods. In both seasons the stress-induced analgesia was higher in the LPT rats, and lower in the HPT rats. In the HPT group the habituation in repeated tail-flick test was slower. The most significant result after the treatment with N-feruloylserotonin was improving of habituation in repeated tail-flick test in the HPT group. In December group, anxiety was tested in the elevated plus maze. The frequency of visits and the duration of stay in the open arms were higher and longer in the animals with the low-pain threshold. N-feruloylserotonin had anxiolytic effect in the high-pain threshold rats only. The precise pharmacological effect of N-feruloylserotonin is not yet known (it does not bind to melatonin receptors), probably it might influence serotonin, melatonin and glutamate transmission.

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P15-08

CERAMIDE AND NITRIC OXIDE MODULATE THE NICOTINIC ACTIVATION OF PREVERTEBRAL GANGLIONIC NEURONS
Fasano C., Miolan J.P., Niel J.P.

Our study has been performed in vitro on the coeliac ganglion in the rabbit. The electrical activity of the ganglionic neurons has been recorded with intracellular microelectrodes. The nicotinic synaptic activation of the neurons is triggered by stimulating thoracic splanchnic nerves. A stimulation with a single pulse elicits an excitatory postsynaptic potential (EPSP) in the absence of any neuromodulatory mechanisms. On all the neurons tested, C2 ceramide (a permeant analogue of ceramide) triggered a statistically significant increase in the EPSPs amplitude. Thus, in the absence of neuromodulations, C2 ceramide directly facilitates the nicotinic synaptic transmission. During iterative stimulations of the splanchnic nerves, neuromodulatory mechanisms of the nicotinic activation are called into play. Indeed, during 10 sec supramaximal stimulations the nicotinic transmission is progressively inhibited. On all the neurons tested, C2 ceramide has reinforced this inhibitory modulation of the nicotinic activation, likely through an indirect effect. It has been previously demonstrated that nitric oxide (NO) exerts a dual modulation, facilitatory or inhibitory, of the nicotinic synaptic activation of the neurons in the coeliac plexus. During our study, on all the neurons tested, the indirect inhibitory effect of C2 ceramide is abolished in the presence of carboxy PTIO (NO scavenger). This result demonstrates that the indirect inhibitory effect of C2 ceramide on the nicotinic activation is exerted through the NO pathway.
Our study demonstrates that ceramide exerts complex modulations of the nicotinic synaptic activation of the pre-vertebral ganglionic neurons : a direct facilitation and an indirect inhibition involving the NO pathway.

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P15-09

PHOTOPIC AND SCOTOPIC COMPONENTS OF THE ELECTRORETINOGRAM IN POSTNATAL RATS
Yinon U., Gurshumov N.

The functional maturation of the rat retina continues until about one month postnatally, when all nerve connections and visual cortex achieve their maturation. We have studied the development of the scotopic and photopic components of the rat electroretinogram (ERG), as well as the initiation of their excitability. Thirty normal pigmented rats (DA) were divided into three postnatal age groups: 0-10, 11-20 and 21-30 days. Animal older than 30 days old were considered as matured group. The electroretinograms were recorded from both dark- (scotopic conditions) and light (photopic conditions) adapted rats. The schedule of the visual stimulation (single light flashes of different intensities) was computer controlled, as well as the processing of the various ERG parameters. The results indicate that the first scotopic response could be detected from the pigmented rat's retina at the 8th postnatal day, whereas the first photopic response is delayed by one week. The shaping of both photopic and scotopic components continues until 20-25 days postnatally, when the ERG obtain its matured pattern. It is concluded that the development of the scotopic mechanism precedes the development of the photopic mechanism.

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P15-10

THE INFLUENCE OF GLUCOCORTICOIDS HORMONES ON NEUROLOGICAL DEVELOPMENT DURING PRENATAL PERIOD
Clichici S., Joanta A., Filip A., Puica C., Rusu M.

Glucocorticoids hormones are involved in neurological development and maturation. High doses could lead to neurological and motor disorders due to brain lesions.
We investigated the effects of synthetic glucocorticoid, prenatal administered, in different manners: for a long time and in a single dose. Experiments were performed on white rats, Wistar race.
We explored at off springs: spontaneous motility, emotivity and learning assesment(shuttle box method)at 3 months old and brain samples stained with Kluver- Barera, histological disorders at 3 days old and 3 months old.
Our results suggest that a single prenatal dose of glucocorticoids hormones, like in clinical therapy, has no influence on learning skill, emotivity and spontaneous motility. A high dose, long time administered, significantly decreased just spontaneous motility and learning skill in offspring.
Long time administration leads to brain morphological disorders, shown in off springs at 3 days postpartum. These disorders were reversible till the age of 3 months.
Single dose administration determined least disorders of the brain in off springs, 3 days old which disappeared till the age of 3 months.

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P15-11

TRANSPLANTATION OF BONE MARROW STROMAL CELLS (MSCS) INTO A CORTICAL LESION
Glogarová K., Jendelová P., Herynek V., Hájek M., Syková E.

MSCs are pluripotent progenitor cells that have the capability to migrate towards lesions and induce or facilitate site-dependent differentiation in response to environmental signals. Using in vivo MR imaging, we studied MSCs transplanted into adult rats with a cortical photochemical lesion. MSCs were isolated from rat bone marrow by adherence to plastic. After in vitro expansion, the cells were co-labeled with superparamagnetic iron-oxide nanoparticles (Endorem, Guerbert Laboratories, France) and BrdU (5 mM) 48 hours prior to transplantation and administered either intracerebrally into the contralateral hemisphere (0.3 million cells in 3 ml PBS; n=12) or i.v. into the femoral vein (2 million cells in 0.5 ml PBS; n=8). Lesions were induced by rose bengal / light beam interaction 24 hours prior to transplantation. MR images were taken weekly using a 4.7 T Bruker spectrometer. Rats were sacrificed 4 weeks following transplantation, and the fate of transplanted cells in the CNS was analysed immunohistochemically. The cells preferentially migrated into the lesion, and subsequently some of the cells expressed the neuronal marker NeuN. In animals without a lesion, the majority of intracerebrally injected cells remained in the close vicinity of the needle track. Starting 7 days after transplantation and persisting for 4 weeks, MR images showed a hypointense signal in the lesion. Anti-BrdU and Prussian blue staining confirmed the presence of iron-oxide-labeled cells in the lesion site. The study demonstrates that iron-oxide nanoparticles can be used as a marker for the long-term non-invasive MR tracking of implanted stem cells. Supported by: J13/98:111300004, LN00A065 and AV025039906

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P15-12

PROTECTIVE CAP OVER CA1 SYNAPSES:EXTRASYNAPTIC GLUTAMATE DOES NOT REACH THE POST-SYNAPTIC DENSITY
Lozovaya N., Melnik S., Tsintsadze T., Grebenyuk S., Kirichok Y., Krishtal O.

Astroglia controls the extracellular level of glutamate (Glu) and is capable of releasing it in concentrations sufficient to activate and desensitize ionotropic Glu receptors. When reaching receptors in the post-synaptic density (PSD), Glu could affect the synaptic transmission. We have tested this possibility in the hippocampal CA1 synapses of rats, either by applying exogenous Glu to the CA1 neurons or by activating non-vesicular release of Glu from intracellular compartments induced by disruption of Glu transporter activity.
L-Glu (200-400 mkM) was directly applied to the hippocampal slices acutely isolated from the rats. It produced a strong inhibition of both ortho- and antidromically elicited action potentials fired by CA1 neurons, while the excitatory postsynaptic current (EPSC) measured in these neurons remained totally unaffected. The optical isomer of L-Glu, D-Glu, which is not transported by the systems of glutamate uptake inhibited not only orthodromic and antidromic spikes (10 +/- 10%, n=5 and 53 +/- 9%, n=6 correspondingly), but also EPSC (57 +/- 17%, n=5).
Non-specific glutamate transporter inhibitor (THA, 400 mkM) mimicked the effects of exogenous Glu and produced strong inhibition of both orthodromic (23 +/- 6%, n=4) and antidromic spikes(46 +/- 10%, n=6), without any influence on the amplitude of EPSCs (105+/-8%, n=5) .
Dihidrokainate (DHK, 300 mkM), selective inhibitor of GLT-1 subtype of glutamate transporter, exerted a significant inhibitory action on the orthodromically evoked spikes (24+/-8% n=5), and also on the EPSC (60+/-12% n=5), while antidromic spikes in the presence of DHK were only slightly depressed: 90+/-6%,n=5.
Our results indicate that extrasynaptic and PSD membranes of CA1 neurons form separate compartments differing in the mechanisms and efficiency of processing external Glu. This allows separate regulation of the synaptic transmission and electrical excitation of pyramidal CA1 neurons.

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P15-13

PSYCHOTIC DRUGS AND ENDOCRINE SYSTEM
Hadj-Bekkouche F., Bouchenak O.

The aim of this work was to assess the effect of the psychotic drugs on the endocrine system.Three groups of drugs were tested : neuroleptic (Nozinan and Largactil), antidepressant (Anafranil) and anxiolytic (Diazepam). Each of them was injected peritoneally to ten maleWistar rats. The control animals received the same volume of vehicle (NaCl 0,9%). The body weight of all the animals was regularly registred.
The duration of the treatment was five weeks. After that the animals were killed by decapitation. The blood samples were collected with EDTA, centrifuged and plasma was stored at –20°C for subsequent hormone determination. The organs (thyroid, testis)were processed for structural study. Serum testosterone, FT3 and FT4 were measured by radioimmunoassay using kits “Immunotech”.
The body weight was significantly lower in treated rats with the anxiolytic drug (P <0,01) than the control animals. The testosterone and FT3 increased (P<0,05) in treated rats with Diazepam.
Serum FT4 was increased (P<0,05) with the Largactil and (P<0,01) with the Diazepam and the Anafranil.
Our results show that the three groups of the drug influence the thyroid system, whereas only the anxiolytic drug seems to be involved in the testicular activity and body weight. The mechanisms remain to be elucitated.

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P15-14

THE INFLUENCE OF OSTEORECEPTOR IRRITATION AND ELECTROSTATIC FIELD ON THE RAT BRAIN NEUROTRANSMITTERS
Praulite G., Jankovskis G., Porozovs J.

The brain monoaminergic and other neurotransmitter systems have significant role in the process of adaptation of organism to the influence of environment factors. Various factors influence different receptors and so different nervous structures are induced into the reaction. The influence of electrostatic field (ESF) on organism in vivo is supposed to begin from skin receptors and then through nervous system is transmitted to inner organs. The visceral and somatic reactions of organism are influenced by viscereroreceptors. Human organism obtains notion about gravitation, vibration and other factors through the bone sensor system. The determination of the content of biogenic amines: norepinephrine (NE), dopamine (DA), serotonin (SER) and inhibitory neurotransmitter gamma-amino butyric acid (GABA) in the rat brain corpus striatum, hypothalamus and medulla oblongata was performed according to the method of Earley, Leonard. Male Wistar rats were used in these experiments. In the experiment of osteoreceptor stimulation experimental animals group was submitted to 5 procedures of injection of 0,2 ml isotonic solution in the osteoreceptive zone of femur bone during two weeks. In the experiment of ESF influence the animals were exposure to ESF for 2 or 4 hours daily over a period of 1, 3 or 14 days. The ESF used had an intensity of 60 - 200 kV/m. Results of the investigation showed that the stimulation of osteoreceptors reduces the content of GABA in hypothalamus and SER in corpus striatum. ESF causes a significant influence on the content of rat brain monoamines. Brain noradrenergic system is the most sensitive to this factor. Increased intensities of ESF cause the reaction of dopaminergic system but longer exposure – of serotoninergic system. The research results showed that irritation of osteoreceptors and ESF cause different changes in rat brain mediator systems. Obviously, physiological mechanisms of the influence of these two factors on organism are different.

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P15-15

ELECTRICAL LESION OF PARAGIGANTOCELLULARIS NUCLEUS ALTERED ACUTE PAIN PERCEPTION IN PRESENCE OF A2 ADRENERGIC RECEPTOR
Fathi Moghaddam H., Kesmati M., Rezaie S., Mohammed Pour Kargar H.

Paragigantocellularis lateralis nucleus (LPGi) involves in several functions such as cardiovascular regulation, sexual behavior, withdrawal syndrome and pain. In addition, the role of a2 adrenergic in analgesia has been reported as well. Here we are studied the role of a2 adrenergic receptor in LPGi on acute pain.
45 NMRI rats were used. These animals have been divided in 6 groups: 1. Control, 2. Sham Control, 3. Lesion group, 4. Lesion+Saline group, 5. Lesion+0.2 mg/km group and 6. Lesion+2 mg/km clonidine. Electrical lesion

has been made bilaterally in LPGi. After Surgical process LPGi was lesioned using electrical DC current (1mA, 6 second), with stainless steel electrode placed in stereotaxic coordinates of (AP=11.8, Lat.±1.86 and Depth=10.5). Pain perception tested using standard tail flick test.
1. No significant difference found between the control group and the sham group.
2. There is a significant difference between the control group and the lesion group (P<0.0002).
3. There is a significant difference between the control group and lesion+saline group (P<0.0002).
4. There is no significant difference between the lesion group and lesion+saline group.
5. There is a significant difference between the lesion+saline group and lesion+0.2 mg/km clonidine (P<0.0001).
6. There is a significant difference between the lesion+saline group and lesion+2 mg/km clonidine (P<0.0004).
We conclude that a2 adrenergic receptors of LPGi nucleus has not a major role in the clonidine induced analgesia and clonidine may be affects other parts of CNS to induce analgesia in rat.

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P15-16

AUDITORY EVOKED POTENTIALS IN CHILDREN WITH AUTISM:ELECTROPHYSIOLOGICAL/CLINICAL RELATIONSHIPS
Bonnet-Brilhault F., Gomot M., Barthelemy C., Bruneau N.

Auditory processing at the cortical level was investigated with late auditory evoked potentials in 26 children with autism (AUT) aged 4 to 8 years (mean age +/- SEM = 71 +/-2 months) compared to 16 normal age-matched children (NOR) (mean age +/- SEM = 69 +/-3 months). The stimuli used were 750 Hz tone bursts of 200 ms duration delivered binaurally at different intensity levels (50, 60, 70, 80 dB SPL) with interstimulus intervals varying between 3 to 5 sec. Two negative peaks occurred in the 80-200 ms latency range, the first culminated at fronto-central sites (N1b) and the second at bitemporal sites (N1c, equivalent to Tb of the T complex). The latter wave was the most prominent and reliable response in the NOR group. In the AUT group temporal N1c were of smaller amplitude and longer latency. A particular pattern of asymmetry was recorded in this group at the highest level of intensity (80 dB SPL) with greater N1c amplitude on the right than on the left side (the reverse was found in the NOR group). To evaluate relationships between temporal AEPs and the severity of disorders on verbal and non-verbal communication we used a behavioural scale (BSE-R). Electro-clinical correlations indicated that the greater the amplitude of the right temporal N1c responses, the higher the verbal and non-verbal communication abilities. This suggests a particular pattern of left-right hemisphere functions in autism which concerns the secondary auditory areas situated on the lateral surface of the superior temporal gyri were N1c is supposed to be generated.

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P15-17

EEG AND DOPPLER CEREBRAL VASCULAR EXPLORATION TO A LOT OF WORKERS EXPOSED TO A PHONIC POLLUTION
Gusti S., Gusti A., Georgescu D., Berila I.

We studied 29 healthy workers with normal audibility (the working of compressor in the tehnological process of obtaining oxigen) 30-50 year-old, with over 7 years lengh of service. We registered EEG on the BIOSCRIPT 2000. We determined the cerebral irrigation with noninvasive Doppler (D) method, using a sonde of 4 MHz (Vasodop type, made in INNOMED, Hungary) connected to a PC with facilities of registration and calculation of D. curves parameters. We made these tests after 16 hours of rest, in order to catch the remanent effects of the noise influence above the cerebral circulation and we compared the values to a control lot of 40 healthy people with no phonic exposure.
We remarked a decrease of the systolic speed with 11%, a delay of the flux and an increase of the resistence index with 28% that signified a hipertonicity in cerebral circulation. These modifications were not significantly correlated with the q wave presence a 14% in the EEG to the studied lot. These results could be explained by the noise stimulation of the reticular formation and mainly of the sympathetic nervous system with hypersecretion of catecholamines.

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P15-18

THE STUDY OF CAROTIDIAN DOPPLER VELOCIMETRY TO A LOT OF PATIENTS WITH DIABETES MELLITUS OF TYPE 1
Gusti A., Gusti S., Georgescu D

The authors have studied the cerebral irrigation to a lot of 26 patients with diabetes mellitus of type 1, 15-60 year-old, of which 11 new-diagnosed and 15 over one-year diagnosed. We used the non-invasive method with Doppler (D) ultrasounds. We used a Sonopan U.D.P.-10 with pulsatile emission of 8 MHz connected to a polyinscriptor 6NEK-4 (made in Germany) for the registration of carotidian D. curves, E.C.G. and phonocardiogram simultaneouslly. We analyse and statistically processed the D. curves parameters and we remarked:
-to the new-diagnosed patients, a decrease of the diastolic speed with 21% and a decrease of the resistence index with 25%; it means the presence of hypotonicity in great and middle vessels of the cerebral circulation;
-to the over one year diagnosed patients, an important decrease of the systolic speed with 24% on right primitive carotid, respectively with 15% on the left one, and a decrease of the diastolic speed with 20% (respectively 16%); that noticed a significant decrease of the irrigation in cerebral territory, modifications determined most of the coexisting atherosclerosis and less of the diabetes mellitus.

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P15-19

THE EFFECT OF FEEDBACK ON VISUAL EVENT RELATED POTENTIALS DURING TIME-PRODUCTION TASK
Khodanovich M., Bushov Y., Vyachistaya Y.,.Ivanov A.

The perception and production of short intervals of time and correction of this production by feedback is a fundamental perceptual process that is relatively poorly understood. We hypothesized that reproduction of time intervals will be corrected due to feedback presence, and this correction will be manifested in changes of event related potentials (ERP). Present study explored effect of the feedback on visual ERP of 10 healthy male students. Procedure included presentation of visual stimuli of 200 and 800 ms duration in random sequence and reproduction of these durations by double pressing a key. In series with feedback relative error of reproduction in percents was presented to the subject after the each reproduction. ERP were averaged on presentation of visual stimuli of 200 and 800 ms duration. Components of ERP were identified by Principal Component Analysis. In the presence of feedback for both 200 and 800 ms durations we found increase of N1 amplitude and negativity of slow positive-negative component with latency from 480 to 540 ms for 200 ms duration and to 1000 ms for 800 ms duration. Also we observe an appearance of negative component with latency of 230 ms in the presence of feedback. The effect of feedback on the components with 230 and 480 ms latencies was significantly greater when 800 ms visual stimuli were presented in comparison with 200 ms stimuli. Also we found increase of P3b amplitude for 800 ms duration independently of feedback presence. We suppose that negative component with 230 ms latency is related to collation of the presented stimulus duration with internal time standard and is similar to the processing negativity (PN). Later negative component with 480 ms latency probably indicates the correction of internal time clock. The differences between reproduction of 200 and 800 ms durations we account for differences of mechanisms functioning during time reproduction of these intervals.

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P15-20

KAINIC ACID LESIONS TO THE PONTINE RESPIRATORY GROUP ELIMINATE COUGH AND EXPIRATION REFLEX IN CATS
Poliacek I., Jakus J., Stransky A., Barani H., Halasova E., Tomori Z.

The importance of neurons in the pontine respiratory group for generation of the cough and expiration reflex was studied.

Experiments were performed on 12 non-decerebrate spontaneously breathing cats under pentobarbitone anaesthesia.
The cough reflexes were regularly evoked during control inspiration by mechanical stimulation of both the tracheobronchial and the laryngopharyngeal mucosae membranes. Similarly, the expiration reflex was induced by mechanical stimulation of the glottis.

Dysfunction of the neurones in the pontine respiratory group (the rostral-dorsal lateral pons) was produced by bilateral microinjection (150 - 200 nl) of excitotoxin kainic acid. This lesions regularly abolished any signs of the cough reflex in all 114 tests of tracheobronchial and 79 tests of laryngopharyngeal stimulation. Similarly, the expiration reflex was absent in all 213 tests of glottal stimulation.
The pontine respiratory group seems to be an important source of the facilitatory inputs to the brainstem circuitries that mediate cough and expiration reflex. Our results indicate the significant role of pons in multilevel organization of brainstem networks in central integration of aformentioned reflexes.

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P15-21

IN VIVO EXAMINATION OF FLUORESCENT BONE MARROW CELLS USING A NEW FOCAL CEREBRAL ISCHEMIA MODEL
Kubis N., Pinard E., Tomita Y., Lévy B.I., Seylaz J.

Focal cerebral infarction was induced in C57/Bl6 mice through a cranial window in order to visualize in vivo remodelling following the administration of fluorescent bone marrow cells.
A closed cranial window (Æ 3 mm) was chronically implanted over the left parietal cortex of young male adult C57/Bl6 mice under isoflurane anesthesia. Focal ischemia was induced by fine cautherization of 2 branches of the middle cerebral artery across the cranial window. Local blood flow was recorded in 7 equidistant areas over the window using laser-Doppler flowmetry before, immediately after ischemia and at days 1, 5, 10 and 14. Stromal bone marrow cells (BMC) from male age-matched transgenically modified GFP (Green Fluorescent protein) mice were injected intravenously immediately after the induction of ischemia. Fluorescent cells were regularly investigated through the superficial layers of the cortex using laser-scanning fluorescence confocal microscopy. After mice sacrificing, brains were processed for histology and sections examined under confocal microscopy to detect GFP+ BMC and to analyze their possible modification in phenotype expression.
In all mice, local blood flows were reduced by 50% after induction of ischemia and were close to 0 the day after. Initial pre-ischemic values were attained between day 10 and day 14 for 50% of mice. Infarcts were located in all cortical layers throughout the dorsal hippocampus. Confocal microscopy showed that during this period, fluorescent cells invested the ischemic area and the ipsilateral hippocampus, that is a well-known site for neurogenesis. Some of these fluorescent cells evidenced numerous and long processes resembling neurons.
The present model provides in vivo information of possible brain remodelling occurring after BMC injection. Ongoing studies are evaluating the short and long-term consequences of cerebral ischemia treated with BMC in terms of angiogenesis, gliogenesis and neurogenesis.

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P15-22

HIGH PREVALENCE OF THE ASSOCIATION OF OSAS AND ALTERATION OF ANS AT THE AGE OF 65. THE PROOF STUDY
Roche F., Pichot V., Duverney D., Costes F., Chomienne S., Garet M., Barthelemy J.-C.

Up to 5% of adults in Western countries are likely to have undiagnosed obstructive sleep apnea syndrome. OSAS had be found to be particularly highly prevalent in people older than age 65 years. The identification of OSAS as a risk factor for increased morbidity and mortality is needed in such older population since they should become candidates for treatment.
The autonomic nervous system (ANS) activity, a recognized marker of cardiovascular and all cause mortality in a general population, is precociously altered in OSAS : the degree of autonomic imbalance appears correlated with sleep fragmentation, inspiratory efforts and hypoxia.
We thus evaluated in a large cohort (n=1011) of 65±0.4 years old men (40%) and women (60%) free of cardiovascular of cerebrovascular event or of diagnosed SRBD, the prevalence of unexpected OSAS and its relationship with ambulatory blood pressure, spontaneous cardiac baroreflex sensitivity and basal cardiac autonomic activity.
According to the presence of nocturnal cyclical heart rate variability quantified using validated algorithm (VLFi, Roche et al. PACE 2002), the probability of OSAS was retained in 40% and 14% of this population with a VLFi treshold responding to, respectively 10 and 30 brady/tachycardia cycles per hour of sleep.
Using logistic regression analysis, the severity of the OSAS was highly correlated with spontaneous baroreflex sensitivity ($p<0.01$), and heart rate variability parameters (parasympathetic indicators, $p<0.0001$). Neither BMI, nor ambulatory blood pressure were significantely associated with OSAS.
Thus, the presence of OSAS and the alteration of ANS appears highly in a general 65 years old population. The follow-up of this parameter to determine the clinical implications of such findings in the occurrence of cardiovascular events is now proposed.

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P15-23

AUTONOMIC ACTIVITY EQUALS FRAMINGHAM RISK FACTORS IN PREDICTING CARDIOVASCULAR EVENTS. PROOF STUDY
Barthelemy J.-C., Roche F., Duverney D., Costes F., Joubert P., Garet M., Gaspoz J.-M., Pichot V.

A prospective cohort of 1011 subjects aged 65 years old at the entry of the study was selected from the electoral register from the town of Saint-Etienne, France.
They were free of cardiovascular event events. Their cardiovascular risk assessment was evaluated from the classical Framingham weighted risk factors which include age, body mass index, presence of diabetes, of hypertension, level of total and HDL cholesterol, triglyceride, and smoking habits.
The subjects were then classified according to these data from the highest to the lowest global risk. Nocturnal heart rate variability (HRV) was simultaneously assessed in the whole cohort. Subjects were again classified according their high frequency (HF) component power spectral density (Wavelet decomposition) from the lowest to the highest value.
The order of the classification of the two lists was then compared using rank correlation.
The pearson coefficient reached 0.995 ($p<0.000$) demonstrating an equivalent ability to summarize cardiovascular risk factors by HF or the complex set of Framingham risk factors.
The nocturnal HF PSD measurement should thus become an easy to use tool to assess global cardiovascular risk and monitor the benefits of medical intervention.

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P15-24

ALTERED FLOW-DEPENDENT VASODILATION IN GREAT ARTERIES DURING CEREBRAL LACUNAR INFARCTIONS
Bonnin Ph., Lavallée Ph., Amarenco P., Levy B.I.

Patients with cerebral lacunar infarction present an actual arteriolar dysfunction enhanced by decrease in the vasomotor reserve in the transcranial Doppler-CO_2 test, there is no data concerning endothelium dysfunction of the great arteries. We investigated alteration in flow-dependent-vasodilation in the brachial and common carotid arteries in 10 men and 7 women (60±2 years) with lacunar infarctions (stroke group: SG). A control age and sex matched group (n=20, 60±2 years) was investigated (control group: CG). In the brachial artery, blood flow velocity and diameters (Doppler and Echotracking Systems) were measured, before and during post-ischaemic hyperaemia. In the common carotid artery, they were measured, before and during hyperemia consecutive to a CO_2 breathing test. Basal diameter of the brachial artery were higher in men than in women in both groups (SG : 4.67±0.14 vs 3.67±0.01 mm, $p<0.001$, CG: 4.14±0.13 vs 3.31±0.10 mm, $p<0.001$). Furthermore, it was higher in men in SG versus CG (4.67±0.14 vs 4.14±0.13 mm, $p<0.05$). Basal diameter of the common carotid artery presented no gender difference but it was higher in SG (6.49±0.21 vs 5.75±0.12 mm, $p<0.01$). In both arteries, during reactive hyperemia, the relative increase in the diameter was lower in SG (brachial

artery: +4.22±0.51 % vs +6.71±0.71%, p<0.01, common carotid artery: +4.09±0.58 vs +11.36±1.32%, p<0.001). After nitroglycerin administration, the increase in diameter was lower in men and woman in the SG in the both arteries (brachial artery: +18.16±2.08 vs +28±1.47 %, p<0.05, common carotid artery: +10.62±1.16 vs 18.29±1.33 %, p<0.01). Patients with cerebral lacunar infarction presented a initial vasodilation in muscular and elastic arteries. As well as the vasomotor reserve is altered representative of a cerebral arteriolar dysfunction, the great arteries presented an actual endothelial dysfunction in patients with cerebral lacunar infartions.

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P15-25

DISTRIBUTION OF THE LOCUS COERULEUS (LC) AXONS WITHIN THE CEREBELLUM OF THE RAT.
Serapide M.F., Cicirata F.

Previous research evidenced that the cerebellum and the cerebellopetal projection of the LC are implied in the learning and memory processes. Despite of the importance of these processes, very few and conflicting data in the literature report a lack of organization in the LC fiber terminals which appeared aspecifically distributed on the cerebellum. The aim of the present study was to reinvestigate the organization of this pathway. Following iontophoretic injections of 10% biotinylated dextrane amine (BDA) solution into the unilateral LC, the distribution of the labeled fiber terminals in the cerebellar cortex (CC) and nuclei (CN) were studied. Labeled fiber terminals were found in the CC, prevalently. About 26% of them were found in the molecular layer; about 68% of them were found in the granular layer and about 6% on them were found on the border of the two layers. The LC fiber terminals appeared morphologically different from the climbing and mossy afferents; in fact, they were very thin and showed very fine varicosities no organized in rosette-like formations. Labeled fiber terminals were found in the granular layer of all cerebellar lobules with a vermal prevalence; in some cases segregated projections to the molecular layer of a few lobules (crus I and II, copula pyramidis) were found. Sometimes a single fiber innervated both layers. The projections appeared bilateral and simmetric; in some cases a banding-like pattern of the fiber terminals was observed in the granular as well as in the molecular layer. Bilateral projections were also found in all the CN; in a few cases the CN appeared innervated by the same fibers ascending to the CC. These results indicate that LC projects to the cerebellum with a precise and complex projection pattern. The distribution of these fibers in different layers of the CC as well as in the CN suggests that learning processes supported by LC may occur contextually in different links of the cerebellar machinery.

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P15-26

CHRONIC MDMA INDUCES PSYCHOTIC-LIKE MOTOR SYMPTOMS IN MICE, WITHOUT ALTERING SENSORIMOTOR GATING
Galan B., Flores J.A., El Banoua F., Caraballo I., Fernández Espejo E.

MDMA (3,4-methylenedioxymethamphetamine), a recreational drug of abuse, is thought to induce psychosis in humans. The present study was undertaken to discern if chronic MDMA administration (10mg/kg daily i.p. for 14 days) induce a psychotic-like state in mice. Animals were evaluated in different behavioral paradigms the last four days of treatment with MDMA alone or cotreatment with the antipsychotic clozapine (0,1 and 2 mg/kg i.p.) before tests, to confirm the psychotic-like nature of symptoms. The psychotic-like profile was established through several behavioural paradigms as follows: spontaneous locomotion (10 min-test), apomorphine-induced stereotypies (30 min after 1.5 mg/kg apomorphine i.p.), social interaction test with a conspecific mouse (10 min-test), and prepulse inhibition (acoustic stimulus; pulse, 120 dB; prepulses of 70 and 80 dB). The findings revealed that MDMA-treated mice presented an increased spontaneous locomotion (p<0.05 vs controls) and they moved faster (p<0.01). Apomorphine-induced stereotypies (head-bobbing) were also enhanced (p<0.01). In the social interaction test, the time spent exploring the cage was significantly increased (p<0.05), but social interaction time with conspecific was not significantly affected. Prepulse inhibition was not disrupted at the MDMA dose tested. Locomotor hyperactivity and enhanced exploration, but not stereotypies, were attenuated by clozapine (2 mg/kg). In conclusion, these findings suggest that chronic MDMA induces some psychotic-like motor symptoms since they are selectively ameliorated by clozapine, although sensorimotor gating (as measured through prepulse inhibition) was not affected.
Study supported by Ministerio de Ciencia y Tecnologia and Fundacio Marato de TV3(Barcelona).

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P15-27

ROLE OF GLUCOCORTICOID RECEPTOR ON MODULATION OF ACUTE PAIN: INTERACTION WITH OPIOID SYSTEM IN MICE
Vafaei A.A., Taherian A.A., Rashidy-Pour A., Miladi-Gorgi H., Jarrahi M.

Previous finding indicated that glucocorticoids might be effective in modulation of peripheral pain and this effect probably modulated by opioid system. The present work investigated the interaction of glucocorticoid receptors and opioid system on modulation of acute pain in Hot plate and Tail flick tests. Male albino mice (25-30 gr.) were used for the experiments. Dexamethasone (0.5 and 1 mg/kg) as a glucocorticoids agonist or saline were used 30 min and Naloxone (1 mg/kg) were used 10 min before the HP or TF test. Result indicates that dexamethasone in both doses has analgesia effect with comparison to control group and this effect modulated by opioid system (P<0.01). The finding provides further evidence for interaction between opioid system and glucocorticoids receptor on modulation of pain.

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P15-28

AUTONOMIC NERVOUS SYSTEM ACTIVITY AND PERFORMANCE IN COMPETITIVE SWIMMING: A FIELD STUDY
Garet M., Pichot V., Tournaire N., Roche F., Duverney D., Barthélémy J.C.

The aim of this study was to assess the potential use of heart rate variability (HRV) analysis, a sympathovagal indice, as a biomarker in the control of the effect of physical training loads on performance at an individual level as it has already been demonstrated in groups of elite sportsmen.
Seven regional-level swimmers (4 male, 3 female; 16.6±0.5 years; 169.3±5.9cm, 59.3±6.3kg, Tanner stages: S4) with a history of 6.4±0.9 years of practice, swimming 9 hours per week during the ongoing season. Maximal aerobic power performance (400-m freestyle) was performed before (P1), and after 3 weeks of intensive training (P2) and 2 weeks of tapering (P3). HRV (time and frequency domain) was recorded during the night before each performance as well as twice a week along the training program. Weekly training loads were individually quantified (sum of daily mileage*intensity*perceived exertion) and heart rate, perceived exertion, stroke rate and distance per stroke were monitored on each performance assessment. Data were compared using a two factors ANOVA, weeks and subjects for group analysis.
Mean and individual training loads along with perceived exertion increased from wk1 to wk3 and decreased to wk5 (P=0.018 to <0.0001). Global HRV and parasympathetic indices evolved individually, decreasing from wk1 to wk3 and increasing to wk5 in 5 swimmers, while they continuously decreased or increased in 2. All performances were maximal. Best and worst performances were respectively realized when global and parasympathetic indices of HRV were highest (P3 in 5 swimmers and P1 in 2), and lowest (P2 in 6 and P3 in 1). Excepted in 1 swimmer, the higher total power spectrum, pNN50 and RMSSD indices of HRV were, the better the performance.
Thus, HRV evolution is highly individual in response to training loads and is a powerful predictor of physical performance, a high global and parasympathetic activity being required for better performance achievement.

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P15-29

INTERRELATIONS OF PROTEASOME AND NEUTRAL PROTEINASES ACTIVITY CHANGES IN AGED RATS
Dosenko V., Zagoriy V.

Turnover of cell proteins is provided by lysosomal and proteasomal proteolysis. Compensative, age-related proteolysis activation may be caused by increase in the level of modified proteins. There are no clear notions

about changes of proteasomal proteolysis in aging. The aim of this work was to study possible interrelations of proteasomal and lysosomal proteolysis activity in blood leukocytes, tissues of brain and aorta. Experiments were performed on male rats in the age of 5 (control) and 18 (experiment) months. Monocytes, lymphocytes and polymorphonuclear leucocytes (PMN) were isolated from blood using centrifuging in density gradient of Percoll. Tissues of brain and aorta were homogenized in Tris-HCl buffer (pH 7,4). Activity of proteasome was evaluated by intensity of fluorogenic substrate hydrolysis with spectrophotometer Hitachi 4000. Specificity of proteolysis was confirmed by inhibition of reaction with selective proteasome inhibitor clasto-lactocystin-beta-lactone. Activity of neutral proteinases was established by protamine sulfate degradation intensity. It was shown that level of activity of neutral proteinases in the tissues of brain and aorta was decreased in old rats comparing with control, and the activity of proteasome is increased. Activity of proteasome in monocytes and lymphocytes in aged animals is higher then in control. Such a controversial character of changes in activity of neutral proteinases and proteasome is a manifestation of adaptive mechanisms: activation of proteasome proteolysis in cortex and aorta prevents abnormal protein accumulation and development of neurodegenerative and angiosclerotic changes; on the other side, decrease of proteasome activity in lymphocytes and monocytes prevents expression of autoantigenes, and, thus autoimmune pathology in animals of given age. Decrease of neutral proteinases activity in cells with increased proteasomal activaty can reflect interrelation between proteasomal and lysosomal proteolysis.

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P15-30

DOPAMINE NEURONS OF THE PERIAQUEDUCTAL GREY PARTICIPATES IN REWARD AND HYPOALGESIA AFTER HEROIN

Flores J.A., Galan B., EL Banoua F., Caraballo I., Fernandez-Espejo E.

The dopaminergic network of the mesencephalic periaqueductal grey (mPAG) was described several years ago (Lindvall and Bjorklund, Handbook of Psychopharmacology. New York, Plenum Press, 1974). However, considering the key role of dopamine in opiate addiction, it is surprising that this network has been ignored in drug reinforcing studies. Besides, the mPAG is critical mediating opiate-induced analgesia, but the role of dopamine mPAG neurons on nociception is not known. The objectives were: i) to further describe the morphological characteristics of this dopaminergic network, ii) to discern if these dopaminergic cells are activated after heroin administration, and iii) to establish the effects of selective lesions of DA mPAG neurons on heroin-induced reward (evaluated through conditioned place preference) and locomotor sensitization, as well as on opiate-induced analgesia. Nociception was evaluated through the tail-immersion (spinal pain reflex) and the hot-plate tests (integrated pain-related response). Immunohistochemical findings revealed that the dopaminergic mPAG network was composed of scattered dopaminergic cells of 15-30µm in diameter (estimated number=129.4±31.3). These cells were activated following heroin treatment, because they expressed c-Fos. Following dopamine depletion (61.9% dopamine cell loss, 80.7% reduction of dopamine content), conditioned place preference to heroin was abolished ($p<0.05$, Wilcoxon test), but not heroin-induced sensitization. Besides, analgesia after heroin was significantly attenuated by lesion in the hot-plate test. The present study provides evidence that the dopaminergic network of the mPAG is activated after opiate treatment and: i) mediates the rewarding effects of heroin, and ii) participates in integrative nociceptive responses after opiate administration.

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P15-31

EFFECT OF SODIUM FLUORIDE AND MAGNESIUM SULFATE ON MAN'S BRONCHIAL HYPERRESPONSIVENESS

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Initial definitions of asthma focused on a primary defect in airway smooth muscle as the cause of asthma. Thus, therapy focused on relaxing airway smooth muscle constriction. Because of the common resistance to beta 2 memitics, the research of new products appears necessary. Magnesium sulfate (MgSO4) have been used to treat sever bronchoconstriction with its competitive action on calcium channel. Sodium fluoride (NaF) have been tested on rats ; it had a relaxing effect on muscle constriction. So, the purpose of this study is to determine and compare the effects of NaF and MgSO4 on man bronchial hyperresponsiveness. Method : Three groups of volunteer subjects (aged between 20 and 30 years) had received inhalation of : The first group NaF (0.5M)(10 subjects), the second group, MgSO4 (12 subjects) and the third, beta 2 agonist (16 subjects, as a control group). All subjects had a similar initial flow expiratory volume in one second (FEV1). After a bronchial challenge with a significant decrease on FEV1, NaF, or MgSO4 or beta 2 agonist were given by an inhalation. Than, FEV1 was measured at 5, 10, 15 and 20 minutes later. Statistical analysis by non parametric tests (Friedman ANOVA, and Wilcoxon test) was done. Results : All subjects have a bronchial hyperresponsiveness. MgSO4, gives an unconstant relaxing effect wich is obtained after 20 minutes. NaF (0.5M) as Beta 2 mimetic give a constant relaxing effect after 5 minutes. A significative difference is constated between three groups at 5, 10, 15 and 20 minutes specially between beta2 agonist group and MgSO4 group. NaF and MgSO4 have a relaxing effect on man's bronchial constriction. The first, by the inhibition of the glycolysis and the second by its competitive action on calcium channel. So, their association can be benefic for asthmatic subjects.

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P15-32

NEURONAL DISINTEGRATION IN SCHIZOPHRENIA

Strelets V.B.

The work is aimed at the study of neuronal assemblies integrity disturbances in schizophrenic patients.
The methods of coherence and spectral covariation were used.
The results obtained by the former method revealed that during performance of the task on visual imagery and mental arithmetic in the normal subjects there were many interhemispheric connections mostly in anterior cortical regions at the high frequency beta-rhythm. In schizophrenic patients the interhemispheric connections in this situation were absent. The data got by the second method used showed the decrease of the spectral power distribution coincidence in different cortical areas at the high frequency beta-rhythm in schizophrenic patients, this decrease corresponding to the severity of their clinical manifestations.
The functional interhemispheric disintegration and spectral power distribution discrepancies in schizophrenic patients could be the most important link of their defected neuronal clusters, complexity and Gestalt.

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P15-33

CENTRAL EFFECTS OF STRESS-RELATED NEUROPEPTIDES ON HEART RATE AND HEART RATE VARIABILITY IN TROUT

Mimassi N., Lancien F., Mabin D., Conlon J.M., Le Mével J.C.

Corticotropin releasing–hormone(CRH) and urotensin-I (U-I) are two members of a family of stress-related neuropeptides. These neuropeptides share partial sequence identity, pharmacological properties and physiological functions. The present study was conducted to compare the central effects of these two peptides on heart rate (HR) and heart rate variability (HRV), a marker of vagal input to the heart, in the unanesthetized trout Oncorhynchus mykiss. In addition, their actions were compared with the effects of angiotensin-II (ANG-II), a neuropeptide which is known to affect HR and HRV in trout. The trout were equipped with two ECG electrodes and an intracerebroventricular (ICV) guide was inserted within the third ventricle of the brain. The fast Fourier transform was used to analyse the HRV. The ICV injections of vehicle (0.5 µl) or CRH(1 pmol) had no effect on the recorded parameters. However, ICV injection of 5 pmol of CRH caused a 12 % ($P < 0.01$) decrease in HR during the 15-20 minutes post-injection period. Furthermore, HRV was increased by 120 %. Injection of CRH-(9-41)-peptide (a CRH antagonist) had no effect on HR or HRV but blocked CRH-induced bradycardia. ICV injections of urotensin-I (5 pmol) produced no significant change in HR and HRV. By contrast, ICV administration of ANG-II (5 pmol) elicited a highly significant 33% ($P < 0.001$) increase in HR and a marked 64% reduction in HRV. The results demonstrate that CRH and U-I exhibit differential effects on HR and HRV. CRH may produce

bradycardia by a mechanism that involves the enhancement of the parasympathetic drive to the heart.

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P15-34

MODULATORY ROLE OF NORADRENALINE ON GLUTAMATE ELICITED EXCITATION OF VESTIBULAR SECONDARY NEURONS

Caldera M., Barresi M., Li Volsi G., Licata F., Santangelo F.

It is known that noradrenaline (NA) depress the spontaneous firing rate of vestibular secondary neurons and this effect is mediated by alpha2 noradrenergic receptors. It remains to ascertain if and at which extent NA action on vestibular neurons is also exerted by a modulation of the responses evoked by classic neurotransmitters. The present study was aimed to determine whether NA is able to modulate neuronal excitatory responses evoked by glutamate (glu) in the vestibular complex (VC). In male Wistar rats anesthetized with urethane (1.3 g/kg) we recorded the firing of single VC neurons and applied NA, glu and related drugs in situ by microiontophoretic technique, to check the specificity of the effects exerted by the amine on glu-evoked excitations. Long lasting (up to 10 min) applications of NA at low intensity currents (2-20nA), producing weak or no effect on the spontaneous firing rate, modified the glu-elicited excitations in 98% of the tested neurons. NA application depressed the intensity of glu-evoked responses in about 78% of the neurons. Effects were significantly higher in lateral and medial nuclei than in superior vestibular nucleus. Glu-evoked responses appeared enhanced in a minority of cases (less than 20%). The depressive effects of NA on glu-evoked excitations were mimicked by applications of clonidine, alpha2 noradrenergic receptor agonist. Anyway, neither NA nor clonidine induced a complete block of glu-evoked responses. These results indicate that in the presence of NA, and then during stress, the responsiveness of VC neurons to the activation of their most important input, the primary vestibular fibers, is strongly attenuated.

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P15-35

A STUDY OF MODIFICATIONS IN ELECTRORETINOGRAM AND VISUAL EVOKED POTENTIALS IN COLOR STIMULATION

Ramboiu S., Iancau M., Georgescu D., Badea P., Rosoiu C., Donoiu I., Voican C., Enescu-Bieru D., Georgescu M., Nestianu V., Rusca N.

The extreme complexity of the process of percepting color means that there is little information on the tests referring to this subject in the specialized literature, and thus motivates our study.
Healthy, volunteer subjects have been investigated, with the recording of electroretinogram (ERG) and visual evoked potentials (VEP), obtained both through TV reversal pattern stimulation: white-black, green-black and red-black, and LED reversal pattern stimulation: yellow-green.
ERG potentials were obtained through the use of active electrodes placed on the lower eyelid, and VEP were sampled through the Fz-Oz derivation.
The amplification of potentials was made with a Beckman R611 polygraph, with the time constant of the amplifier of 0.3 seconds, and the frequency filter at 30 Hz. The sampling of biosignals was made with a frequency of 1000 Hz and a resolution of 12bit, and the extraction of ERG and VEP through the mediation of 100-150 stimulations.
The latencies, the amplitudes and the duration of ERG and VEP waves were measured and retained for the statistical processing.
The analysis of the results allows the relevation of high-significant differences between the answers obtained through TV monitor LED stimulation and between those obtained through red and white light stimulation.
The increase of the studied group and the completion of testing with another luminous radiations is necessary for the establishment of own standard values.

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P15-36

PROJECT FOR IMPROVEMENT OF PHYSIOLOGY PRACTICAL SESSIONS IN THE HACETTEPE UNIVERSITY MEDICAL FACULTY

Balkancı D., Rıdvanağaoğlu A.Y., Yörükan S., Pehlivanoğlu B., Dikmenoğlu N., Finci S., Duman O., Yılmaz G., Durmazlar N., Tuncer M., Erdem A., Ergönül Z., Duran F.

Objectives:The HUMF Physiology Department carries out 31 practical sessions in 12 subjects in parallel with the lectures of the hybrid teaching system for medical students. In this project, carried out during the 2000-2001 and 2001-2002 academic years, we aimed to introduce contemporary laboratory materials reflecting recent technological advances, carry out human experiments, increase student interest in laboratory studies and achieve student motivation by updating our course. Methods: Due to the large student classes and limited sources, the Muscle, Peripheral Nerve and Circulation practicals were reviewed, and experiments with new content and instruments were organised. The students were in groups of 23-27 and experiments were carried out as demonstrations on volunteer students using a computerized data acquisition and analysis system (Biopac MP 100). Following the session, student feedback was obtained using a 5 point evaluation scale (Likert) questionnaire. After the ECG demonstration, the ECG reading skills of 32 randomly selected students were evaluated using the checklist. Results: The views of 339 participating students were obtained. Interest in Physiology lectures increased in 86.7 % and in laboratory research in 82.7 %, while 74.8 % found use of modern instruments stimulating, 91.0 % stated that the experiments had strengthened their theoretical knowledge and 90.3 % indicated that they wished to carry out the experiments individually. ECG demonstrations were generally regarded as inadequate for gaining skills. Conclusion: Practical sessions are known to be an integral part of Physiology courses. Introduction of contemporary technology, and experiments the students can perform themselves, will strengthen motivation and increase preclinical skills, thus contributing to the training of highly qualified physicians.
This project was supported by the H.U. Research Fund project no. 00.01.101.017.

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P15-37

SYNTHESIS OF CONTROLLER USING THE NEURAL MODEL

Iancu I., Iancu E., Sfredel V.

The work of Hodgkin and Huxley and their equations for ionic channels have created the natural starting point for the discipline of computational neuroscience. This led to various types of mathematical models of neurones. The authors propose a model, described by the input-output relation, who use:
ui(t)- the excitatory afferents; vj(t)- the inhibitory afferents; ui0(t)- the values of the thresholds for excitatory afferents; vj0(t)- the values of the thresholds for inhibitory afferents; ai, bj- the weight of synapse i or j; fe(t)-the efferent signal; t0-an input-output delay time constant.
Using this model, we have demonstrated the possibility to create different structures with special functions like amplification, attenuation, integration, etc.
Because the control structures are essential for biological systems, we have shown in this paper how to synthesise a controller for PI law with five neurones. Two neurones, N1 and N2, together with their synaptic connections act like an integration module. The neurone N3 makes the sum and the neurones N4 and N5 amplify the signal. By choosing the appropriated values of ai and bj we can modify the parameters of the controller.
In conclusion, we assert that, with a minimum number of neurones, the brain can create local controllers, which are also subordinated to the hierarchical system. Because the signals are frequency modulates, the controllers are less perturbed and have a high degree of robustness. The authors use these structures to automatic control of robotic arms and have significant results in control of mobile robots.

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P15-38

AMPLIFICATION OF SYNAPTIC EXCITATION IN SPINAL MOTONEURONES BY PERSISTENT INWARD CURRENT
Hultborn H., Enríquez Denton M., Wienecke J., Nielsen J.B.

Spinal motoneurones may display prolonged plateau potentials and self-sustained firing following short lasting depolarisation or synaptic excitation. These plateau potentials are due to persistent inward currents through voltage dependent, non-inactivating Ca^{2+} channels (Hultborn, Prog. Br. Res.1999;123:39-48; Powers & Binder, Rev. Physiol. Biochem. Pharmacol. 2001,143:137-263). These channels appear mainly to be located in the dendritic area, which also receives most of the synaptic input. The present study tests the hypothesis that one of the functions of the persistent inward Ca^{2+}-currents may be to boost synaptic excitation. Intracellular recordings were made from spinal motoneurones in decerebrate cats. A strikingly non-linear summation occurred, when synaptic excitation by pyramidal tract stimulation or muscle stretch was added to the firing caused by current injection through the intracellular microelectrode. The additional firing frequency induced by the synaptic excitation increased stepwise as the motoneuronal activity induced by current injection was increased within the primary range. We interpret this as a sign of a stepwise increase of the synaptic excitation by the persistent inward current. Recurrent inhibition induced by stimulation of motor axons caused a constant reduction of firing frequency when added at different firing frequencies induced by current injection along the primary range. However, when the current injection alone was strong enough to force the firing into the secondary range the reduction in firing frequency by recurrent inhibition increased dramatically. We interpret this as due to effective reduction of the voltage-dependent dendritic persistent inward current by the synaptic inhibition. We propose that the motoneuron´s dendritic area, because of the persistent inward currents, serves to amplify the net synaptic depolarisation, which is then converted into a frequency code in the soma and initial segment.

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P15-39

A BIOLOGICALY PLAUSIBLE NEURAL-NETWORK FOR SOUND-SOURCE LOCALIZATION
Polevaya S.A., Tikidji – Hamburyan R.A.

A biologicaly plausible neural-network model of human auditory systems is constructed to simulate production localization of a virtual sound source under dichotic stimulations. Modified "Integrate – and – Fire" neurons are used for designing of two populations with global projections, making analogy between the model and good known physiologically identified neurons. The model simulates auditory responses by ensembles of cortical neurons in the right and left hemispheres under conditions of an interaural time delays, with limits from 0 ms to 10’000ms and a pitch of 200ms. The model allows suggesting mechanism of detection of short time delays by slow elements and shows the several phenomena associated with auditory spatial illusions.

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P15-40

ELECTROPHYSIOLOGICAL INVESTIGATION OF VOLLEYBALL: PARTICULARITIES AND INFLUENCE OF PERFORMANCE LEVEL
Cravéa C., Magnié M.N.

The current study aimed at evaluating the particularities of Visual Evoked Potentials (VEPs), Brainstem Auditory Evoked Potentials (BEAPs), and the P300 component in volleyball players at professional and regional levels. Evoked potentials were recorded from 30 right-handed 18-35 year old men. They were divided in three age-matched groups: 10 professional volleyball players, 10 regional volleyball players, and 10 sedentary subjects. VEPs were obtained using a reversal pattern and BEAPs according to the standard protocol. The P300 component was elicited in both visual and auditory modalities with the classical oddball paradigm. We failed to find differences between volleyball players and sedentary subjects for VEPs and BEAPs. In the visual modality, volleyball players from both groups showed shorter P300, P3a and P3b latencies than sedentary subjects. In the auditory modality, volleyball players from both groups differ from sedentary subjects by larger N2 and P300 amplitudes. No significant differences were found between regional and professional volleyball players neither for VEPs, BEAPs nor for the P300 component in both modalities. Nevertheless, professional players differ from sedentary subjects by shorter N2 latencies, larger P300 and P3b amplitudes in the visual modality, and also by larger P3a and P3b amplitudes in the auditory modality. Moreover, regional players presented larger P2 amplitudes than sedentary subjects in visual modality. Present data suggest that volleyball players are enable to process faster visual information at cognitive level without particularities of the sensory pathways. In addition, they might allocate a greater amount of attentional ressources than sedentary subjects in the auditory modality. Finally, electrophysiological studies are needed to further track particularities in volleyball as a function of the performance level since we found different P300 patterns in regional and professional volleyball players.

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P15-41

THE EFFECT OF GLUTATHIONE DEPLETION ON LEARNING
Ocakcioglu A.B., Parlak F.

The oxidant/antioxidant balance involves in regulation of brain function and the antioxidants may play an important role in prevention of the progressive cognitive impairments. An endogenous tripeptide glutathione (GSH) has been suggested to have neurotransmitter and neuromodulatory functions besides its antioxidant role. GSH has been found in great amounts in brain. Therefore we aimed to investigate the effect of depletion of glutathione on an aspect of learning procedure. For this purpose, the rats were classically conditioned and diethylmaleate (DEM), a glutathione depletor, was administered to the test group at a dose of 6 mmol/kg an hour before the test. The rats were trained as 25 trials/day for three consequtive days by using two ways active avoidance shuttle box in the classical conditioning test. In the test, a light (60 W) for 6 sec as conditioned stimulus and an electrical shock (0.8mA) for 4 sec as unconditioned stimulus were used. The electrical shock was applied through the grid floor and ended if the rat cross to the other compartment of the box. If the rat cross during the light flashing, this was called as avoidance response. In the control group (n=6), the conditioned avoidance response (CAR) of the rats gradually increased on three consequtive days since there were significance between first and third day’s, and second and third day’s CAR values (p= 0.028, and 0.027, respectively). However a significant inclement was not observed in DEM administered group (n=8). Each day’s CARs of the test group were significantly low compared to the control group’s responses. For instance, p=0.006, p= 0.001 and p=0.001 for the first, second and third day, respectively. Our results indicate that GSH involves in learning processes since its depletion disrupts learning. In conclusion, the glutathione affects learning processes through its effects on oxidant homeostasis and/or as a signalling mediator in brain.

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P15-42

THE EFFECTS OF CHRONICAL $MnCl_2$ APPLICATION ON LEARNING AND MEMORY OF RATS
Bastug M., Zaloglu N., Ficicilar H., Karaorman G., Aydin A.

Manganese (Mn)is an essential trace element at low concentrations.When it is present in excess quantities in tissues,it may be toxic.The neurotoxic effects are seen in deep brain tissues.Changes in central nervous system are considered as a critical health effect.In this study we aimed to investigate over dose (30mg/kg/day) effects of Mn application on learning and memory of rats.30 Wistar rats were used in the experiments. Nine of the rats were injected $MnCl_2$ for 50 days and kept as experimental group.9 of the rats were injected saline and kept as sham operated group. 12 rats were kept as control group.The rats were trained by using automated two ways active avoidance shuttle box to test the learning and memory on the 31st days. The acquisition tests (AT) were terminated with training the rat from each group to be 25 trials per day during 3 days.Ten days after the last AT,the retention test was performed and the acquisition of the conditioned avoidance responses (CAR) of the rats were evaluated.At the end of the experimental period,under the light anesthesia blood samples were collected for the measurments of plasma and erytrocyte Mn levels.Brain was removed for the measurement of the brain Mn and malondialdehid(MDA)levels.The Mn levels increased significantly. There were not considerable differences between the brain MDA levels.The CAR of all rats from three groups showed a significant increase in three consecutive days while the differences observed in CAR of same sessions were not significantly different among three groups.The

memory process of these rats also was not affected significantly.According to our results we suggest that, the learning and the memory mechanisms were not affected by the chronical $MnCl_2$ application.There were no differences between the brain MDA levels suggesting that, chronical $MnCl_2$ application has no effect,on the brain,and also,on learning and memory mechanisms due to antioxidative adaptation and other possible regulatory mechanisms.

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P15-43

INHIBITION KINETICS AND EXPRESSION OF GLUTAMATE TRANSPORTER IN RETINAL PIGMENT EPITHELIAL CELLS
Mäenpää H., Tähti H.

Retinal pigment epithelial (RPE) cells form the blood-retina barrier, and their glutamate transporters are essential for retinal homeostasis. Glutamate is the main excitatory neurotransmitter in the retina. The toxicity of glutamate is connected to the dysfunction of glutamate transporter. Our objective was to study the expression and kinetics of glutamate transporters in the RPE cells in vitro. The second aim was to clarify the effect of tamoxifen and toremifene on the glutamate transporter. These compounds are used in the breast cancer therapy and tamoxifen has caused retinal changes as a side effect.
The pig RPE culture and two human RPE cell lines, D407 and ARPE-19, were used. The cultures were solubilised in buffer containing 1% Triton X-100, 0.1% deoxycholate and 0.1% SDS and separation of proteins was done with SDS–PAGE. Proteins were blotted onto nitrocellulose and the binding of five known glutamate transporter antibodies was detected with ECL. Glutamate uptake inhibition was investigated by using L-[3H]glutamate as a tracer. The cells were exposed to 0.1-5 μM tamoxifen for 7 days (western blots) and to 7.5 μM tamoxifen/ toremifene for 10 min (uptake assays).
The transporter subtypes EAAT4 and EAAC1 were found in RPE cells. EAAT4 was expressed only in the cell lines. The EAAC1 signal was stronger in the cell lines compared to the pig RPE cells. Tamoxifen did not change the EAAT4 expression. In contrast, in the kinetic analyses tamoxifen and toremifene increased the Km constant for glutamate transport, which indicates that inhibition evoked by them is competitive. Both drugs were more effective in the human RPE cell line than in the pig RPE cells. This result showed for the first time that the antioestrogens tamoxifen and toremifene hamper glutamate transport by replacing glutamate as the substrate.

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P15-44

VIGILANCE STATES AND CIRCARDIAN RHYTHMS OF TEMPERATURE IN THE MPTP MICE.
Monaca C., Laloux C., Gelé P., Kreisler A., Bordet R., Destee A., Derambure P.

Introduction : Sleep troubles and vigilance disorders (excessive daytime sleepiness and sleep attacks) are frequently observed in Parkinson's disease. Among the animal models of Parkinson's disease, MPTP mice have been used in biochemical, pharmacological studies but not in behavioural analysis. No study have explored vigilance states in this model. In this present study, we aimed to explore sleep and circadian rhythms (locomotor activity and temperature) in MPTP mice, compared with wild-type mice.
Methods : MPTP was injected at two doses : in the first group of mice at 25mg/kg/jr during 5 days and in the second group at 15mg/kg/2h x 4 in one day. After recovery, polysomnography were recorded during 48 hours and circadian rhythms during 7 days in " light-dark » conditions and during 7 days in " dark-dark » conditions. The dopaminergic lesions of substantia nigra were evaluated in all mice by using immunohistochemical markers.
Results : Compared with wild-type, the 2 groups of MPTP mice presented abnormal temperature circadian rhythms : a global hypothermia along the 24 hours (mean of temperature was 37.8°C in wild-type and 36.4°C in MPTP mice) and a decrease of the temperature amplitude. Sleep analysis revealed a decrease in paradoxical sleep amounts in MPTP mice compared with wild type (109.4 ± 5.6 min versus 83.4 ± 8.4 min). Finally, the immunohistochemistry analysis showed a dopaminergic neurons loss (inferior to 50% in substantia nigra).
Conclusion : 1) These mice have a loss in substantia nigra like parkinsonian patients. 2) We have observed an increase of paradoxical sleep in MPTP mice. This result could be compared with the fact that parkinsonian patients have paradoxical sleep during the day. 3) MPTP mice have an hypothermia, like parkinsonian patients. In view of these results, the MPTP mice could be used as a behavioural model of sleep disturbances in Parkinson's disease.

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S16 ROLE OF GAP JUNCTIONS IN VASCULAR TISSUE

ORAL SESSION

S16-1

VASCULAR CONNEXINS AS TARGETS FOR MODULATION OF GAP JUNCTION COMMUNICATION

Pohl U., Khandoga N., Kameritsch P.

Gap junctions (GJ) are clusters of channels that allow transfer of low molecular weight substances and electrical currents between neighbouring cells. In the vascular system these channels are formed by various combinations of four membrane-spanning proteins, the connexins (Cx). GJ allow the signal exchange within endothelial and smooth muscle layer and in addition there is evidence that coupling exists also between both cell types. The different expression of the Cx in endothelial cells (Cx 37, 40, 43) and smooth muscle cells (Cx 40, 43, 45) may be the basis for the observations of rectified dye transfer between endothelial- and smooth muscle cells and apparent differences between exchange of currents and small solutes. Moreover, recent data indicate that transmigration of leukocytes can be altered significantly by precedent gap junctional coupling with endothelial cells. Though our understanding of the coupling between endothelial cells and smooth muscle cells or leukocytes is still incomplete it is obvious that the transfer of signals via GJ is regulated. The endothelial autacoids NO and PGI2 have already been shown to act as modulators of gap junctional communication. While PGI2 increases coupling via a cAMP dependent pathway of pre-existing gap junctions, NO is decreasing it. There is, however, also some evidence that NO, under certain conditions, can improve coupling by increasing the formation of new gap junctions. In transfected HeLa-cells this different action of NO is based on selective effects on different connexins. NO increases the membrane incorporation of Cx40-GJ and reduces the permeability of already incorporated GJ formed by Cx43. These data suggest that the coupling between the vascular cells is subject to modulation by endothelial- and leukocyte derived compounds. The functional significance of this modulation for vasomotor responses and vascular growth is yet fully understood and subject of further investigations.

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S16-2

INFORMATION NETWORK IN THE ARTERIAL WALL

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Introduction: The main task of the arterial system is to secure an adequate supply of oxygen to organs. For this purpose, the diameter of the arteries is finely adjusted to allow an adapted blood flow. The arteries receive information from nerves and hormones. In addition, the blood vessels are under the influence of local metabolic, autocrine, paracrine and physical factors. The convergence of these factors implies the integration of multiple signals in the vascular wall. What is the role played by gap junction communications in this integration?
Method: Gap junctions, the structural support for intercellular communication were shown by electron microscopy. Electrical coupling was demonstrated by intracellular microelectrodes. Chemical coupling was observed by microinjection of fluorescent dyes. Inter-cellular calcium diffusion was detected by calcium imaging.
Results: Two networks of homocellularly coupled cells were demonstrated: the excitable smooth muscles forming the media and the non-excitable endothelial cells forming the endothelium. Myoendothelial bridges connect these two networks. Electrical and chemical signal spreads between these two networks in a symmetrical way in small vessels but in an asymmetrical way in large vessels. Electrical signals that are efficiently conveyed in these two networks are not the same. An action potential propagates in the media but is not regenerated in the endothelium. At the opposite, an electrotonic signal spreads more easily in the endothelium than in the media.
Conclusion: The reactivity of the arterial wall to an agonist cannot be deduced by the extrapolation of the response of one cell, but it results from the interaction of the different cell type responses through the information network described above.

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OC16-1

RHO-ASSOCIATED KINASES MODULATES MICROVASCULAR PERMEABILITY IN CAT SKELETAL MUSCLE IN VIVO

Lundblad C., Bentzer P., Grände P.-O.

The size of the intercellular gaps of the capillary wall is suggested to be an important factor for regulation of microvascular permeability. The formation of the intercellular gaps are dependent on the shape of the endothelial cells, in turn influenced by the contractility of intraendothelial filaments. Rho associated kinases are supposed to be involved in modulation of intracellular actin-myosin contraction, and may thereby affect both protein and hydraulic microvascular permeability. Hydraulic and protein permeability may be determined by partly different mechanisms, as protein molecules pass the capillary wall through the large pores located at the venous side of the capillary bed, whilst fluid passes through small pores along the whole capillary bed. The aim of the study was to investigate whether, and to what extent Rho kinases influence hydraulic and protein microvascular permeability.
The study was performed on the autoperfused cat skeletal muscle. A capillary filtration coefficient (CFC) technique was used to evaluate a change in hydraulic permeability, while alterations of protein permeability were evaluated by estimation of the change in the reflection coefficient for albumin. In the first part of each experiment, the effects on CFC of three doses of the Rho kinase inhibitor Y-27632 of 0.35, 0.70 and 1.05 mg/h per mL plasma flow were determined.
There was a significant reduction in CFC at the lowest dose, and a tendency to further reduction at the higher doses used, reaching a decrease in CFC of 20%. The effects on CFC of the high and the middle dose did not differ. The reflection coefficient for albumin was increased by 31% following infusion of the highest dose of Y-27632.
We conclude from these results that hydraulic and protein microvascular permeability increase by Rho kinase activation, and that Rho kinases may be involved in the general regulation of microvascular permeability.

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OC16-2

TRANSIENT DEVELOPMENTAL APPEARANCE OF MYOENDOTHELIAL GAP JUNCTIONS AND EDHF IN RAT SAPHENOUS ARTERY

Sandow S.L., Goto K., Rummery N.M., Hill C.E.

Myoendothelial gap junctions (MEGJs) are integral for endothelium-derived hyperpolarizing factor (EDHF) activity in a number of vascular beds. In the femoral artery of the mature rat an absence of MEGJs accounts for a lack of EDHF activity and of electrical coupling between endothelial cells (ECs) and smooth muscle cells (SMCs). However, no studies have examined the incidence of MEGJs and EDHF activity during development. In the present study, arterial morphology and MEGJ incidence were examined in the saphenous artery of the juvenile and adult WKY rat, using connexin immunohistochemistry and serial section electron microscopy, while acetylcholine (ACh) induced EDHF activity (in the presence of L-NAME, 100uM, and indomethacin, 10uM) was assessed with intracellular microelectrodes and myography. Lumen diameter, the number of SMC layers and EC area were significantly smaller in juvenile than in adult arteries (n=4; 133±4um, 3.9±0.5, 346±16um2 versus 233±16um, 7.1±0.1, 392±9um2). On the other hand, MEGJs were prevalent in the saphenous artery of the juvenile (n=4), but absent in the adult. While ACh did not evoke an EDHF relaxation in the adult, ACh elicited a dose-dependent hyperpolarization and relaxation in juvenile arteries. In these vessels, EDHF was abolished by the combined application of apamin (0.5uM) and charybdotoxin (60nM; n=5), or apamin and the IKCa antagonist, TRAM-34 (50nM; n=4), or significantly attenuated by the Cx-mimetic peptide combination 43Gap26, 40Gap27 and 37,43Gap27 (100uM each; n=4). Barium (30uM) and ouabain (0.5mM; n=3) in combination, nitric oxide scavengers (n=4), catalase (1250U/ml; n=3), or 14,15-EEZE (10uM; n=4) had no effect. We conclude that in the rat saphenous artery during development, ACh-induced relaxation and hyperpolarization is due to the transient appearance of MEGJs. The presence of MEGJs and EDHF during development provides further support for the involvement of heterocellular coupling in EDHF activity.

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OC16-3

EFFECTS OF CONNEXIN-MIMETIC PEPTIDES ON RENAL EDHF-RESPONSE, RENAL BLOOD FLOW AND BLOOD PRESSURE

Van de Voorde J., De Vriese A.S.., Lameire N.H.**

In vitro studies support a role for gap junctions in EDHF-mediated signal transmission. The present study aimed to examine the contribution of gap junctional communication to the EDHF-mediated responses in the rat renal microcirculation in vivo and to address the potential physiological role of EDHF. Therefore the effects of intrarenal administration of connexin-mimetic peptides on the L-NAME- and indomethacin-resistant renal blood flow response to acetylcholine, on basal renal blood flow and on systemic blood pressure were examined. 43Gap 27 (3.91 mg), a peptide homologous to the second extracellular loop of connexin 43, partially inhibited the L-NAME- and indomethacin-resistant renal blood flow response to acetylcholine, whereas 40Gap 27 (3.87 mg), homologous to the second extracellular loop of connexin 40, abolished the response. A control peptide (3.62 mg), with a replacement of two amino acids in the motif SRPTEK present in the second extracellular loop of connexins 40 and 43, was without effect. None of the peptides affected the vasodilator responses to DETA-NONOate, pinacidil or papaverine. Intrarenal infusion of 43Gap 27 or 40Gap 27 decreased basal renal blood flow and increased mean arterial blood pressure, both in the presence and absence of systemic infusion of L-NAME and indomethacin. The influences of the peptides on acetylcholine-response, basal renal blood flow and blood pressure were reversible since they disappeared 30 min. after administration. It is concluded that inhibition of gap junctional communication with connexin-mimetic peptides blocks EDHF-mediated signal transmission in vivo, as suggested by the abolishment of L-NAME- and indomethacin-resistant renal vasodilatation to acetylcholine. The peptides also decrease basal renal blood flow and increase blood pressure, supporting a role for tonic EDHF release in the control of tissue perfusion and vascular resistance.

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OC16-4

ANTIOXIDANT VITAMIN C INCREASES VASCULAR GTP CYCLOHYDROLASE I ACTIVITY IN MOUSE AORTA

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It has been shown that vitamin C (Vit C) increases nitric oxide synthase (NOS) enzyme activity via chemical stabilization of its co-factor tetrahydrobiopterin (BH4) in cultured endothelial cells. Our aim was to determine the in-vivo effects of Vit C on eNOS function and BH4 turnover in the aorta of C57BL/6J mice treated for 7 months without and with Vit C (1%/ kg chow). NOS enzyme activity was determined by L-[14C]citrulline assay. BH4 levels and GTP cyclohydrolase I (GTPCH I) activity were determined by RP-HPLC. Isolated aortic rings were suspended in organ chambers (37°C; 94% O_2/6% CO_2; pH 7.4) and isometric forces were recorded. Chronic Vit C treatment increased plasma levels of L-ascorbic acid from 2.0±0.1 to 5.5±0.8 mg/dL ($P<0.05$; n=5). Calcium-dependent NOS activity was increased in the aorta of Vit C fed mice (978±204 fmol/mg/min $P<0.05$ vs. wild-type mice: 548±74 fmol/mg/min; n=7). In addition, calcium-independent NOS activity was augmented after Vit C treatment ($P<0.05$; n=7). Significantly higher GTPCH I enzymatic activity were detected in aortas after Vit C treatment (1.1±0.2; $P<0.05$ vs. control: 0.6±0.1 pmol/mg of neopterin; n=4-5) and this was associated with increase in vascular BH4 levels (37±14 vs. 11±3 pmol/mg; $P<0.05$). On the other hand, aortic 7,8-dihydrobiopterin levels, the oxidized form of BH4, were unchanged. In contrast, endothelium-dependent relaxations to acetylcholine (0.001-10 µmol/L) were reduced in aortas of Vit C treated mice (78±3% vs. 91±1% for control mice; $P<0.05$; n=9-10). Endothelium-independent relaxations to NO donor diethylamine-NONOate (0.001-10 µmol/L) were also reduced and concentration-dependent curve was shifted to the right ($P<0.05$; n=8). Lucigenin-enhanced chemiluminescence assay showed no change of superoxide anion levels. Our results demonstrate that long-term Vit C treatment increased both GTPCH I and NOS enzymatic activities. This is associated with reduced vascular reactivity to both endogenous and exogenous NO.

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S16-3

GAP JUNCTIONS AND ENDOTHELIUM-DEPENDENT RELAXATION

Griffith T.M., Chaytor AT., Edwards D.H.

Synthetic peptides possessing homology with the extracellular loops of the major vascular connexins (Cxs 37, 40 and 43) have been used to investigate the role of direct cell-cell coupling in arterial relaxations that are independent of NO and prostanoids and widely attributed to a putative endothelium-derived hyperpolarizing factor (EDHF). Such peptides interrupt intercellular communication in a highly connexin-specific fashion, and in rabbit iliac and ear arteries EDHF-type relaxations evoked by acetylcholine (ACh) are inhibited by 37,43Gap27, a peptide homologous to the Gap 27 domain of Cxs 37 and 43. In iliac arteries diffusion of the fluorescent dye calcein from the endothelium into the media and transmission of ACh-evoked endothelial hyperpolarizations into the subintimal smooth muscle layer are both blocked by 37,43Gap 27, confirming that the EDHF phenomenon involves electrotonic signalling via myoendothelial gap junctions rather than a freely transferable EDHF. Although 37,43Gap27 only partially inhibits EDHF-type responses in rabbit cerebral arteries, in this vessel relaxation can be abolished by 37,43Gap27 in combination with 40Gap27, a peptide homologous to the Gap 27 domain of Cx40, indicating that there may be regional heterogeneity in the nature of the participating connexin subtypes. We have also shown that ACh promotes a prostanoid-independent increase in endothelial cAMP synthesis that plays a critical role in the EDHF phenomenon. Indeed, in rabbit arteries the adenylyl cyclase inhibitor 2/,5/-dideoxyadenosine abolishes ACh-evoked smooth muscle hyperpolarizations and the associated relaxation. This role of cAMP appears to be permissive and reflects modulation of electrotonic signalling via gap junctions, as EDHF-type relaxations, EDHF-type hyperpolarizations of smooth muscle cells remote from the endothelium and diffusion of calcein from the endothelium into the media are each potentiated by inhibition of cAMP hydrolysis with isobutylmethylxanthine.

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S16-4

COMMUNICATION IN THE MICROCIRCULATORY NETWORK – GAP JUNCTIONS AND VASOMOTOR CONTROL

de Wit C.

Intercellular communication via gap junctions contributes to the coordination of arteriolar behaviour within the microcirculation. This communication is reflected by the conduction of vasomotor responses (dilations or constrictions) along the arterioles after locally confined application of vasomotor substances. Upon localized acetylcholine (ACh) stimulation in vivo, a hyperpolarization is initiated in both endothelial (EC) and vascular smooth muscle (VSM) cells at the local stimulation site that travels along the vascular wall. Blockade of Ca^{2+}-dependent K^+-channels (charybdotoxin, ChTx) abrogated VSM, but not EC hyperpolarization. If ChTx was applied at the site of ACh stimulation, local and remote dilations were inhibited. In contrast, ChTx treatment at remote sites reduced neither local nor remote dilations. The intercellular gap junction channels are formed by different connexins (Cx37, Cx40, Cx43). Cx40 is expressed in the endothelial cell layer and the conduction of dilations is reduced in mice deficient for Cx40. Interestingly, these mice are hypertensive (117±10 vs. 92±4 mm Hg). Despite blockade of NO-synthase or increasing dosages of an AT1-receptor antagonist, the pressure differences between genotypes were still observed. Systemically applied boli of ACh and a NO-donor were equally potent and effective to induce pressure decreases in both genotypes. However, Cx40-deficient arterioles exhibited an irregular vasomotion pattern consisting of a localized constriction which eventually led to a complete but intermittent flowstop.

Taken together, we conclude that EDHF initiates a local hyperpolarization which spreads along the vascular wall and the hyperpolarization itself induces remote dilations. Cx40 is important to support this conduction pathway. The loss of Cx40 not only alters conduction along arterioles, but is also associated with arterial hypertension. Thus, we propose that Cx40 has a significant role in the control of peripheral vascular resistance.

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S16-5

CONNEXINS IN ENDOTHELIAL INJURY AND REPAIR
Kwak B.R.

Gap junction channels allow the direct exchange of ions and small metabolites between neighboring cells thus coordinating physiological processes such as tissue homeostasis and cell differentiation. These channels are formed by members of a family of related proteins called connexins (Cx). Each type of gap junction channel has unique gating properties and permeabilities to various molecules and ions. The endothelium, a regulatory organ that mediates hemostasis, blood coagulation, vascular tone and leukocyte interactions in the vessel wall, is known to express multiple Cx. Gap junctions in arterial endothelium in situ consist mainly of Cx40 and Cx37. Cx43 is moderately expressed or absent in quiescent endothelium but is induced under conditions associated with endothelium injury or dysfunction. Hypertension, hyperlipidemia, smoking and local hemodynamic forces induce endothelium dysfunction, an early event in atherosclerosis. Interestingly, high levels of Cx43 in the rat aortic endothelium are exclusively localized in areas facing turbulent blood flow and increased expression of Cx43 has been demonstrated in endothelial cells at the shoulder region of atherosclerotic lesions. Several in vitro and ex vivo studies also show a positive correlation between endothelial Cx43 expression and oscillatory shear stress. Direct mechanical injury to endothelial cells in culture is known to increase Cx43 and decrease Cx37 expression in cells close to the wound. Such an inverse in Cx expression suggest that these two types of gap junction channels may differentially regulate the cell-to-cell transfer of factors controlling endothelial repair. Indeed, endothelial wound repair is delayed when gap junctional communication has been reduced by dominant negative Cx. In summary, current evidence suggests that gap junctional communication may have a regulatory function in endothelial injury/dysfunction and repair, this way affecting the evolution of atherosclerosis.

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POSTER SESSION

P16-01

SEVERE ACUTE PANCREATITIS AND REDUCED ACINAR CELL APOPTOSIS IN MICE DEFICIENT FOR THE CX32 GENE
Frossard J.L., Rubbia-Brandt L., Benathan M., Ott T., Willecke K., Chanson M.

The early events leading to acinar cell injury during acute pancreatitis are poorly characterized. Signalling through gap junction channels contributes to the homeostasis of the exocrine pancreas by coordinating acinar cell activity within an acinus. To explore the role of gap junctional communication (GJC) in acinar cell response to injury, we analysed the course of acute pancreatitis induced by injection of cerulein in mice deficient for Cx32, the major gap junction protein expressed in the exocrine pancreas. The severity of pancreatitis was evidenced by measuring serum amylase activity, pancreatic edema, acinar cell necrosis, pancreas TNF-alpha concentration and myeloperoxydase activity. Acinar cell apoptosis was detected by TUNEL, caspase-3 activity and Bax/BCl-2 expression. Connexin expression and function were evaluated by immunofluorescence and dye coupling. Cx32-deficient mice exhibited a deleterious course of acute pancreatitis with increased necrosis, edema and inflammation of the exocrine pancreas. In addition, the exocrine pancreas of Cx32-deficient mice showed decreased number of TUNEL positive acinar cells, decreased caspase-3 activity but no change in Bax or BCl-2 pancreatic expression. Interestingly, chemicals known to induce apoptosis in vivo had no effect on Cx32-deficient pancreatic acinar cells. Deficiency of a pancreatic connexin converts a mild reversible form of acute pancreatitis into a severe disease and decreases the sensitivity of acinar cells to apoptotic stimuli. The results demonstrate that acinar cell-to-cell communication plays a key role in the modulation of acute pancreatitis severity.

Geneva University Hospitals – Switzerland

P16-02

HIGH PHENYLEPHRINE (MM) INDUCES TRANSIENT ENDOTHELIUM-DEPENDENT RELAXATION OF PIG FEMORAL ARTERIES
Woodley N.

Phenylephrine (Phe) dose-response experiments (10 nM to 1 mM) conducted on in vitro pig femoral arteries, revealed that 1 mM Phe induced a transient relaxation of endothelium-intact (n=6) but not denuded (n=6) arteries. To determine the mechanism behind this relaxation, the responses of endothelium-intact arteries to Phe (0.1 and 1 mM), acetylcholine (ACh 1 mM), and sodium nitroprusside (SNP 1 mM) were assessed in the presence and absence of: Nw-nitro-L-arginine (NNA 10 mM, n=8), a nitric oxide synthase inhibitor; and the combination of nordihydroguaearetic acid (NDGA) and indomethacin (INDO), inhibitors of 5-lipoxygenase and cyclooxygenase respectively (both 10 mM, n=6). Contraction to 0.1 mM Phe (11.5 ± 1.3 g) and relaxation to SNP (6.9 ± 0.6 g) were not affected by any blocker tested. By 1 min, 1 mM Phe induced a 28 ± 4% relaxation of the 0.1mM Phe tension; which reversed to a contraction (9 ± 4%) by 10 min. NNA significantly attenuated the relaxation induced by 1 mM Phe (20 ± 8% of control) and ACh (control: 106 ± 13% of SNP relaxation; NNA: 44 ± 13%) indicating the contribution of endothelium-derived nitric oxide in both conditions. Although NDGA and INDO significantly attenuated the ACh induced relaxation (72 ± 10%), the relaxation to Phe was unaffected (90 ± 31% of control). Since modification of Phe-induced tone in arterioles has been attributed to intercellular communication between the smooth muscle and endothelium, the high doses of Phe used in this study may have allowed sufficient communication between arterial smooth muscle and the endothelium to facilitate increased nitric oxide release that then buffered the contraction.

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P16-03

SYNCHRONIZATION OF ARTERIAL SMOOTH MUSCLE CELLS
Lamboley M., Bény J-L., Schuster A., Meister J-J.

Objectives: Our goal was to investigate how the physiological behavior of individual smooth muscle cells integrates into microscopic arterial wall behavior to leads to contraction and spontaneous diameter oscillations of arteries, called vasomotion.
Method: We have improved a method that allows studying the correlation of calcium oscillations (flashing) of individual SMCs with mean calcium

variations and arterial contraction using confocal microscopy. Endothelium-stripped rat mesenteric arteries were cut open, loaded with dual calcium fluorescence probes. Intracellular calcium concentration was expressed as a fluorescence intensity ratio obtained from Fura red acetoxymethyl ester (AM) and Fluo-4 AM fluorescence. Loaded strips were stimulated by increasing concentrations of the vasoconstrictors phenylephrine (PE) and potassium chloride (KCl).
Results: We demonstrated that the number and synchronization of cells where $[Ca^{2+}]i$ transiently increases (flashing cells) depends on vasoconstrictor concentration. At low vasoconstrictor concentration, few cells flash asynchronously and no local contraction is detected. At medium concentration, recruitment of cells is complete and synchronous, leading to strip contraction after KCl stimulation and to vasomotion after PE stimulation. High concentration of PE leads to synchronous calcium oscillations and fully contracted vessels, whereas high concentration of KCl leads to a sustained non-oscillating increase of calcium and to fully contracted vessels.
Conclusion: Our results suggest that the number of simultaneously recruited SMCs is an important factor, controlling rat mesenteric artery contraction and vasomotion rather than simply the mean SMCs' $[Ca^{2+}]i$. Independently from the Ca^{2+} source (induced by PE or KCl), the cell displays Ca^{2+} oscillations, but in our experimental conditions vasomotion only occurs after stimulation with PE.

EPFL and University of Geneva – Switzerland

P16-04

ROLE OF GAP JUNCTIONS IN THE MECHANISM UNDERLYING CEREBRAL VASOMOTION
Haddock R.E., Brackenbury TD., Hill C.E.

Spontaneous oscillations in vessel diameter are an inherent feature of many blood vessels in vivo and in vitro and may play a role in regulating blood flow. We have investigated the mechanism underlying vasomotion in caudal cerebellar arteries of young rats (14-17 days). Changes in membrane voltage were measured using intracellular microelectrodes, while changes in global muscle calcium or intracellular calcium within individual smooth muscle cells (SMCs) were assessed using the ratiometric dye Fura 2AM and either photometry or an intensified cooled CCD respectively. Vessel diameter was monitored with video microscopy. Spontaneous contractions were preceded by spontaneous depolarizations, oscillations in global calcium and synchronized oscillations in calcium in adjacent SMCs. Nifedipine (1uM) abolished vasomotion, depolarized SMCs and desynchronized calcium oscillations in SMCs. The PLC inhibitor U73122 (10uM) also abolished vasomotion, but hyperpolarized SMCs and abolished intracellular calcium oscillations. Removal of the endothelium resulted in irregular contractions and asynchronous calcium oscillations in adjacent SMCs. This was not due to loss of nitric oxide-induced cGMP as L-NAME (10uM) increased the frequency and amplitude of vasomotion without a change in membrane potential. The gap junction uncoupler 37,43Gap27 (100uM) produced a loss of vasomotion and hyperpolarization of the SMCs, while 40Gap27 resulted in irregular contractions and desynchronization of calcium oscillation in SMCs. Serial section electron microscopy confirmed that myoendothelial gap junctions connected the endothelium to the SMCs. Using immunohistochemistry, connexins (Cx)37, 40 and 43 were found in the endothelium, while Cxs37 and, to a lesser extent, Cx43 were expressed in SMCs. We conclude that the IP3 store is essential for intracellular calcium oscillations in SMCs and these are synchronized by calcium influx through L-type calcium channels and cell coupling with the endothelium.

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P16-05

TRANSDIFFERENTIATION OF PREADIPOSE CELLS INTO SMOOTH MUSCLE CELLS/ ROLE OF ACLP
Abderrahim-Ferkoune A., Bezy O., Astri-Roques A., Ailhaud G., Amri E.Z.

ACLP (aortic Carboxypeptidase Like Protein) is a secreted protein that associates with the extracellular matrix (ECM) and is highly expressed in vascular smooth muscle cells of blood vessels. Our results show that ACLP is expressed in the stroma vascular fraction of the adipose tissue (containing preadipocytes) but not in mature fat cells. During differenciation of preadipose cell lines, ACLP is expressed at the adipoblast and preadipose stage and its expression decreases gradually during adipose conversion. Ectopic expression of ACLP by retroviral infection of 3T3-F442A preadipocytes inhibit their ability to undergo adipogenesis under conditions permissive for adipose conversion observed at both morphological and molecular level. Further more these cells are able to transdifferentiate into smooth muscle cells, exhibiting specific markers such as smooth muscle-alpha actin, and SM22alpha. Transdiferenciation of adipose tissu precursor cells into smooth muscle cells could open a new area of cell therapy for musclar diseases

Institute of Signaling, Developmental Biology and Cancer Research CNRS 6543 Nice

S17 ALDOSTERONE: SYNTHESIS, TARGETS AND FUNCTIONS

ORAL SESSION

S17-1

SYNTHESIS OF ALDOSTERONE IN NORMAL AND PATHOLOGIC HEART

Delcayre C., Heymes C., Bendall J., Garnier A., Fuchs S., Oliviero P., Robidel E., De Angelis N., Nehme J., Ambroisine M.L., Swynghedauw B., Milliez P.

The RALES trial has shown that aldosterone (Aldo) inhibition decreases mortality in patients with heart failure (HF), and part of this effect is due to reduced cardiac fibrosis. This points to a crucial role of Aldo in cardiovascular pathologies.
In rat, Aldo induces pericoronary inflammation and cardiac fibrosis. Production of Aldo has been evidenced in the heart of rodents and man. In rat heart, the key-factors steroidogenic acute regulatory (StAR) and Aldo-synthase (AS) are present and Aldo is produced at low level. The heart thus has a system of mineralocorticoid synthesis that is regulated by Ang II alike the adrenal steroidogenic system. Aldo production is increased in the non-infarcted zone of left ventricle after myocardial infarction and is involved in the ventricular remodeling. In man, activated cardiac Aldo production and AS mRNA upregulation has been described in patients with HF or with essential hypertension. In mice overexpressing the AS gene in heart, females have increased cardiac AT1-receptor and cardiac fibrosis, and male have a major coronary dysfunction. These observations support the idea that Aldo from cardiac origin may have detrimental effects in pathological conditions. However, the mechanisms of Aldo action in heart are not totally clear since cardiac specific inducible expression of a mineralo-corticoid receptor antisense mRNA in mice induces interstitial fibrosis and congestive HF.
The function of the cardiac steroidogenic system in physiological conditions remains to discover, and the mechanisms of Aldo actions in heart to understand. Namely, the mineralo-corticoid receptor selectivity for Aldo and the molecular targets of this system remain an open question. It appears however that increased Aldo production, as observed in several pathological states, may participate to cardiac dysfunction. Beneficial effects of anti-Aldo treatment in heart failure may thus be secondary in part to blockade of cardiac Aldo action.

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S17-2

MINERALOCORTICOID RECEPTOR: AGONISM AND ANTAGONISM

Rafestin-Oblin M.E., Fagart J., Pinon G., Bens M., Vandewalle A.

In the kidney, corticosteroid hormones regulate sodium absorption by binding to the mineralocorticoid receptor (MR) that belongs to the nuclear receptor superfamily. The crystal structure of the ligand-free and agonist-bound ligand-binding domains (LBD) has been solved for several nuclear receptors. The major difference between the two structures is linked to the positionning of the helix H12 that allows the recruitment of transcriptional coactivators in the active structure. The crystal structure of the MR-LBD has not been yet established. However, an homology model of the human MR-LBD has been constructed. By using a site-directed mutagenesis approach, we have shown that the contact between Asn770 of the MR and the 21-hydroxyl group of aldosterone is crucial to trigger the active receptor conformation state. Glucocorticoid hormones are also characterized by a 21-hydroxyl group but differ from aldosterone by their substituents at positions 11, 17 and 18. Cis-trans cotransfection assays revealed a preferential activation of MR by aldosterone over glucocorticoid hormones, a feature related to the impairement of glucocorticoid hormones accomodation within the MR ligand-binding cavity. Due to its inability to contact Asn770, progesterone, as well as 17a-hydroxyprogesterone and 20a-hydroxyprogesterone, acts as mineralocorticoid antagonists, as assessed in the immortalized mouse mpkCCDcl4 collecting duct cells by using the short-circuit current method. In contrast, we found that 11b-hydroxyprogesterone activates MR, whereas it displays antagonist activity when bound to MRA770, a mutant MR in which Ala is substituted for Asn at position 770. These findings provide lines of evidence that the mineralocorticoid agonist/antagonist feature of a steroid is depending upon its contact with MR-Asn770.

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OC17-1

SPECIFICITY OF TRANSACTIVATION BY RAINBOW TROUT MINERALOCORTICOID RECEPTOR

Sturm A., Rafestin-Oblin M.-E., Flouriot G., Fagart J., Prunet P.

Despite absence of aldosterone in fish, a recent study identified a partial cDNA sequence of a putative mineralocorticoid receptor in trout (rtMR). The rtMR bound cortisol at a higher affinity than aldosterone, in agreement with the osmoregulatory role of cortisol in fish (Colombe et al., 2000). However, binding does not necessarily reflect hormonal selectivity on the level of transactivation. This study investigates the potential of hormones to stimulate transactivation by the rtMR. The complete coding sequence of the rtMR was obtained by screening a genomic DNA bank followed by 5'RACE. Sequence analysis of the rtMR revealed that it is a homolog of the human MR (hMR). The ligand binding pocket of the rtMR strongly ressembles that of the hMR, suggesting a similar hormone selectivity between the two receptors. The transactivation properties of the rtMR were analysed in COS-7 cells co-transfected with receptor construct and a reporter gene under the control of the MMTV promotor. Among a series of corticosteroids, the order of transactivation capacity is similar between rtMR (this study) and hMR (Hellal-Levy et al., 1999). Aldosterone and 11-deoxycorticosterone (DOC) were the most potent activators of rtMR transactivation with EC50 = 0.1 nM. Glucocorticoids such as cortisol, corticosterone, dexamethasone were at least 10 times less potent. Surprisingly, the MR antagonists spironolactone and progesterone displayed agonist activity on rtMR, an effect also observed with a reporter construct using the TK promoter. In conclusion, (i) rtMR shows high ressemblance to hMR, but an unusual behavior with antagonists (ii) Cortisol is probably not the only ligand of the rtMR (iii) DOC, being the most activator of the rtMR in vitro, is detected in trout plasma, and hence a likely candidate to constitute a selective physiological ligand of the rtMR.

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OC17-2

THE DIFFERENT EFFECTS OF STEROIDS ON HEART MUSCARINIC RECEPTORS AND BETA-ADRENOCEPTORS

Myslivecek J., Ricny J., Trojan S.

Steroid hormones are known to change the expression of huge amount of proteins including receptors. We studied the effects of hydrocortisone and dexamethasone on muscarinic receptors, b2- and b1-adrenoceptors, on some subsequent steps of signal transduction mediated via these receptor types and on the effects of dexamethasone on muscarinic receptors, b2- and b1-adrenoceptors. Adult male Wistar rats were: 1) injected daily by hydrocortisone for 1 to 6 days, or 2) infused by dexamethasone for 1, 3 and 7 days using Alzet osmotic minipumps implanted subcutaneously and the receptors have been characterized by radioligand binding studies. All differences mentioned bellow were significant (unpaired two-tailed Student's t-test). Hydrocortisone enhanced the densities of muscarinic receptors in the atria and both ventricles. The density of bl-adrenoceptors became enhanced in the atria, and that of b2-adrenoceptors was raised in the atria and ventricles. According to the effects of GTP on the binding of carbachol, the coupling of muscarinic receptors with G proteins was not affected. The activity of adenylyl cyclase (determined by HPLC) and its stimulation by isoprenaline and inhibition by carbachol were not significantly altered. Dexamethasone caused dose-dependent increases in muscarinic receptors and bl-adrenoceptors in atria and left ventricles (preceded by decreases), but also the dose-dependent decrease in the right ventricles. b2-adrenoceptor number first decreased than increased in left ventricles and decreased in the right ventricles. Preliminary experiments using RT-PCR have showed the increase in M2 and b1 mRNAs after 7-day infusion of dexamethasone in atria. The findings raise the question of possible roles of different glucocorticoids in the control of the expression of neurotransmitter receptors.

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OC17-3

RAPID INHIBITION OF VASOCONSTRICTION IN RENAL AFFERENT ARTERIOLES BY ALDOSTERONE

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We have reported that the ability of 100 mM KCl to induce vascular contraction in microdissected perfused rabbit renal afferent arterioles was reduced after exposure to aldosterone (10^{-9} M). The present series of studies was undertaken to elucidate the signaling and regulatory mechanisms involved in the rapid effects of aldosterone on vascular tone
Aldosterone in physiological concentrations (10^{-9} mol/L) inhibits depolarization-induced vasoconstriction in small renal resistance vessels through the classical mineralocorticoid receptor, but probably through non-genomic action. The effect could not be blocked by mifepristone (10^{-6} mol/L). Expression of mineralocorticoid receptors and 11-beta-hydroxy steroid dehydrogenase 2 was demonstrated by RT-PCR and immunohistochemistry on rat preglomerular renal vasculature and cultures of rat renal vascular smooth muscle cells. Aldosterone did not affect depolarization-mediated increases in calcium concentration in non-perfused rabbit afferent arterioles as measured by fluorescence microscopy. Inhibition of phosphatidylinositol (PI)-3 kinase with LY 294002 (3×10^{-6} mol/L) restored sensitivity to K^+ in the presence of aldosterone and the catalytic PI-3 kinase subunit p110 a was demonstrated in afferent arterioles by immunohistochemistry. Inhibition of nitric oxide formation by L-NAME (10^{-4} mol/L) also restored K^+-induced vasoreactivity in the presence of aldosterone. The nitric oxide donor SNP (10^{-6}- 10^{-4} mol/L) reduced the K^+-induced vasoreactivity.
We conclude that aldosterone inhibits depolarization-induced vasoconstriction in renal afferent arterioles by a rapid non-genomic mechanism initiated by mineralocorticoid receptor activation and involving PI-3 kinase-mediated stimulation of NO-generation.

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OC17-4

ALDOSTERONE MODULATES PHOSPHOLIPIDS IN COLONOCYTES

Jindrichova S., Mrnka L., Novakova O., Tvrzicka E., Lisa V., Novak F., Pacha J.

Previous studies have shown that aldosterone treatment of amphibian epithelium results not only in stimulation of Na^+ absorption but also in changes of phospholipids that are necessary for the mineralocorticoid action of aldosterone. The purpose of this study was to determine whether aldosterone (ALDO) has similar effect in mammals and whether this effect represents a direct effect of the hormone on colonocytes. Phospholopid and fatty acid composition was examined in colonocytes and thymus of rats which had received ALDO by miniosmotic pump and in confluent Caco-2 colonic cells incubated in the presence of ALDO or dexamethasone (5.10^{-7} or 5.10^{-8} M, 4 days). In colonocytes ALDO changed the percentage distribution of fatty acids, whereas the percentage of phospholipid classes was not changed. Phospholipids increased the content of polyunsaturated, PUFA, (arachidonate) and decreased the content of monounsaturated, MUFA, fatty acids (oleate, palmitoleate). No changes were observed in thymus. In Caco-2 cells ALDO treatment caused a decrease in triglyceride and an increase in phospholipid content without relative changes in phospholipid species. Fatty acid composition of phospholipids was associated with a decrease of MUFA and an increase in PUFA. The incorporation of [3H]arachidonate to phospolipids was increased. Dexamethasone treatment was without any effect. The ALDO-dependent changes in phospholipids may reflect a physiologically important phenomenon with long-term consequences for membrane structure and function.
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S17-3

MOLECULAR MECHANISMS IN THE REGULATION OF ALDOSTERONE BIOSYNTHESIS

Capponi A.

In the zona glomerulosa of the adrenal cortex, the classical tissue producing mineralocorticoids, and in less conventional tissues, such as heart and vascular wall, aldosterone is synthesized, like all steroid hormones, from cholesterol. The regulation of acute aldosterone biosynthesis by one of its main physiological activators, the octapeptide hormone angiotensin II (Ang II), is exerted at various levels along the cascade leading from cholesterol to the final product, aldosterone. First, AngII activates the calcium messenger system and generates marked alterations in cytosolic and mitochondrial calcium homeostasis. Second, AngII regulates cholesterol supply and storage within intracellular lipid droplets, by increasing HDL-cholesterol uptake into the glomerulosa cell, as evidenced by fluorescence videomicroscopy. This is achieved through an increased expression at the cell surface of the receptor for HDL, scavenger receptor class B type 1 (SR-B1). Third, AngII activates the enzyme that hydrolyses cholesterol esters from lipid droplets, cholesterol ester hydrolase (CEH), to generate free cholesterol. This activation of CEH occurs via an ERK2/1-mediated phosphorylation of the enzyme in response to AngII challenge. Fourth, the hormone increases transcription of the gene for the Steroidogenic Acute Regulatory (StAR) protein, which is the key regulatory factor in steroidogenesis, and its importation into the mitochondrial matrix. This process is accompanied by increased cholesterol transfer from the outer to the inner mitochondrial membrane, thus augmenting cholesterol supply to the intramitochondrial steroidogenic enzymes. The transcriptional effect of AngII on StAR gene expression involves the repression of DAX-1, a transcription factor which is known to repress StAR. The concerted response to these multiple actions of AngII on various intracellular effectors leads to an increased biosynthesis of aldosterone.

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S17-4

IDENTIFICATION OF GENE INVOLVED IN ALDOSTERONE SIGNALLING PATHWAY IN THE HEART

Muller O.

Aldosterone is a mineralocorticoid hormone that plays an essential role in the regulation of sodium reabsorption in the kidney. Experimental evidences suggest that aldosterone may also exert direct effects on the heart. According to the current model for aldosterone action in the heart, it is proposed that aldosterone alters the physiological and morphological state of cardiac tissue by transcriptional modulation in cardiomyocytes, or other cardiac cells, of gene networks which may comprise up to a few hundreds of different genes. The characterization of the aldosterone-induced/repressed gene network, which is largely unknown, is the principal aim of this study. Using Serial Analysis of Gene Expression (SAGE) we have characterized the transcriptomes (or complete list of expressed genes) of untreated (Control) and Aldosterone-treated (4 hours) neonatal mouse cardiomyocytes. Comparison of the transcriptomes has allowed us to establish the set of genes that are up- or down-regulated by aldosterone. 40 transcripts were upregulated and 20 downregulated. Northern blot analysis has allowed us to confirm 3 SAGE candidates. We have performed the initial characterization, which will be discussed in details, of two genes that are strongly (> 5-fold) induced by aldosterone.

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S17-5

REGRESSION OF THE ARRHYTHMOGENIC REMODELING OF THE LEFT ATRIA WITH TREATMENT OF HEART FAILURE IN RAT

Milliez P., DeAngelis N., Rucker-Martin C., Leenhardt A., Beaufils P., Samuel JL., Delcayre C., Hatem S.

Background. The arrhythmogenic substrate of atrial fibrillation (AF) is composed of marked functional and structural abnormalities of the atrial myocardium including fibrosis (F) and dystrophic myocytes (DM). This atrial remodeling is also seen in hemodynamic overloaded atria (A) and during heart failure (HF). Methods. Here, we used the rat model of myocardial infarction (MI) in HF to study the reversibility of the atrial remodeling. Echocardiography, invasive hemodynamic measurements and Holter monitoring procedures were used to characterize the cardiomyopathy. Histological analysis of the LA was performed with Masson's trichrome and picrosirius red assays. Three months after MI, all rats were in HF with hemodynamic (left ventricle end disatolic pressure, LVEDP>15 mmHg), and echocardiographic signs of LV dysfunction with dilated A. After one month, all rats were sacrified (controls and treated). Results. There was a marked F at the periphery of trabeculae and surrounding hypertophied atrial myocytes with extensive myolysis. In addition, enlarged A with an increase of its weight/body weight ratio was observed. There was a correlation between atrial F and LVEDP (n, 30; r2=0.37; P<0.001) and A weight (n=30; r2=0.44; P<0.001). Eighteen rats were treated against HF using ACE inhibitor, □-

blocker and spironolactone. After 1 month of treatment, rats without any more signs of LV dysfunction (n=7) showed a full regression of the atrial remodeling with the disappearance of F and normalization of the atrial myocyte size. Conclusion. Changes in the hemodynamic loading conditions of A is a major factor for the constitution of the atrial myocardium remodeling which, consequently, appears very sensitive to the treatment of HF.

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POSTER SESSION

P17-01

COUNTER-REGULATORY MECHANISMS OF THE LPS-INDUCED INFLAMMATORY CELL RESPONSE

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Under the influence of different stimuli (infection, trauma, surgery, burns) bacterial endotoxin initiates a global activation of inflammatory pathways. Glucocorticoids (GCs) inhibit the expression of adhesion molecules, the recruitment of leukocytes to the site of inflammation, the release of inflammatory mediators induced by endotoxin. In the present study we investigated the effect of the GC Oradexon and the GC antagonist RU 38486 on the endotoxin-induced inflammatory cell response.
MATERIALS AND METHODS: male CFLP mice (20-25 g, n = 5 per group) were injected i.p. with 10 µg (10 g body weight)-1 endotoxin (E. coli 026:B6 LPS, Difco Lab, Detroit, lot 110273JB). Dexamethasone (Oradexon, N.V, Organon Oss, The Netherlands) was administered i.p., i.v. or s.c. in a dose of 0·1 mg (10 g body weight)-1, alone or concomitantly with endotoxin. Other groups were given 0·5 mg (10 g body weight)-1 RU 38486 (Russel Uclaf, France) i.p. or s.c., either alone or concomitantly with LPS. RESULTS: Bacterial endotoxin increased the total cell count at 24, 48 and 72 h, due to neutrophilia at 24 h and, due to increases in the number of macrophages and lymphocytes 48 and 72 h after treatment, respectively. The i.p., s.c. and i.v. injection of Oradexon, concomitantly with endotoxin, reduced the total cell count and the macrophage count at 48 and 72 hours. The number of lymphocytes and neutrophils decreased at 24 and 72 hours. The i.p. and s.c. injection of RU 38486 increased the total cell count, due to increases in the number of macrophages, lymphocytes and neutrophils - at 24, 48 and 72 hours.
Endotoxin at 24 hours decreased, but at 48 and 72 hours increased the WBC count and the lymphocyte count. CONCLUSION: The mortality from gram-negative sepsis and multiple organ failure remains high. Our studies may contribute to the elucidation of the pathophysiology of these severe conditions.

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P17-03

DIRECT AND INDIRECT ESTRADIOL ACTION IN THE REGULATION OF GLUCOCORTICOID FUNCTION OF ADRENAL CORTEX

Kovzun O.

Castration and treatment with estrogens can affect the secretion and metabolism of corticosteroids in some species. However, the processes that underlie these changes are not studied.
In this work the regulatory effects of estradiol on the function of adrenal gland cortex were studied. Analysis of the mechanisms of realization of estradiol effects was carried out in vivo and in vitro. It was determined that steroidogenesis estimated with labelled cholesterol transformation in corticosteroids by dispersed adrenocorticocytes, intensified under the influence of estradiol in vivo (25 µg/100 g, 3 days). Production of 11-hydroxycorticosteroids and biosynthesis of DNA assessed by incorporation of the labelled thymidine increase as result of in vitro addition of estradiol (final concentration 0,00037- 3,7 µM) to the cultivated adrenal cells. There are the evidence for direct action of hormone on adrenocorticocytes. We have obtained some data which suggest that proliferative effect of estradiol may be mediated at least in part by prolactin. Specific binding of 3-[125I]iodotyrosyl23)ACTH(1-39) by adrenocortical microsomal fraction decreased sharply in consequence of ovariectomy (8 weeks after operation) and increased considerably after 3-day replacement therapy with estradiol (25 µg/100 g). Some fundamental biochemical characteristics: biosynthesis of DNA, RNA and proteins are changed under the influence of estradiol; this pointed to the proliferative effect of this hormone in adrenal glands. Thus, regulatory interrelations ovaries-adrenal cortex can be more complicated than it was believed till now.

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P17-04

THE EFFECT OF DIFFERENT HYPERTENSION MODELS ON ACTIVE AVOIDANCE LEARNING

Hacioglu G., Agar A., Ozkaya G., Yargicoglu P., Gumuslu S.

This study tested the effects of different hypertension models on active avoidance learning in rats. Three month-old male Wistar rats were divided randomly into six groups as follows: control (C), sham operated (sham), two kidney-one clip (2K-1C), one kidney-one clip (1K-1C), deoxycorticosterone-salt (DOCA) and N-omega-nitro-L-arginine-methyl ester (L-NAME) groups. Mean arterial blood pressures were significantly higher in four hypertensive groups compared with control and sham groups. The active avoidance training results indicated that hypertension state is associated with learning impairment. Thiobarbituric acid-reactive substances (TBARS) were determined as an indicator of lipid peroxidation in brain and hippocampus. Additionally, brain and hippocampus nitrite levels were studied.

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P17-05

SYSTEMIC EFFECTS OF ANGIII IN CONSCIOUS DOGS DURING ACUTE DOUBLE BLOCKADE OF THE RENIN SYSTEM

Gammelgaard I., Wamberg S., Bie P.

Background. The heptapeptide angiotensin III (AngIII, des-asp1-angiotensin II) may possess specific biological effects. The study aimed to determine (i) whether AngIII exerts effects similar to those of angiotensin II (AngII) under conditions of identical plasma concentrations of the two peptides, and (ii) whether the effects of AngIII are mediated through AT1 receptors.
Methods. Effects of AngII (3 pmol/kg/min = 3.1 ng/kg/min) and AngIII (15 pmol/kg/min = 14 ng/kg/min) were investigated in trained conscious dogs during acute inhibition of converting enzyme (enalaprilate, 2 mg/kg) and aldosterone (canrenoate, 6 mg/kg plus 1 mg/kg/h). This inhibition allows measurement of the effects of the peptides with minimal interference from endogenous angiotensin and aldosterone. Arterial plasma concentrations of AngII and AngIII (pAng-ir) were determined by AngII radioimmunoassay using an antibody which cross-reacts 100% with AngIII. During ongoing peptide infusion, candesartan (2 mg/kg) was used to block the AT1-receptors.
Results. Increases in pAng-ir with the AngII and AngIII infusions were very similar, 44 ±4 pg/ml and 43 ±8 pg/ml, respectively. Infusion of AngII caused significant increases in mean arterial blood pressure (12 ±5 mmHg) and plasma aldosterone (177 ±21 pg/ml) while plasma renin activity (29 ±12 mIU/L) and sodium excretion (60 ±9 µmol/min) were reduced. Infusion of AngIII mimicked all these effects and the magnitude of AngIII responses was statistically indistinguishable from those of AngII. All measured effects of both peptides were blocked by candesartan.
Conclusion. At the present concentrations around 50 pM, AngIII is equipotent to AngII with regard to effects on blood pressure, aldosterone secretion, and renal functions. These AngIII effects are mediated through AT1 receptors.

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S18 PHYSIOLOGY OF EATING BEHAVIOUR AND ENERGY EXPENDITURE: CONTROL BY CENTRAL AND PERIPHERAL MECHANISMS

ORAL SESSION

S18-1

INTEGRATED MECHANISMS CONTROLLING EATING BEHAVIOUR

Motta M.

The last decade has seen an increasing scientific interest in the elucidation of the complex physiological mechanisms controlling energy metabolism and food intake. Many reasons underly this huge effort, because of the continuous increase of the epidemic diffusion of diseases, like obesity and the metabolic syndrome, in particularly in the Western society. As a consequence, a strong need to clarify the complex physiology regulating these processes has emerged.
It is well established that the hypothalamus plays a pivotal role in the control of food intake and energy metabolism. However, several novel observations suggest that the regulation of these functions might be better described as a dialogue between central (i.e. hypothalamus) and peripheral organs (i.e. adipose tissue), instead of just a hypothalamus-driven process. In this respect, one major observation appears the discovery of the adipose-produced hormone leptin. Leptin acts centrally stimulating the sense of satiety, and thus it represents a clearly identified factor linking a peripheral tissue (in this case, the adipose) to central areas like the hypothalamus. Recently new other factors have been identified (resistin, adiponectin, ghrelin, etc.), the role of which has to be clearly defined yet. There is a need to investigate the mechanisms through which each hormone involved acts (ligands, receptors, intracellular signals), in order to clarify the specific role of each agent in the complex mechanisms controlling the different components of feeding and energy expenditure, such as: signals of initiation and termination of meals, nutrient preference, regulation of caloric intake, regional fat accumulation, regulation of energy expenditure, and control of locomotor activity, just to mention some of them.
Supported by MIUR (COFIN 2000) and by University of Milan (FIRST).

Institute of Endocrinology - University of Milano – ITALY

S18-2

CENTRAL AND PERIPHERAL SIGNALS INTERACT TO CONTROL FEEDING BEHAVIOUR AND ENERGY METABOLISM

Magni P., Dozio E., Ruscica M.

The mechanisms controlling feeding behaviour and energy metabolism imply the reciprocal interaction of central (i.e., the hypothalamus) and peripheral (i.e., adipose and gut) structures and the involvement of a growing series of neurotransmitters and peptide and steroid hormones. Since a few years, an important role has been attributed to the adipose-secreted hormone, leptin, which appears to inform the central structures controlling energy metabolism on the status of energy stores, represented by fat mass, thereby inducing satiety and increased energy expenditure. Leptin is involved also in the control of reproduction, which in turn is dependent on energy availability, and thus it is regarded as a possible link between modulation of energy expenditure/food intake, and the reproductive function. Leptin actions on food intake are transduced by hypothalamic neurons expressing specific membrane receptors and different neuropeptides, including neuropeptide Y (NPY), Agouti-related protein, proopiomelanocortin, and cocaine-amphetamine-related transcript. Leptin has been shown for example to downregulate the expression of NPY in rats and in human neuroblastoma cell lines. These neuronal systems appear at least in part to be targeted also by other hormones involved in these processes, like ghrelin, a 28 aa acylated peptide secreted mainly by the stomach and able to elicit a marked increase of food ingestion in humans and in experimental animals.
Future studies will extend the knowledge by identifying novel secreted molecules participating to the regulation of energy metabolism and clarifying their physiological effects. These discoveries may also suggest novel therapeutical approaches for the treatment of diseases related to food intake and energy metabolism, such as obesity and disorders of eating behavior.
Supported by the University of Milano and MIUR.

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OC18-1

LUMINAL ACIDIFIED NITRITE INCREASES GASTRIC MUCOSAL BLOOD FLOW IN THE RAT

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Background: Dietary nitrate is reduced to nitrite in the oral cavity. When the nitrite meets the acidic environment of the stomach, nitric oxide (NO) is formed. The aim of this study was to investigate if this NO affects the gastric mucosal blood flow.
Methods: Rats were anesthetized with inactin and the gastric mucosa was exteriorized for intravital microscopy. Acid secretion was stimulated with pentagastrin (40µg kg-1 h-1) Gastric mucosal blood flow was measured with laser-Doppler flowmetry, before, during and after topical administration of 0.1 mM NaNO2 0.5mM NaNO2 1.0 mM NaNO2 and 5.0 mM NaNO2 in HCl (10mM) in ten minutes periods. To investigate the influence of endogenous enzymatic NO production, we blocked this NO production with an intravenous bolus injection of N-Nitro-L-Arginine (L-NNA) (10 mg kg-1) followed by a continuous intravenous infusion (3 mg kg-1 h- 1).
Results: The gastric mucosal blood flow increased dose-dependently by luminal NaNO2 pH2 (121 ± 17 % by 0,1mM NaNO2, 129 ± 8 % by 0,5mM NaNO2, 150 ± 8% by 1mM NaNO2 and 172 ± 12% by 5mM NaNO2) meanwhile the mean arterial blood pressure was not altered. We found a similar dose-dependent increase in mucosal blood flow in the L-NNA treated animals. In control rats, gastric blood flow was not altered by luminal pH2 alone.
Conclusion: Nitrite given luminally increases gastric mucosal blood flow in rats. The increase in mucosal blood flow is not altered by inhibition of the endogenous NO production. Our results thus indicate that the dietary nitrate enhances an important gastric mucosal defense mechanism.

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OC18-2

EFFECTS OF LEPTIN AND INTERLEUKIN-1ß ON CAT INTESTINAL VAGAL AFFERENT NERVE FIBRES

Gaigé S., Vitton V., Abysique A., Bouvier M.

Vagal afferents are involved in transmitting information on food intake and immune reactions to the central nervous system (CNS). In a previous study, we established that leptin (10µg), administered intra-arterially (i.a), activates 68% (type 1 units) and inhibits 32% (type 2 units) of the vagal afferent fibres, with a short latency. The excitatory effects of leptin are blocked by the interleukine-1ß receptor antagonist (Il-1ra, 250µg, i.a.), indicating that these effects involve interleukin-1ß (Il-1ß).
Aims: To investigate how leptin and Il-1ß interact, we studied the effects of this drug on vagal afferent activity from intestinal mechanoreceptors.
Methods: Vagal afferent activity was recorded in the nodose ganglion via an extracellular glass microelectrode. Using amplitude discrimination and shape recognition procedures, it was possible to select the electrical activity of a single afferent fibre, and thus to work under unitary recording conditions.
Results: Il-1ß (0.1, 1 and 10µg, i.a) induced light activation in the discharge frequency of the type 1 units and stronger activation in that of the type 2 units. When Il-1ß was administered 20 min after CCK (10µg i.a.), its excitatory effects were enhanced on the type 1 units, but blocked on the type 2 units. CCK-A and CCK-B receptor antagonist pre-treatment blocked the effects induced by CCK pre-treatment on the Il-1ß effects in both types of units. Previous administration of Il-1ra (250µg, i.a.) blocked both the excitatory effects of Il-1ß after CCK and those of leptin on the type 1 units, while enhancing the inhibitory effects of leptin on the type 2 units.
Conclusion: It can therefore be concluded that (i) leptin acts on intestinal vagal mechanoreceptors via Il-1ß on the type 1 units and independently of Il-1ß on the type 2 units, and (ii) each type of units transmits different information on ingestion or inflammation to the CNS, depending on chemical environment.

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OC18-3

SHORT FASTING MODULATES DUODENAL MUCOSAL SECRETORY RESPONSIVENESS TO SECRETAGOGUES

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Introduction By tradition, most studies of the neurohumoral control of gastrointestinal functions in intact animals and humans are conducted after an overnight fast. Our aim was thus to investigate whether short (overnight) fasting influences the intestinal secretory response to some secretagogues. The bicarbonate secretion by the duodenal mucosa, a main mechanism in duodenal protection against gastric acid, was chosen for study.
Method All animals had free access to drinking water. Overnight fasted as well as fed animals were anesthetized (Inactin) and a 12-mm segment of proximal duodenum with intact blood supply was cannulated in situ. Bicarbonate secretion (pH stat) and mean arterial blood pressure were continuously recorded. All secretagogues were administered to the duodenum by close intra-arterial infusion, minimizing any central nervous actions of the compounds.
Results Infusion of the appetite-regulating peptide orexin A (60, 240 and 600 pmol·kg-1·h-1) dose-dependently increased the secretion (~100%) in fed rats, but had no significant effect in fasted rats. The muscarinic agonist bethanechol (50, 500 and 5,000 nmol·kg-1·h-1) induced a significant rise in secretion in fed rats already at a dose 50 nmol·kg-1·h-1 but the highest dose tested (5,000 nmol·kg-1·h-1) was required for stimulation in fasted animals. Melatonin (20, 200 and 2000 nmol·kg-1·h-1) and vasoactive intestinal polypeptide (50, 250 and 1,000 pmol·kg-1·h-1) caused marked increases in secretion in fasted as well as in fed animals. No significant differences in blood pressure were observed between the fed and fasted animals.
Conclusions Overnight fasting, a standard procedure in experimental studies of intestinal function thus rapidly and profoundly down regulates the response to some secretagogues. The observations suggest that the many studies of the neuroendocrine control of gastrointestinal secretion and effects of drug therapy may require re-evaluation with respect to feeding status.

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S18-3

HORMONAL REGULATION OF ENERGY PARTITIONING
Rohner-Jeanrenaud F.

Body weight homeostasis is maintained via a series of complex interactions between the brain, particularly the hypothalamus, and the periphery. Leptin, a hormone synthesized in adipose tissue, plays an important role in such interactions. Secreted leptin has some direct peripheral effects, but it mainly exerts its action within the brain, where it inhibits many of the orexigenic neuropeptides, while favoring many of the anorexigenic ones. Due to this, leptin decreases food intake and body weight. However, leptin is also known to centrally exert a series of metabolic effects resulting in the depletion of fat stores and, in some instances, in improvement of insulin resistance. The main pathways involved in such leptin actions are the sympathetic nervous system and thyroid hormones.
As mentioned above, the hypothalamic neuropeptides that are targets for leptin action can be divided into two main categories: those that increase food intake (orexigenic factors), and those that inhibit this process (anorexigenic factors). The effects of these neuropeptides on food intake are accompanied by metabolic actions that either favor fat storage and development of insulin resistance for the orexigenic factors, or like leptin itself, do the contrary as is the case for the anorexigenic peptides. As examples, the obesity-inducing effects of neuropeptide Y and the fat stores-depleting action of the melanocortin system will be described. While, under normal conditions, a dynamic equilibrium exists between the orexigenic and the anorexigenic factors, obesity and type 2 diabetes can follow from alteration therefrom.
Glucocorticoids also play an important role in the maintenance of normal body weight homeostasis. Indeed, by acting centrally, these hormones modulate both the expression and the effects of various neuropeptides. Overall, their effects are opposite to those of leptin as they favor fat deposition and the occurrence of insulin resistance.

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S18-4

IMPORTANCE OF DIFFERENT NUTRIENTS IN THE REGULATION OF EATING BEHAVIOUR.
Louis-Sylvestre J.

The basic mechanism of the control of eating behaviour is the "hunger-satiety" mechanism. Satiety duration is explained by glucose dynamics and insulin secretory pattern. By combining behavioural and metabolic data, a strong and causal relationship has been demonstrated between declines in blood glucose concentration and spontaneous meal onset. The preprandial transient drop in blood glucose is thought to be the signal of a shortage in immediately available glucose detected by central gluco-sensitive. After an eating episode the delay of occurrence of the next drop in available glucose is dependent on the rate of glucose utilization (depending on absorption, neoglucogenesis, glucose oxidation and storage). A meal usually provides the three macronutrients. Proteins increase the glucose availability very slowly and lately (however differently according to the induced insulin secretion): they could be considered as "extra slow carbohydrates". About carbohydrates and fat, the availability of both glucose and fatty acids would determine their respective oxidation rates by simple competition of substrates via the mass action law but carbohydrates and fat induce insulin secretion (differently according to their nature), and insulin stimulates glucose oxidation and storage, favours lipogenesis, inhibits lipolysis. In the early part of the postprandial interval, hyperglycemia and hyperinsulinemia favour glucose utilization and lipogenesis. Later on, with declining levels of glucose and insulin, lipolysis resumes and the plasma concentration of fatty acids increase, favouring their oxidation. The faster and more intense the secretion of insulin just after the meal and thereafter, the slower and more moderate the fat utilization. Glucose dynamics ? Satiety lasts as long as glucose is available: glucose is both a satiety factor and an initiation signal. Insulin secretory pattern ? It determines the temporal evolution of glucose and vice versa.

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POSTER SESSION

P18-01

DIETARY PATTERN AND LIPID PARAMETERS OF COLLEGE STUDENTS IN SLOVAKIA

Gabašová E. (1), Babinská K. (1), Béder I. (1), Béderová A. (3), Uhlíková E. (2), Turecký L. (2)

This study was designed to identify the beliefs, motivation and personal and environmental influences shaping eating habits of a group of college students in Slovakia. We studied 167 students of medical faculty- 43 men and 124 women, average age 21.5years, who provided information on demographic and socio-economic variables, responded to an interviewer-administered, food-frequency questionnaire that assessed the consumption of more than 100 food items and 24-hours recalls of food intake. Study included measuring of blood pressure, anthropometric and lipid parameters as well as lipid peroxidation levels- conjugated diens. Higher level of total cholesterol has been found in 26% of students, high levels of LDL cholesterol in 13,6%. Almost a half of students did not take food regularly, in most of the cases they replaced the main meal with the fast food. One third of the investigated group, takes vitamin and mineral supplements. However, there were variations between individuals, with specific practices being influenced by personal food preferences, time availability, health beliefs and concern, food availability, and the physical and social environment. Results indicate that, in general, the study group was reasonably well nourished. However, fat consumption was 30% higher than the recommended intake, for both males and females. The percentage of energy derived from carbohydrates was below the guideline value in both sexes. Relatively low iron and fiber intakes were found for females. Based on these results, some concern about the dietary habits and the related health consequences in medical students appears justified.

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P18-02

FUNCTIONAL FEATURES AND CARBOHYDRATE DETERMINANTS OF ERYTHROCYTES MEMBRANES IN WOMEN WITH OBESITY

Kireev R.A., Kurmacheva N.A., Sayapin S.V.

The objective of our investigation was the studying of the erythrocytes acid resistance (functional changes) and the structural changes of the membranes complexes carbohydrate components in women with abdominal obesity.
Subjects and methods: We have studied the erythrocytes of 52 women (group 1) with abdominal obesity (the average age 29±0,98). The control group consisted of 40 women (group 2) with simple obesity (the average age 29±1,1). The acid resistance was studied by means of the method of erythrograms (the effect on erythrocytes 2N CH3COOH in vitro). To determine carbohydrate determinants and structural changes of the membranes complexes, erythrocytes were incubated together with lectins. We have used the following preagglutination concentration of lectins: the b-galactose specific, peanut lectin, PNA (125 mkg/ml); the mannose specific, Canavalia lectins, ConA (125 mkg/ml).
Results: In the 1 group we noted a decrease of the erythrocyte membranes resistance than in group 2: (the total hemolysis 342 sec contrasted 420 sec) and an increase of low-resistant erythrocytes. In erythrocyte membranes of women in group 1 there was discovered an increase of carbohydrate determinants of b-galactose, ready to react with lectins PNA; with the help of ConA there was observed an increase of the quantity of the accessible receptory locations of D-mannose. In group 2 there was not observed an interaction between lectins and carbohydrate determinants of erythrocytes membranes. The fact, that there are certain locations on the erythrocytes membranes, which are ready to react with lectins, in women with abdominal obesity, is an evidence of the cialic acids loss processes. In its turn, this preconditions the aggregation increase of fibrinogens and b-lipoproteins on the erythrocytes surface; it also leads to the changes of the membranes electrostatic properties, which can be viewed as one of the risk factors of aterosclerosis.

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P18-03

EFFECTS OF PERIWINKLES INTAKE ON THE ANTIOXIDANT STATUS IN RATS

Millán R., Míguez I., Taboada M.C.

Common cellular metabolism is included among the biological sources of the potentially deleterious reactive oxygen species (ROS). The extent to which ROS produce damage depends on the effectiveness of antioxidant defenses. These include specific enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) or catalase (CAT), in addition to non-
enzymatic defenses. In general, sea foods are a rich source of easily digestible protein that also provides polyunsatured fatty acids, vitamins and minerals for human nutrition. However, up to now, how antioxidant enzymes in mammalians can be influenced by sea food has received scant attention in spite of these enzymes can be affected by several factors including diet. Thus, the aim of the present study was to determined the effect of periwinkles (Monodonta lineata) intake on the antioxidant status in kidney and liver of rats. SOD, GSH-Px and CAT activities in liver and kidney were measured in rats fed for 4 weeks with periwinkles as source of protein in comparison to casein. Although the body weight was similar in both groups, in rats fed with periwinkles the food intake was significantly higher than in the casein group, probably because marine species has high contents of glycine and arginine that increase the palatability. In this study, renal and hepatic GSH-Px and the renal CAT activities were increased in treated rats compared to control rats. A significant increase in liver CAT/SOD and liver and kidney GSH-Px/SOD ratio could indicate an activation of the antioxidant enzymes due to an increase in ROS. However, hepatic and renal SOD activities were not modified by feeding with periwinkles and that in consequence, the endogenous H2O2 production was not altered. In conclusion, periwinkles intake can maintain the antioxidant status of cell by increasing catalase and glutathione peroxidase activities at least in liver and kidney and thus can protect against oxidative damage by reducing peroxides.

Dept. Physiology. Faculty of Pharmacy. University of Santiago de Compostela – SPAIN

P18-04

ROLE OF L-ARGININE ON SOME SERUM PARAMETERS AND INTESTINAL ENZYMES OF EXCESS-FAT-DIET FED RATS

Míguez I., Rodríguez B., Taboada M.C.

Nitric oxide (NO) mediates diverse aspects of feeding and gastrointestinal function. Administration of L-arginine, the biological NO synthase (NOS) substrate and an essential nutrient for adult mammalians, reverses the appetite suppresion induced by NOS inhibitors. This study was designed to test the effect of oral supplementation of dietary L-arginine (2%) on body weight and food intake in young rats. Animals were given free access to either standard laboratory diet or a saturated high fat diet (15% lard) and both diets plus arginine for 4 weeks maintained in individual metabolic cages. Serum cholesterol, triglycerides and protein were measured as well as intestinal maltase, sucrase, lactase and leucine aminopeptidase activities. The weights of the rats did not show significant differences between groups and the food intake was lower in the animals fed with the diet containing saturated high fat plus arginine, probably because the diet composition could affect the center of satiety. Serum results showed that total cholesterol, LDL-cholesterol and triglycerides were higher and HDL-cholesterol values were reduced in groups taking fat. L-arginine supplements did not improve these parameters. Previous results showed that plasma cholesterol of hypercholesterolemic rabbits was unaffected by arginine but plasma arginine levels were increased in the arginine-treated group and there was no progression of intimal thickness. Indeed, it seems that the antiatherogenic effects of arginine is not due to an alteration in plasma lipids but intimal vascular thickness during hypercholesterolemia was reduced by inhibiting smooth muscle cell proliferation. In our study, we also founded increased protein serum levels in both groups receiving L-arginine. Furthermore, sucrase and lactase activities were lower in the high-fat diet, specially when arginine was also added, reflecting the importance of fat and amino acids in the structure and function of microvilli.

Dept. Physiology. Faculty of Pharmacy. University of Santiago de Compostela – SPAIN

P18-05

DIETARY EFFECTS OF FIBRE ON SERUM LIPIDS AND INTESTINAL DISACCHARIDASE ACTIVITIES
Taboada M.C., Rivas M.C., Míguez I.

The fibre consumption in humans is of particular interest given that a fibre-rich diet is thought to reduce the risk of cardiovascular disease, diabetes and cancer. However there is still a great disagreement on some aspects such as source of fibre. It seems that physiological effects depend on the properties of the individual fibre sources. Fibre supplements or fibre-rich foods may function as normal dietary agents by modulating the digestive and absorptive process which can be conclusive factors. This study has been carried out with the aim of determining the effects of soluble fibre (apple and orange pectin) and complex mixture of fibres from cereals (wheat bran and rye bran) on cholesterol and triglycerides in serum as well as on intestinal disaccharidase activities in growing rats. Feed intake and body weight were not evidently affected after three weeks feeding period. Except rats fed rye-containing diet, an hypocholesterolemic effect of fibre-rich diet was obtained compared with the control diet. Moreover, a reduction of serum triglycerides was observed with both types of pectin. A decrease on the absorption of fats, changes on enzymes involved in the hepatic cholesterol metabolism, as well as short-chain acids originated in colon by soluble fibre fermentation, could be some of reasons for reduction of serum lipids. In general, feeding fibre produced a decrease on intestinal disaccharidase values, especially when soluble fibre was used. These changes may be due to effects on the growth and differentiation of enterocites or to a decrease on the disaccharidase substrate availability, which would inhibit the dietary induction. In the case of pectins, alterations of bacterial intestinal microflora which affects the substrate, metabolism and maduration of mucosal cells, may be involved.

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P18-06

METABOLIC FEATURES IN A NEW OBESITY-RESISTANT RAT (LOU/C) DURING AGEING
Perrin D., Soulage C., Géloën A., Pequignot J.M.

Ageing is associated with metabolic alterations characterised by changes in energy expenditure, fat distribution, obesity, leptin and insulin resistance. The Lou/C rat, an inbred strain of Wistar origin, is presented both as an obesity-resistant rat with a lower fat accumulation and as a model of healthy ageing. To characterise the mechanisms underlying obesity resistance in Lou/C rat, we measured food intake and energy expenditure by indirect calorimetry at 1, 6, 12, 18, and 24 months of age. Moreover, plasma insulin and leptin concentrations were determined by radioimmunoassay in the two strains throughout lifespan. At each age, Lou/C rats exhibited no differences compared to Wistar rats concerning food intake and few differences have been elicited concerning energy expenditure (+21% and +14% compared to Wistar rats at 6 and 12 months of age). Whereas 18- and 24-month-old Wistar rats presented a marked increase in insulin concentration, it remained stable during ageing in Lou/C rats. These data bring evidence that insulin resistance is closely related to the excess of adipose tissue and that Lou/C rats did not develop insulin resistance as confirmed by a lower glucose infusion rate during hyperinsulinemic-euglycemic clamp compared to Wistar rats. Lou/C rats exhibited a progressive and limited increase of leptin concentrations throughout lifespan while Wistar rats exhibited a sustained increase that led to higher leptin concentrations in comparison with Lou/C rats (four-, seven-, five- and threefold higher at 6, 12, 18, 24 months of age, respectively). These data suggest a non-development of leptin resistance with ageing in Lou/C rats. Not only Lou/C rats live longer than Wistar rats, but they display also a healthy ageing considering certain metabolic features. This strain could be a relevant model to discriminate between the process of ageing and the effect of fat mass accumulation on the installation of metabolic syndrome during ageing.

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P18-07

A NUTRIENT SENSOR MECHANISM CONTROLS DROSOPHILA GROWTH
Colombani J., Raisin S., Pantalacci S., Radimerski T., Montagne J., Leopold P.

Organismal size and tissue growth are controlled by developmental and nutritional cues. We still understand little about how nutrition controls growth during development. We used a P-element insertion Drosophila line to down-regulate, in a tissue-specific manner, the slimfast (slif) gene which encodes a cathionic-aminoacid transporter. Down-regulation of slif mimics all physiological aspects of nutriment starvation and provokes important tissue growth defects. This allowed us to assess both cellular and humoral response to aminoacid starvation in a developping organism.
Ours results show that cells respond autonomously to starvation by a rapid down-regulation of protein biosynthesis pathways, that could be rescued by increasing cellular levels of eIF4E or S6-kinase. By directing slif down-regulation in the larval fat body (a secretory organ wich cumulates liver and adipocyte functions), we observed a strong developmental delay and a general reduction of adult size, suggesting that this organ participates in a non-autonomous humoral response to starvation that restrains overall organismal growth.

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P18-08

METABOLIC RESPONSES TO REPEATED FOOD DEPRIVATIONS IN BIRDS
Laurila M., Hohtola E.

We examined how birds adapt to repeated food deprivations. We subjected pigeons (Columba livia) and Japanese quails (Coturnix coturnix japonica) to repeated fasts (four 3-d fasts, interval between fasts 3-10 days for pigeons, 2-5 days for quails) at an ambient temperature of 22°C. Body weight was measured by using perch-scales for pigeons and manually for quails. Deep body temperatures (Tb) were continuously recorded with intraperitoneal radio transmitters in both species. Also, rates of O_2 consumption were recorded by indirect calorimetry in quails. Both species reacted to fasting with deeper nocturnal hypothermia: nocturnal Tb during the fasts was 0.5-3°C lower than during the ad lib-feedings, but nocturnal hypothermia did not increase with repeated fasts. However, in both species diurnal Tb during fasts decreased, the decrease being more evident in quails. Pigeons responded with a linear increase in body mass between fasts. In quails, body mass decreased with repeated food deprivations although food consumption increased between fasts. The rate of nocturnal O_2 consumption was lower during the fasts than during the feeding. In addition, the quails reacted to food deprivations with a slightly lower diurnal rate of O_2 consumption during the fasts. Nocturnal hypothermia is an adaptive response to food deprivation in birds. In addition, pigeons, in which the crop is an important part of the digestive system, can store extra food and increase their body mass as a response to repeated fasts. On the other hand, although quails eat more between food deprivations, they may adapt to fasts by decreasing actively their body mass. With active decrease in body mass and low diurnal Tb during the fasts, the energetic demands become less and survival may be enhanced.
Supported by the Academy of Finland.

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P18-09

SEASONAL ACCLIMATIZATION IN SEDENTARY AND ACTIVE PIGEONS
Saarela S., Hohtola E.

Thermal and metabolic responses of pigeons maintained outdoors throughout the year in small cages (sedentary birds) or in large aviaries (active birds) were measured at three ambient temperatures (20, -10, -40°C) in midwinter and midsummer in a strongly seasonal environment (Oulu, Finland, lat. 65°N). In winter, all birds maintained a lower heat production (measured as oxygen consumption and shivering), body temperature, and conductance than in summer at the two lower test temperatures. All birds maintained homeothermic body temperatures down to -40°C. The activity regimen of the birds had no effects on these thermoregulatory variables. Body mass was higher in winter birds, and pectoral mass was correspondingly increased in winter. An additional specific increase in pectoral muscle mass was seen in sedentary birds. Muscle cytochrome oxidase levels were higher in active birds, especially during winter. In winter, the birds had a higher plasma triiodothyronine level than in summer, and lowest values were found in active summer birds. We conclude that seasonal thermal acclimation in pigeons takes place to a great extent by insulative adjustments and that the

capacity for thermogenesis is sufficient without specific adaptations. Activity-induced changes in the oxidative capacity of the main heat producing organ, the pectoral muscle, or plasma thyroid hormone levels do not influence thermal responses. Thus, there seems to be little interaction between activity- and acclimatization-induced metabolic responses in pigeons.

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P18-10

ANAEROBIC ENERGY EXPENDITURE OF IN THE FIELD RECTANGULAR EXERCISES ESTIMATED BY ASTRABIO MODEL

Eclache J.P., Botton F.

In real time measurement of the anaerobic energy expenditure participation during the displacement activities is impracticable in the field. The purpose of this work is to test a simplification of the Astrabio model and a method making it possible to estimate in real time the intensity of the lactate production and transfers between compartments. Astrabio is composed of the descriptive equations of the adjustment aerobic and anaerobic kinetics and the lactate transfers between circulatory space and production and extraction spaces. Its parameters are fitted to the characteristics of the subjects having carried out one of the 3 types of rectangular tests imposing a level of energy expenditure EE infra transitional (it), supra-transitional (st) or supra-maximal (sm). EEit is obtained in laboratory by direct measurement of gas exchanges; EEst and EEsm are obtained by in the field speed recording (V) after establishment of the effectiveness function V-DE. The Astrabio lactate kinetics are compared with the experimental values. The agreement between the model blood lactate (La), the experimental and/or literature values is good. The EEit tests are characterized by a non significant fall of ATP, a mean fall PCr, an overshoot of anaerobic glycogenolysis occurring before 20''of exercise and a peak La at about 10 minutes exercise; EEst by an introduction lactate flow higher than the extraction flow and a drift of La; EEsm by a rise of the ATP-PCr fall and La drift. These results confirm the good predictibility of the metabolic model Astrabio previously published and its interest to estimate anaerobic metabolism during supra-maximal activities in the field.

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P18-11

EFFETS DE METHYLXANTHINES, D'ISOPROTERENOL ET DU SYNACTHENE SUR L'ADIPOCYTE ISOLE DU RAT

Othmani-Mecif K., Benazzoug Y., Amraoui L., Jacob M.P.

L'étude est réalisée sur des rats Wistar mâles (poids 220 gr.). Le diabète est instauré par deux injections de streptozotocine (45 mg/K.de poids corporel) à un mois d'intervalle. Le groupe des rats témoins reçoit de l'eau physiologique. Tous les animaux sont soumis aux mêmes conditions de température (25°C) et de photopériode (10L/14D).Après installation de l'état diabétique, apprécié par l'hyperglycémie, le tissu adipeux périrénal est prélevé pour l'étude morphométrique et l'analyse de la lipolyse. Les adipocytes sont isolés selon la méthode de Rodbell (1964), modifiée par Lafontan et al. (1979). L'évaluation des lipides totaux (Dole et Meinertz, 1960) et du glycérol (Wieland ,1957) libérés par les adipocytes permet d'exprimer la lipolyse en micromoles de glycérol/ 100mg de lipides totaux/ 60mn d'incubation.Comparé à l'adipocyte de rat normal, l'adipocyte du rat diabétique présente une importante augmentation de la lipolyse basale(417%) ainsi qu'une réduction de la taile cellulaire(48%). La lipolyse de l'adipocyte des deux groupes de rats est évaluée en présence de synacthène (SYN), d'isoprotérénol IPR,et de deux méthylxanthines(caféine et théophylline). Connues pour augmenter la lipolyse du tissu adipeux du rat normal, ces différentes drogues stimulent de façon spectaculaire la lipolyse de l'adipocyte du rat streptozotocino-diabétique. En présence de théophylline,à 10-5M la stimulation enregistrée est de 380% et à 10-3M elle atteint 660%. La caféine à 10-4M triple l'activité lipolytique de l'adipocyte. Mieux encore, dés 10-7M, IPR stimule cette dégradation de 833%. A trés faible dose(10-8M) SYN entraine une élevation de la lipolyse adipocytaire de l'ordre de 900%. Ces importantes stimulations de la lipolyse enregistrées peuvent s'expliquer par la grande sensiblisation des récepteurs adipocytaires conséquente de l'état diabétique.

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P18-12

INTERACTIONS BETWEEN NEUROPEPTIDE-Y, LEPTIN AND INSULIN DURING PROLONGED DIURNAL FASTING IN RAMADAN

Kassab S., GhaffarT.-A., Das N.S., Sachdeva U., Nayar U.

Fasting during the month of Ramadan for Muslims is a unique metabolic model that include abstinence from food and fluid intake during the period from dawn to sunset as well as reduction in meal frequency and alterations in the sleep wakefulness cycle. Leptin, neuropeptide-Y and insulin are thought to play a role in long-term regulation of caloric intake and energy expenditure. However, the long-term changes and interactions between these hormones during this pattern of fasting are not known.

The study was conducted on 73 healthy volunteers (age = 22 ± 2 years, BMI = 25.8 ± 0.6 kg/m2). Fasting serum levels of neuropeptide Y, leptin, insulin and glucose were estimated at baseline (day 1), days 14 and 28 of the month of Ramadan and 2 weeks after Ramadan. Baseline serum levels of leptin were correlated positively with body fat ($r = 0.87$, $p = 0.0002$). Serum leptin levels exhibited a significant increase by approximately 41 % and NPY levels were decreased by 30.4 % throughout the month of Ramadan. In addition, a significant correlation ($r = 0.63$, $p = 0.0001$)) was found between changes in serum Leptin and serum insulin. However, changes in serum NPY levels did not correlate with those changes in leptin or insulin

Conclusions: Ramadan Fasting is associated with significant elevations in serum leptin and reduction in serum NPY. The elevations in leptin levels appear to be mediated by the parallel changes in serum insulin. These data support the role of insulin in the long-term regulation of leptin secretion during chronic diurnal fasting followed by nocturnal eating during the month of Ramadan. It also indicate that NPY is not likely to be involved in long-term regulation of leptin secretion during this type of fasting

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P18-13

THE FIBRINRESYSTOMETRIC STUDY IN THE POSTPRANDIAL PERIOD

Sfredel V., Trăilă A., Sfredel D., Matcas H.

Objectives. In the frequence of vascular accidents, the second spike is represented by the absorptive period, the first being the first hours of the morning. The responsible mechanism is the state of hipercoagulability, due especially to the change of the plasmatic lipoproteins specter.
We want to explore the change of hemostasis during the absorptive period by fibrinresistometry, method which reveals the clot biophysical properties.
Method. We studied a group of healthy subjects, each being taken 3 fibrinresystometric determination: one in the morning, and two in different moments of the absorptive period (after lunch). In the same time we also investigated the hemostasis by the usual tests.
Results. For the absorptive period we founded a high resistance for the breaking of the fibrin clot, comparative with the values measured in the morning, but these values didn't passed the normal superior limit.
Conclusions. The method we proposed showed the tendency of hipercoagulability in the absorptive period, in terms that the usual tests for hemostasis, especially the "temporals" one didn't showed anything while passing throw the hipercoagulability state.
Key words: hemostasis, postprandial, lipoproteins.

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P18-14

HEMODYNAMIC CHANGES DURING BOTH ACTIVE AND PASSIVE RECOVERY FROM A SUPRAMAXIMAL EXERCISE

Crisafulli A., Melis F., Tocco F., Santoboni U., Lorrai L., Pittau G.L., Caddeo M., Lai C., Concu A.

The aim of this study was to investigate hemodynamic response during two modes of recovery (active and passive) from repeated bouts of supramaximal, cycle ergometer exercise and to verify whether the active recovery led to a lesser hemodynamic perturbation than the passive one.

Seven male volunteers with mean ± SE age of 28.7±1.4 years, height of 177.8±2.3 cm, and weight of 72.8±2.1 kg, were recruited. Before entering the study, the subjects performed a preliminary cycle ergometer incremental test (20W/min, 60 rpm), up to exhaustion, to assess the maximum workload achievable (Wmax). In separate days each subject underwent the following study protocol, randomly assigned: I) a warm-up of 3 min pedaling at 60 rpm against a resistance of 40W followed by 5 bouts of supramaximal intermittent efforts pedaling at the maximum speed possible against a resistance equivalent to 150% of Wmax for 30 seconds, spaced by 1 min of active recovery pedaling against 40W at a rate of 60 rpm, and followed by 10 min of the same active recovery after the exercise bouts ceased; II) the same protocol as above but the subjects stopped on the bicycle without moving their legs during the recovery between and after the bouts of supramaximal exercise; III) subjects sat quietly on the cycle-ergometer for 22,5 minutes in order to obtain reference control rest values. Cardiac output (CO), heart rate (HR) and stroke volume (SV) were assessed by using an impedance cardiograph (NCCOM 3, BoMed Inc., Irvine, CA) connected to the subject thorax by arranging eight commercially available spot electrodes. Active recovery, with respect to passive recovery, induced higher increases in HR (+76.3 ± 11.6 vs. +60.4 ± 19.8 bpm, $p<0.05$), SV (+28.7 ± 15.3 vs. +15.8 ± 11.7 ml, $p<0.05$), and CO (+3.8 ± 3.1 vs. +0.4 ± 0.6 l*min-1, $p<0.01$).These results showed that motionless recovery from repeated bouts of this kind of exercise induced a reduction in cardiac performance with respect to the active one.

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S19 PHYSIOLOGY OF REPRODUCTION

ORAL SESSION

S19-1

IODIDE TRANSPORTERS IN THE REPRODUCTIVE ORGANS

Pourcher T. [1], Ferhat O. [1], Flachon V. [1], Basquin C. [1], Bouskila N. [1], Leblanc G. [1], Schlumberger M. [2], Bidart J.-M. [2], Lindenthal S. [1]

Iodide is an essential constituent of thyroid hormones. These hormones are essential for proper development of the nervous system and bones. Thyroid hormone biosynthesis depends directly on the supply of iodide. As iodide is a rare element in the biosphere, mammals have developed efficient ways to concentrate it in thyroid colloids and to provide the fetus and the newborn with iodide for their own thyroid hormone synthesis. Several groups reported iodide transfer occurring across the placenta. Transport and concentration of iodide into the milk in the lactating mammary gland has been known for decades.
Current understanding of the mechanism involved in iodide transfer across epithelia mostly relies on studies performed on the thyroid tissue or derived cell lines. It has now been well established that in thyrocytes iodide uptake is mediated by NIS (Natrium Iodide Symporter) localized in the basolateral membrane. Two other membrane proteins are involved in iodide transport across the apical membrane : Pendrin, the protein product of the Pendred Syndrome gene, and the recently identified AIT (Apical Iodide Transporter).
The molecular identification of these transporters allowed the characterization of the proteins involved in iodide transport across extra-thyroidal tissues, in particular across epithelia of the reproductive organs. NIS expression was detected in the mammary gland and in the placenta. In the mammary gland, NIS is up-regulated during lactation and catalyzes iodide accumulation in milk. In the placenta, NIS is only expressed in cytotrophoblast cells and therefore does not participate in iodide transfer across this epithelium. Pendrin expression has been reported in both organs. In the placenta, pendrin was detected by immunolocalization in the villous syncytiotrophoblast cells but its role in iodide transfer is still unclear. AIT expression was found in reproductive organs and could also play a key role in iodide transfer in these tissues.

1 CEA LRC16V, UNSA, Villefranche-sur-mer and 2 Gustave Roussy Institute, France

S19-2

ROLE OF PROLACTIN IN THE REGULATION OF FEMALE REPRODUCTION AND MAMMARY GLAND DEVELOPMENT

Binart N.

Prolactin (PRL) exerts pleiotropic physiological, and is mainly considered as a regulator of reproduction and cell growth. Null mutation of the PRL receptor (R) gene leads to female sterility due to a complete failure of embryo implantation. Implantation and decidualization in the mouse appear to be dependent on ovarian rather than uterine PRLR expression, since progesterone replacement allows the rescue of implantation. To better understand PRL receptor deficiency, we analyzed the successive stages of ovarian development, the ovulation process and subsequent expression of specific mRNAs of enzymes involved in steroid production. We demonstrated that the ovulation rate was unchanged. Formation of the corpus luteum occurs but an elevated level of apoptosis and extensive inhibition of angiogenesis occur during the luteal transition leading to a loss of the enzymatic cascades necessary to produce adequate levels of progesterone which is essential for the maintenance of pregnancy.
Signaling through the prolactin receptor is crucial for functional mammary gland development. We have shown that PRLR heterozygous mice exhibit a severe defect in lactation after the first pregnancy. Transplantation of mammary epithelial cells experiments demonstrate that a direct action of lactogenic hormones to promote alveolar development is confined to the epithelium suggesting that the defect is intrinsic to mammary epithelial cells. PRL and growth hormone (GH), acting through the IGF system have interactive effects to enhance epithelial cell survival. After GH treatment, mammary development is improved in PRLR(+/-) mice demonstrating that PRL is a major repressor of IGFBP expression and that this represents a mechanistic explanation for its ability to inhibit apoptosis. Further characterization of the targets of PRL is needed to more fully explain its specific role in the regulation of female reproduction and mammary gland development.

INSERM U584 Molecular Targets, Faculté de Médecine Necker 75730 Paris, France

OC19-1

EXPRESSION OF EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS 1 AND 2 IS MODULATED BY D-GLUCOSE IN ENDOTHELIUM

Aguayo.C., Sobrevia.L.

Expression and activity of human nucleoside ENT1 transporters (equilibrative, Na^+-independent, nitrobenzylthioinosine (NBMPR) sensitive) is down-regulated in human umbilical vein endothelium (HUVEC) exposed to 25 mM D-glucose. D-glucose increases protein kinase C (PKC), endothelial nitric oxide synthase (eNOS) and mitogen-activated protein kinases (MAPK) p44 and p42 (p42/44mapk) activity in HUVEC. We report the involvement of PKC, eNOS and p42/44mapk on expression of human ENT1 (hENT1) and ENT2 (hENT2) in HUVEC. Cells isolated from normal pregnancies (Ethics committee approval obtained) were cultured in medium 199 with sera (20%). hENT1 and hENT2 mRNA was amplified by reverse transcriptase-polimerase chain reactions on total RNA extracted from cells in 5 or 25 mM D-glucose (24 h), in presence or absence of PD-98059 (10 μM, MAPK kinase inhibitor), NG-nitro-L-arginine methyl ester (L-NAME, 100 μM, eNOS inhibitor), calphostin C (100 nM, PKC inhibitor) and RO-320432 (50 or 100 nM, PKC inhibitor), and phorbol 12-myristate,13-acetate (PMA, 100 nM, 30 min or 24 h, PKC activator). hENT1 and hENT2 mRNA were amplified in HUVEC. hENT2 mRNA was lower (~70%) compared with hENT1. D-Glucose (25 mM) reduced (25 ± 0.7%) the hENT1, but increased (1.4-fold) the hENT2 mRNA level. hENT1 and hENT2 mRNA levels were reduced significantly after incubation of cells with PMA, an effect blocked by calphostin C. The effect of D-glucose on expression of hENT1 and hENT2 mRNA was blocked by calphostin C, PD-98059, L-NAME and RO-320432. These results show that hENT1 and hENT2 mRNA levels are modulated by D-glucose in HUVEC, a process that seems associated with activation of PKC, p42/44mapk and eNOS in this cell type. Support: FONDECYT (1030781 & 1030607), DIUC (201.084.003-1.0)–Chile & The Welcome Trust (UK). C.A. holds a CONICYT (Chile)-PhD fellowship.

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OC19-2

THE INVOLVEMENT OF OXIDATIVE STRESS DURING PREGNANCY

Muresan A., Alb C., Suciu S., Sabau L., Puscas M.

The aerob metabolism offers certain advantages to all life forms. Nevertheless, oxygen- a vital element- is also the source of some very active species, with a destructive potential, called the reactive oxygen species(ROS). Their actions become manifest either when their production is increased or when the antioxidant defense systems are exceeded thus generating the oxidative stress.
The authors have followed on small animals the implication of ROS during the normal pregnancy. We have determined the dinamic of seric lipid peroxides and ceruloplasmine(an extracellular antioxidant)in pregnant female rats, Wistar species and, also, the effect of administration of endogenous antioxidants-vitamin E- on the generation of ROS. These parameters were determined in 2 study groups: first group- pregnant female rats, second group- pregnant rats given vitamin E during the first, second and third week of pregnancy. The results were compared between the 2 groups and also with the witness group-non- pregnant female rats. The lipid peroxides were determined using the colorimetric method with thiobarbituric acid(TBA), seric ceruloplasmine through the colorimetric method Ravin.
The analyze of the results has revealed a gradual increase of seric peroxides and ceruloplasmine in pregnant animals with a pick point during the last week of gestation. In the second group, protected with vitamin E, the response was a "plateau" type: an increase during the first week, without any increase of ROS production at the end of the period. Seric ceruloplasmine has increased progressively with a maximum value in the final stage of gestation.

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OC19-3

INVESTIGATION OF THE ROLE OF BETA2-ADRENERGIC RECEPTORS IN THE RAT CERVICAL RESISTENCE IN VITRO

Gáspár R., Kolarovszki S.Z., Ducza E., Falkay G.

During pregnancy the cervix has to resist tension and remain closed. At term, however, its function is changed to the opposite by a process called cervical ripening/softening in which the resistance of the cervix is significantly decreased. Our aim was to investigate the beta2-adrenergic receptors (beta2-ARs) as modulators of cervical ripening.
Cervical rings were dissected from SPRD rats on different days of pregnancy, and suspended in an organ bath. The cervix was stretched incrementally; every step was followed by 5 min accommodation. The stretching tension was plotted against the tension detected after accommodation, and a regression line was fitted. The slope of the line represented the resistance of the cervix. The experiments were also carried out in the presence of 10-6M terbutaline. The beta2-ARs mRNAs level was measured by RT-PCR technique.
The cervical resistance remained unchanged till the 15th day of pregnancy (slope ~1.00). From day 18 a continuous decrease can be found towards the term (slope ~0.93-0.78). The in vitro incubation with terbutaline did not cause any change in the resistance till day 18. Significant increases in resistance, however, were observed at day 21 and 22. The mRNA level of beta2-ARs was elevated from day 18 to term (day 22).
Beta2-ARs can enhance the cervical resistance in the late pregnant rat in vitro. This result suggest that, opposed to myometrium, the cervical beta2-ARs are possibly coupled to inhibitory G-proteins eliciting a decrease in intracellular cAMP level, and causing an adrenergic inhibition of the softening process in the pregnant cervix.

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OC19-4

EFFECTS OF NEONATAL HYPOTHYROIDISM ON GROWTH AND ADULT TESTICULAR MORPHOLOGY AND FUNCTION
Hamouli-Said Z., Hamoudi F., Hadj-Bekkouche F.

Several studies indicate that thyroid hormones are essential for post-natal growth and development. The aim of the present study is to evaluate the effect of transient induced hypothyroidism on the growth of Wistar rats and development of the male reproductive system in adult.
Rats were treated with a reversible goitrogen, 6propyl-2thiouracil (PTU) from birth to day 21. Thereafter the pups were given tap water and food ad libitum. At day 100, the animals were decapitated; plasma levels of total androgens, FT3 and FT4 were determined by RIA method. The testes were fixed, embedded in paraffin and 5 µm sections were then cut and stained.
The obtained results show a significant impairment of body development for treated rats. Thyroxin replacement improves body size and weight. However, these variations remain significantly lower than for controls. Moreover, we observe a dramatic increase in testes weights (1.65 ± 0.04 vs 1.34 ± 0.04 and 0.88 ± 0.03 vs 0.58 ± 0.01 g/100g of body weight, $p< 0.001$) and in diameter of seminiferous tubules (1051.20 ± 31.25 vs 829.09 ± 28.89 µm, $p< 0.001$) in PTU for treated animals when compared to controls. Significant reduction of plasmatic total androgens (0.95 ± 0.17 vs 2.74 ± 0.44 ng/ml, $p < 0.01$) and in plasmatic levels of FT3 and FT4 (respectively: 4.34 ± 0.25 vs 5.95 ± 0.21 pM and 17.55 ± 1.13 vs 21.72 ± 0.80 pM) can also be seen. There were no obvious histological abnormalities in testes.
The results are in good agreement with those reported in the literature. This is mainly the case of the reduction in the body weight and plasmatic contents of androgens maintained until the adulthood. A reduction in testes weight followed by a lasting increasing one indicates an early critical influence of thyroid hormones on growth and development of reproductive system.

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S19-3

ADHESION SIGNALS FOR DIFFERENTIATION AND SURVIVAL IN MAMMARY EPITHELIUM
Streuli C.

Differentiation, specifically the activation of tissue-specific transcription factors, in mammary epithelia is co-ordinately regulated by integrin-mediated cell-matrix interactions and soluble signals that activate prolactin and insulin receptor. We have discovered that specific cell-matrix adhesions are essential for prolactin and insulin-mediated signal transduction, and a current focus is to identify the molecular mechanisms of crosstalk. We have also discovered that integrins determine survival potential of mammary epithelia a) directly via a FAK-mediated control on the subcellular distribution of the pro-apoptotic protein Bax and b) indirectly through crosstalk with IGF signalling. We are currently dissecting the molecular links between integrin and Bax. IGFs are central for survival of mammary epithelial cells and we have found that they are involved with complex networks of signals. IGF signalling is ECM-dependent and it operates through both IRS-1 and via crosstalk through the EGF-R.

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S19-4

TRANSPORT OF AMINONITROGEN IN THE RAT MAMMARY GLAND
Shennan D.

The transport of amino acids into mammary epithelial cells is a crucial step in the process of milk secretion.In addition, it appears that amino acids are also required by mammary cells to regulate the cellular hydration state which in turn influences protein and fat synthesis. The functional properties of system L, a sodium-independent neutral amino acid carrier, in the lactating rat mammary gland have been examined. Experiments were performed using isolated tissue explants and the perfused mammary gland. The latter preparation was used in conjunction with a rapid, paired tracer-dilution technique. Functional studies suggest that system L is located in the basolateral aspect of the mammary epithelium. Cis-inhibition studies suggest that most neutral L-isomer amino acids, with the exception of L-proline, are substrates. Some D-isomers may also be substrates suggesting that LAT1 may be the molecular correlate of system L in rat mammary epithelial cells. L-alanine and L-glutamine are transported by system L in the rat mammary gland which indicates that LAT2 may also make a contribution to neutral amino acid uptake. In this connection mRNA for LAT1 and LAT2 as well as CD98 is expressed in the rat mammary gland. One unusual feature of system L in the lactating mammary gland is the lack of trans-stimulation of amino acid efflux. Furthermore, system L in the rat mammary gland was inhibited by pre-treating animals with bromocryptine suggesting that the transport system is regulated by prolactin. Unilateral weaning also inhibited amino acid transport via system L which is consistent with the notion that milk stasis regulates transport activity: this may be a way of matching amino acid supply to the demands of the mammary epithelial cells. In conclusion, system L may play an important role in delivering aminonitrogen for mammary protein synthesis.

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POSTER SESSION

P19-01

INFLUENCE OF NITRIC OXIDE SYNTHASE INHIBITOR ON MURINE OOCYTE MATURATION IN VITRO

Voznesenska T.

We investigated the extent to which NO participates in oocyte maturation using an in vitro culture system adding NOS inhibitor (N-omega-nitro-L-arginine methyl ester, L-NAME).
Cumulus-oocyte complexes (COC) were isolated from ovarian follicles of 8-week-old CBA mice and were cultured in medium without (control) or with different doses of L-NAME (0,02 mM, 0,12 mM and 0,24 mM) at 37 degrees C for 20 hr.
To assess effects of NO deficiency on the kinetics of germinal vesicle breakdown (GVBD) COC and forming the first polar body (PB) were observed for 5 hr and 20 hr, respectively.
After the culture period, cumulus cells were removed, and oocytes were classified as metaphase II (M II), metaphase I (M I) or showing atypical (degenerative) morphology.
Maturation of COC treated with L-NAME resulted in a lower percentage of oocytes at M II stage ($P < 0.01$) and a higher percentage of oocytes at M I or atypical stages ($P < 0.01$) compared with those in medium without L-NAME.
Our data demonstrate that NO is a key modulator of oocyte meiotic maturation and may have crucial roles in oocyte maturation in vitro.
These results support our previous observations in vivo and indicate that NOS/NO has functions in both oocyte maturation and follicular/oocyte development.

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P19-02

INFLUENCE OF NITRIC OXIDE SYNTHASE INHIBITORS ON MURINE EMBRYOS

Blashkiv T., Voznesenska T.

Nitric oxide (NO) has emerged as one of several important intra-ovarian regulatory factors. The pre-implantation period is a critical time during murine development. Although the importance of nitric oxide has been demonstrated during gestation, its role in pre-implantation period has not been fully defined.
We investigated the extent to which NO participates in the developmental competence (embryonic destructions and morphological features of pre- and post-implantation embryos) using an nitric oxide synthase (NOS) inhibitors which oppress all forms of NOS and mice-female which entered inhibitors NOS before their crossing with intact male.
It has been shown the increase of embryonic destruction (pre- and post-implantation embryos death rates) at female groups after their crossing with intact male in early term after introduction of preparations.
Studying of embryonic morphological features has shown, that there is a delay of embryonic development at a stage of division and in a course of gastrulation.
The received results testify, that NO an important diffusive regulator of cellular differentiations, in particular at pre- and post-implantation embryos at mice.

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P19-03

THE ROLE OF REACTIVE OXYGEN SPECIES AND NITRIC OXIDE IN ONSET OF LABOR AT TERM

Sabau L., Suciu S., Costin N., Dorofteiu M.

The exact cause of the increased activity of uterus at the end of the pregnancy that produces the stretching of cervix is not known. The aim of our study was to investigate the implication of reactive oxygen species (ROS) and nitric oxide (NO) in producing increased contractility of uterus at the onset of labour. We included in our study 25 healthy pregnant women with gestational age between 38 and 40 weeks without labour and 25 healthy pregnant women having the same age and gestation age at the beginning of labour. The study has the permission from the Ethical Committee of the University "Iuliu Hatieganu" Cluj-Napoca. We determined for all patients the amount of ROS by measuring the plasma levels of lipid peroxides, using the method with thiobarbituric acid and carbonyl content of proteins, using the method with phenilhydrazine. We also determined the amount of antioxidants by measuring the plasma level of caeruloplasmin, by Ravin method and the hydrogen donating ability, by Hatano method. The plasma level of NO was measured using the Griess method. We found significantly higher ($p<0.01$) values of lipid peroxides and carbonyl content of proteins for patients with labour than in pregnant patients without labour, illustrating a great production of ROS in the first group. It is known that arachidonic acid pathways to form prostaglandins (which produce the contraction of the uterus) can be triggered by ROS. The plasma levels of antioxidants and NO were found significantly lower ($p<0.01$) for patients with labour than in those without labour, probably because they are consumed in the reaction with ROS produced in excess (the antioxidants were used for the neutralization of ROS and NO is consumed in the reaction with superoxide anion). We conclude that the lower level of NO - NO being known to produce the relaxation of the uterine smooth muscle in pregnancy - may contribute to the increased contractility of uterus at term and consequently to the onset of labour.

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P19-04

ALBUMIN STIMULATES HCG AND HPL RELEASES FROM HUMAN TERM PLACENTA EXPLANTS

Lambot N., Meuris S., Delogne-Desnoeck J., Vanbellinghen A.-M., Lebrun P.

The maternal plasma is in direct contact with the placental trophoblast layer. Albumin, the major plasma protein, has been reported to stimulate the release of hCG and hPL from human term placenta explants when increased from interstitial (0.5%) to circulating physiological levels (5%). The purpose of this study was to further investigate the specificity of the albumin effect on the hormonal secretion from placental explants.
The secretory effect of albumin was partly reproduced by colloidal agents such as dextran (4.5%) or polygelin (4%), indicating that a rise in colloidal osmotic pressure can elicit hormonal release by the trophoblast layer. The stimulatory effect of dextran and polygelin was transient (less than 10 min of duration) and followed by a marked but reversible inhibitory phase. This inhibitory phase was not observed with albumin, suggesting that the albumin effect is “partly” specific. The effect of these three agents on hCG and hPL release was not modified in the absence of extracellular calcium.
Concurrently to the stimulation of hormonal release, albumin (5%), dextran (4.5%) and polygelin (4%) provoked a marked increase in 45calcium outflow from prelabelled and perifused placental explants. Furthermore, this rise in 45calcium outfow persisted in the absence of extracellular calcium, suggesting the mobilisation of calcium from intracellular stores.
Taken together, these data support the view that the triggering effect of albumin on hormonal secretion is “partly” specific, “partly” colloidal, and independent on extracellular calcium. The secretory effects evoked by the modifications of the colloidal osmotic pressure seem to involve a mobilisation of calcium from intracellular stores.

Université Libre de Bruxelles - Lab. of Experimental Hormonology Belgium

P19-05

THE MODULATION OF UTERINE CONTRACTILE ACTIVITY BY CAPSAICIN-SENSITIVE AFFERENT FIBERS

Klukovits A., Gáspár R., Sántha P., Jancsó G., Falkay G.

The possible participation of capsaicin-sensitive sensory nerves in the modulation of neurogenic contractions was studied in non-pregnant and term pregnant rat uteri. Neurogenic contractions were elicited by electric field stimulation (40 V, 1-70 Hz, 0.6 ms) in intact uteri and in uteri which were previously exposed to capsaicin in vitro. In capsaicin pretreated preparations obtained both from non-pregnant and term pregnant rats, a dose-dependent increase in the amplitude of uterine contractions was detected. Prior systemic treatment of the rats with capsaicin (130 mg/kg, s.c.) abolished the effect of in vitro capsaicin administration on the amplitude of neurally evoked contractions. These findings suggest that the effect of capsaicin on uterine contractility is of neural origin and may not be related to a direct action on the smooth muscle. Experiments using a specific antagonist of calcitonin gene-related peptide revealed that depletion of this peptide, normally contained in capsaicin-sensitive sensory nerves, may be responsible, at least in part, for the increased responsiveness of uterine smooth muscle to low frequency electric field stimulation.

The findings support the notion that capsaicin-sensitive afferent nerves, by the release of sensory neuropeptides, significantly contribute to the modulation of uterine contractility. It is suggested that uterine sensory nerve activation may be part of a trigger mechanism leading to preterm contractions evoked by, e.g. inflammatory processes.

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-06

ROLE OF SEROTONERGIC ACTIVITY IN THE ALPHA1A-BLOCKADE IN THE PREGNANT RAT MYOMETRIUM IN VITRO
Mihalyi A., Ducza E., Gáspár R., Falkay G.

The adrenergic system plays an important role in the regulation of myometrial contractility. It had been revealed that blockade of the alpha1A-adrenoceptors inhibits contractions of the pregnant rat uterus elicited either by electrical field stimulation (EFS) or norepinephrine. Some alpha1A-adrenoceptor antagonists exert stimulatory effect on 5-HT1A receptors. The aim of the study was to clarify whether this feature has any influence on the uterus relaxant effect.
Uterus rings were taken from 22-day pregnant SPRD rats and mounted in a tissue bath. Concentration-response curve of 5-HT was constructed alone and in the presence of subtype-selective alpha1A-adrenoceptor antagonists, WB4101 and 5-methylurapidil. Next, the concentration-response curves of the alpha1A-antagonists were constructed in the presence of 5-HT1A –antagonist WAY100135, using EFS. RT-PCR was used to determine the mRNA expression of the two receptor types.
The mRNA expression of the alpha1A-adrenoceptors is significantly greater than that of the 5-HT1A receptors. Serotonin increased the contractility of the myometrium dose-dependently. In the presence of the alpha1A-antagonists the concentration-response curve of serotonin was shifted to the right in each case. The uterus-relaxant effect of WB4101 did not change in the presence of 5-HT1A –antagonist WAY100135. The maximal inhibition of 5-methylurapidil increased in the presence of the 5-HT1A –antagonist.
These results suggest that the contractions induced by serotonin are mediated by alpha1A-receptors. Serotonergic activity of WB4101 does not influence its uterus-relaxant effect. Concerning 5-methylurapidil, its serotonin activity depresses its efficacy in terms of uterus relaxation. These findings provide further proofs for the interaction between the adrenergig and serotonergic systems.

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-07

STUDY ON MYOMETRIAL ACTIVITY AS A FUNCTION OF INTRAUTERINE PRESSURE IN PERFUSED RAT UTERUS IN VITRO
Zupkó I., Bokor D., Falkay G.

In spite of the increasing knowledge concerning the regulation of the motor activity of the pregnant uterus the mechanism which is responsible for the initiation of delivery remains unknown. The intrauterine volume and pressure is proved to be one of the crucial factors determining the contractility of the myometrium [1]. Up to now the effect of intrauterine pressure on the activity of the uterine smooth muscle could be investigated only in vivo, arising a methodological limitation to most investigation on drugs with myometrial site of action.
We therefore elaborated a unique system in which the isolated uterine horn is continuously perfused by a peristaltic pump. The longitudinal contraction of the horn and the inner pressure are registrated in the same time. Due to perfusion, non pregnant uterine horns, which had no spotaneous activity in a traditional in vitro chamber, showed a pressure dependent motor activity.
The spontaneous activity of the myometrium was recorded as a function of the perfusing pressure during the time course of the pregnancy of the rat.
We believe that the involvement of intrauterine pressure as an experimental parameter into this in vitro system gives a substantial contribution to the understanding of the initiation of labor as well as to the development of new tocolytic agents and uterotonics.
1. Csapo AI: Model experiments and clinical trials in the control of pregnancy and parturition. Am. J. Obstet. Gynecol. 85: 359-376 (1963)

University of Szeged, Department of Pharmacodynamics and Biopharmacy - Hungary

P19-08

ALTERATION OF ESTROGEN RECEPTOR SUBTYPES OF THE PREGNANT MYOMETRIUM IN THE RAT
Minorics R., Ducza E., Márki Á., Falkay G.

Estrogens exert numerous biologic effect in large number of targets, including the uterus. Two subtypes of estrogen receptors (ERs) have been described to date, ERalpha and ERbeta. The timecourse density of these subtypes in the pregnant rat uterus is not completely examined. Our present aim was to determine the changes in the expression of ER subtypes proteins and mRNA on days 4, 5, 6, 7, 8, 10, 15, 18, 20 and 22 of pregnancy.
To demonstrate the expression of ER subtypes mRNA we used reverse transcription-polymerase chain reaction (RT-PCR), and the densities of receptor proteins were determined by radioligand binding assay (RBA).
This was the first characterisation of ERalpha and ERbeta during pregnancy in the rat myometrium. The first maximum of ERalpha mRNA expression was found on day 5-6, then the receptor expression increased again from day 8 to 22. ERbeta1 and beta2 were detectable from day 7 to 15 only. The maximum levels of ERbeta1 and beta2 mRNA were on day 7 and slowly decreased to day 15. These results were supported by the measurment of RBA.
In light of these facts it can be concluded, that the presence of ERalpha is dominant on the days of pregnancy. The continuous increase in the expression of ERalpha mRNA until the end of pregnancy correlates with the expression pattern of alpha1A-adrenergic receptor mRNA investigated by earlier studies. Therefore the interaction between estrogens and the adrenergic system, a previous hypothesis, might be realized through the ERalpha receptor.

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P19-09

ONTOGENESIS OF MUSCARINIC ACETYLCHOLINE RECEPTORS IN SERTOLI CELLS.
Caviglia D., Angelini C., Scarabelli L., Voci A., Palmero S.

Cholinergic-like molecules have been previously localized in rat testis during postnatal development by immunological assays.
In a recent study all the M1-M5 mAChR mRNA subtypes were detected in Sertoli cell primary cultures from 30-d-old rats.
The aim of the present study was to evaluate by RT-PCR the ontogenesis of mAChR mRNA isoforms during postnatal development in Sertoli cell isolated from prepubertal 8-15-21-d-old rats. Moreover, the localization of the mAChRs at Sertoli cell level was investigated on piglet Sertoli cell primary cultures as well as in a pure mouse clonal Sertoli cell line (42GPA9).The presence of molecules immunologically-related to cholinergic system has been then validated by specific monoclonal antibodies.
Our results suggest an age-dependent expression of the different muscarinic receptor isoforms.Actually, M1 and M2 mAChRs mRNAs appear to be the earliest (8-d-old rats) subtypes expressed in rat Sertoli cells, while all the five isoforms were identified in Sertoli cells from older rats.
The presence of muscarinic receptors in Sertoli cells from piglet testes as well as in a pure mouse Sertoli cell line was demontrated both at mRNA (RT-PCR) and protein (Immuno-cytochemistry) level.
Cholinergic molecules in Sertoli cell might play a role in cell-to-cell communication affecting cell differentiation and co-ordinating cell functions.

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P19-11

ACTIVITY OF BGT: QUALITATIVE AND QUANTITATIVE ANALYSIS OF BILIRUBIN COMPOUNDS THROUGHOUT DEVELOPMENT
Ortiz A.[1], Cantarino M. H.[1], Bustamante N. [1], Cubero F.J.[2], García-Barrutia M.S.[1], Mula N.[2], Maganto P.[2], Arahuetes R. M.[1]

Hepatic bilirubin excretion requires bilirubin UDP-glucuronosyltransferase (BGT)-mediated glucuronidation. Patients with type I Crigler-Najjar syndrome and Gunn rats inherit deficiency of BGT activity towards bilirubin as an autosomal recessive trait and, as a result, exhibit marked hyperbilirubinemia.In fetal life placental clearance of bilirubin of fetal origin is efficient via conjugation in maternal liver. At birth, once the neonatal pup

is separated from the placental circulation, hepatic clearance of the pigment is incomplete until it becomes liver-dependent.AIM:The aim of our work is to assess the variation of the activity throughout development combined with a simple and qualitative method of isolation of the bilirubin glucuronides.METHODS:Fetuses, newborns and adult Wistar rats were used. Liver microsome fraction was obtained and microsome protein concentration calculated using Bradford´s method. Activation of the microsomal aliquots (4 mg/ml of protein) was carried out by the addition of 0,08% Brij-56. Selective diazotization of glucuronides in the presence of bilirubin was also performed to determine the activity of the enzyme. Glucuronides turn into coloured pigments that are easily quantified by spectrophotometry and visualized by thin layer chromatography.
RESULTS:Our results show low BGT activity at 21-days´gestation followed by a rapid neonatal BGT activity is shown up to the first month of extrauterine life when levels become adult. These data are consistent with those obtained by chromatography. CONCLUSION:This work provides a simple and trustworthy standard for bilirubin glucuronidation in normal physiological conditions, which might contribute to detect pathological abnormalities as well to evaluate the therapeutic potential of treatments.
(This work was financed by grants FIS 01/0001-01 and 02)

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P19-12

ANDROGENS CONCENTRATIONS IN RESPONSE TO ADMINISTRATION OF ACTH RATS GENETICALLY PRIVED OF VASOPRESSIN
Kandsi F.

To know the possible influence of vasopressin in the regulation of adrenal androgens production, we compared basal and after ACTH treatment testosterone (T),androstenedione(A) and dehydroepiandrosterone sulfate(DHEA.s)concentration adrenal venous blood of 14 males Brattelboro(BI) and 12 males rat long Evans(LE). The animals are pre-treated 3h and 15 min before injection of 1-24ACTH(0.5mUI/100g body respectively by chlorpromazine(1mg/100mg body) weight). The rats are anaesthetised by pentobarbital(0.5ml/100g body weight). The adrenal vein blood is recupered before (0 min) and 20 min after the injection of o.5mUI of ACTH/100g body weight, the T, A and DHEA-S are measured byRIA. The results show that basal concentration of T, A and DHEA are two fold lower in BI rat than in LE rat ($p<0.001$).
The ACTH don't modify 20 min after the injection the concentration of the three cited hormones they remain twice lower in the BI rat .As a conclusion, the vasopressin hypothalamic could be a stimulant factor implied in the production of the adrenal androgens in the rat.

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P19-13

ACTH EFFECTS ON PLASMA ANDROGENS, CORTICOIDS, ACTH AND ON ADRENAL AND TESTIS TESTOSTERONE IN RABBIT
Soltani Y., Hadj Bekkouche F.

Objectives: The exploration of the relationship between the adrenal and the testicular axis in the domestic rabbit.
Methods: Administration of 10ug/kg of cortrosyn (1mg/ml) through the marginal ear vein, The control animals received the same dose of physiological serum (NaCl 0.9%).The testes and adrenals were moved and the plasma (n=42) collected before, then 20, 40, 60, 80, 100 and 120mn after ACTH administration. Plasma ACTH is evaluated with radioimmunometric assay, glucocorticoids by a competitive binding to transcortine (CBG), while the plasma androgens, testicular and adrenal testosterone are estimated by radioimmunoassay.
Results: Plasma ACTH falls from 12.26± 0.11 to 5 ± 0.1pg/ml (-59.2%;$p<0.05$) after 40 mn in the ACTH group, then rises (+380%;$p<0.05$) to 19±3.59 pg/ml at 120mn; inversely in the control group, ACTH increases (373%;$p<0.05$) in two steps from 5.63± 0.41(0mn) to 21.74± 3.81pg/ml (40mn), then between 60mn and 100mn (+228%).to reach 14.5±2.9pg/ml. Plasma corticosterone don't shows a significant fluctuations, while plasma cortisol increases in the ACTH group (-73.9%;$p<0.05$) after 20mn 789±37.3 pg/ml, then falls at 120mn (-54%; $p<0.05$).to 362.78± 81.9 pg/ml. Plasma androgens increases (+175% ; $p<0.05$) after 20mn but the testicular testosterone shows a concomittent diminution in the ACTH and the control groups after 40mn ($p<0.05$) respectively from 13.06 ± 1.72 to 1.22±0.55 ng/g and from 10.3± 2.49± 0.1ng/g to 2.49± 0.1ng/g, while two opposit plots are respectively recorded between 0 and 60mn for the adrenal testosterone, an increase (+1100%; $p<0.01$) from 0.65± 0.19 to 7.26±0.2ng/g, and a diminution (-83%; $p<0.05$) from 2.61± 0.54 to 0.44±0.05 ng/g.
Conclusion: We suggest that the involvement of ACTH in the adrenal steroidogenesis leads to control the testosterone and the glucocorticoids secretion and thus, to regulate the testis testosterone secretion through a specific glucocorticoid receptors via a feed back on ACTH-LH secretion.

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P19-14

HORMONAL MODULATION OF IMMUNE FUNCTION; ROLE OF NITRIC OXIDE
Pehlivanoglu B., Balkancı D., Durmazlar N., Erbas D.

Objective:Stress is a factor blamed to be responsible in the etiology of many diseases. One of the most consistent findings due to stress is immune system modulation. The menstrual cycle(MC)and changing hormonal profiles during luteal and follicular phases result in redistribution of peripheral blood lymphocytes(PBL)in response to stress, in part due to alterations in nitric oxide levels (NO). Of the effective contraception methods, oral contraceptives(OC) are among the most widely used, we performed the present study to investigate the effects of OC on PBL subsets under stress. Methods:Women using OC(Desolett(n=3)or Myralon(n=5) each including desogesterel 150 Mg and etinil estradiol 30 Mg and 20 Mg respectively) and healthy women (n=10),during the follicular and luteal phases underwent the Stroop colour-word interference and cold pressor tests. Pre and post test immune system responses were determined by cell counts using the flowcytometer. MC phase was ascertained by plasma estrogen and progesterone measurements. Stress response was evaluated by blood pressure and heart rate records throughout the tests and plasma cortisol, urinary metanephrine and vanillylmandelic acid measurements before and after the tests. Plasma and urinary NO determined before and after the tests. All the results were analysed with the appropriate statistical methods. Results; OC users had significantly higher CD3+CD8+ T and lower natural killer cells compared to non users of both phases. Luteal and follicular phases of non OC group differed due to the presence of suppressed immune response to acute stress, including decreased CD4/CD8 ratio and NK cell percentage. NO was elevated in the luteal phase in OC users and increased further in post stress samples. Conclusion; individual reaction towards stress is affected by MC phase and with use of OC. NO appears to be a possible effector molecule for these differences.
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P19-15

EFFECT OF NEONATAL ANDROGENIZATION OF FEMALE RAT ON CARDIOVASCULAR STRESS-REACTIVITY IN ADULT FEMALE
Klimova O., Anishchenko T., Igosheva N., Fetisova Y.

The objective of this study was to establish whether the presence of testosterone in the neonatal period is essential for the determination of gender differences in cardiovascular responsivity to stress in adult rats.
Adult intact males and females (n=24) and neonatally androgenized (NeA) females (n=12) were instrumented with catheters in the femoral artery for direct measure of mean arterial pressure (MAP). Next day, the rats were subjected to 60-min immobilization stress (IS) and MAP and heart rate (HR) were studied during IS and recovery period (60 min).
The basal values of HR and MAP did not differ significantly between sexes, but the cardiovascular responses to IS were gender-dependent. So, in males compared with females, IS caused more significant and prolonged HR increase (38% vs., 25%, $p<0,05$). At 60th min of recovery period HR remained elevated in males and normalized in females. Stress-induced increase in MAP was also greater in males vs. females (26% against 16%, $p<0,05$).
Neonatal androgenization of females resulted in decrease in basal HR values and increase in basal MAP ones. It is very important, that patterns of HR and MAP responses to IS in adult NeA females were similar to that in intact mature males and differed from that observed in females. Actually, in NeA females HR increased by 38% during the first min of IS and remained elevated even to 60 min of recovery. Also, stress-induced MAP increase in NeA females was similar (25%) to that, observed in intact males. So, our data indicate a pivotal role of androgens during neonatal development for the

determination of gender-specific difference in cardiovascular responses to stress in the mature rats.

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S20 DYNAMIC ANALYSIS OF BIOLOGICAL OSCILLATORS

ORAL SESSION

S20-1

CHAOS AND SYNCHRONIZATION IN NEPHRON DYNAMICS

Holstein-Rathlou N-H., Sosnovtseva O.V., Mosekilde E.

Synchronization phenomena play an important role in the regulation and function of normal physiological systems as well as in certain disease states. We have previously shown an interaction between the tubuloglomerular feedback (TGF) mechanisms of nephrons arising from the same cortical radial artery. The interaction leads to an in-phase synchronization of the TGF mediated oscillations in both normotensive and spontaneously hypertensive rats (SHR). In some normotensive rats, a subset of nephrons showed antiphase synchronization. These nephrons belonged to different cortical radial arteries. Computer simulations of a realistic nephron model indicate that antiphase synchronization arises due to socalled hemodynamic coupling. The latter is caused by vascular steal, i.e., the dilation of one arteriole diverts blood away from neighboring vessels sharing the same feed artery.
Besides the slow TGF mediated oscillations (2 - 3 cycles per min), faster myogenic oscillations (6 - 10 cycles per min) can also be demonstrated in individual nephrons. Using a newly developed wavelet based technique we have investigated the simultaneous coupling of the fast and the slow oscillations in interacting nephrons. In the normotensive rats, the typical pattern was one of full entrainment, with synchronization of both the fast and the slow oscillations. A similar pattern could be found in hypertensive rats (SHR) having chaotic fluctuations. However, in SHR the typical pattern was one of partial synchronization where the slow oscillations were synchronized while the fast oscillations demonstrated asynchronous behavior. Similar behavior could be obtained in model simulations by varying the interaction strength.
In conclusion, the present work has shown that significant synchronization occurs between nephrons. Furthermore, different synchronized states can be detected. We hypothesize that transitions between the different states may be of significance in the regulation of renal function.

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S20-2

BAROREFLEX INSTABILITY AND BLOOD PRESSURE OSCILLATIONS

Julien C., Chapuis B.

Every negative feedback control system containing a time lag is prone to oscillate because, as frequency increases, the output signal is progressively delayed from the input signal until, at a particular frequency, it feeds back positively, i.e. the output is in phase with the input. This theoretical consideration has been applied to the arterial baroreceptor reflex, and it could be demonstrated in rats that the resonance frequency of the baroreflex loop (0.4 Hz) is close to the actual frequency of sympathetically mediated oscillations of blood pressure (BP), the so-called Mayer waves. Control theory predicts that, depending on the open-loop gain (Go) at the resonance frequency, the feedback oscillation will vanish (Go<1), continue at a stable amplitude (Go=1) or grow indefinitely (Go>1). Close examination of Mayer waves in conscious rats indicates that their amplitude is highly variable over time, i.e. exhibits a "waxing and waning" pattern. Simultaneous recordings of BP and renal sympathetic nerve activity (SNA) in conscious sinoaortic baroreceptor denervated rats have suggested that the circulatory system is continuously challenged by slow (<0.1 Hz) internal perturbations unrelated to SNA, which appears in the form of a "1/f noise" in the BP spectra. Modelling of the closed loop operation of the baroreceptor reflex suggests that Mayer waves are more transient responses to BP perturbations than true self-sustained oscillations. Their instantaneous amplitude depends both on the underlying perturbation and on the baroreflex gain. In summary, while the gain of the baroreceptor reflex largely determines its ability to provide an efficient correction of slow BP perturbations, this is achieved at the cost of instability at higher frequencies. However, all available evidence indicates that this instability is not detrimental to the cardiovascular system.

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OC20-1

COMPUTATIONAL ANALYSIS OF THE MODULATION OF CALCIUM OSCILLATIONS AND WAVES BY CALRETICULIN

Yano K., Petersen O., Tepikin A.

Sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) is an essential component of intracellular calcium signalling as it removes calcium from cytosol and replenishes the endoplasmic reticulum (ER). It has been shown that calreticulin, a calcium-binding lectin chaperon in ER, modulates IP3-induced calcium oscillations in a SERCA subtype specific manner. In order to understand the effects of calreticulin on SERCA, we have developed a mathematical model for SERCA that considers the effects of both cytosolic and ER calcium. We have incorporated the model into the zero- and three-dimensional whole cell model, which contains cytosolic and the ER compartments as well as calcium release channels. Our computational simulations have successfully reproduced the experimentally observed calcium oscillations and waves in xenopus oocytes expressing subtype 2a or 2b of SERCA as well as the effects of calreticulin. The bifurcation analysis of the mathematical model suggests that calreticulin may inactivate SERCA2b as well as IP3 receptor by modifying the maximum transport rates and the sensitivity to ER calcium.

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OC20-2

A PHYSICAL MODEL BASED STUDY OF PRESSURE WAVE TRANSMISSION

Hayashi K., Shigemi K., Mizobe T.

OBJECTIVES: Aortic pressure waveforms (APW) have important information about cardiovascular conditions. However, direct monitoring is invasive and not practical. The method for estimation of APW from radial pressure wave (RPW) using generalized transfer function (GTF) is not common, since the estimation was not available when circulation changes extremely. In the present study, pressure wave transmission was quantitatively analyzed with 4-element model to elucidate the mechanism of pressure waveform deformation.
METHOD: The model is formed with characteristic impedance (Zc), total systemic inertance (L), peripheral resistance (Rp), and total compliance (C). The transfer function (TF) of 4-element model is expressed in a simple numerical formula: $G(s) = 1 / \{ LC s^2 + (C Rc + L / Rp) s + Zc / Rp + 1 \}$ ($s = \sigma + j$). The interactions between APW and RPW in various conditions were simulated with the model. To confirm the accuracy of the simulations, we simultaneously measured APW and RPW in 9 cardiac surgeries, and TF between them (measured TF) was calculated with 10-order Auto-Regressive Exogenous (ARX) model. We fitted measured TF (<8 Hz) to the model TF to minimize the squared absolute value of the complex number difference between them in the frequency domain, and appropriate 4 elements were calculated.
RESULT: As for 3 cases, RPW form was dull (D-group), and in other 6 cases, RPW was sharp (P-group). With fixed L value (0.0012), parameters in the model were obtained in D and P groups as follows, C: 0.82+/-0.08, 0.53+/-0.16 [$ml.mmHg^{-1}$], Rp: 0.35+/-0.03, 1.28+/-0.82 [$mmHg.ml^{-1}.s$], Zc: 0.037+-0.007, 0.022+-0.009 [$mmHg.ml^{-1}.s$].
CONCLUSION: The simulations using the 4-element model coincided with the clinical data, and will contribute to estimate aortic pressure in various conditions.

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OC20-3

DYNAMIC ASSESSMENT OF CARDIORESPIRATORY COUPLING IN NON-STATIONARY EXERCISE

Blain G., Meste O., Bermon S.

The high frequency (HF) power spectral component of heart period variability (HPV) represents the coupling between respiratory and cardiac 12on a breath by breath basis, and ECG was simultaneously recorded. In order to take into account the non-stationary character of the RR intervals under dynamic exercise, an evolutive model was estimated from the detrended and high pass filtered tachogram. The time-varying frequencies were obtained from a pole tracking algorithm. Then, the instantaneous power of the time-varying HF (IPHF) was calculated with the short time Fourier transform. Correlation coefficients revealed that time respiratory frequency

(RF) and HF evolutions were highly correlated. Mean (SD) value was r=0.89 (0.10) ($p<0.01$). On the other hand, IPHF has been found to vary dynamically with time, but no correlation was found between IPHF and respiratory parameters or exercise intensity. This signal processing method applied to the RR data allowed us to accurately assess the instantaneous influence of RF in the variation of the HF of the HPV during dynamic exercise.

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OC20-4

VARIATIONS IN HEART RATE AT ~0.1 Hz BUFFER OSCILLATIONS IN MEAN ARTERIAL PRESSURE IN HUMANS
Toska K., Elstad M., Walløe L.

The aim of this study was to test the hypothesis that mid-frequency fluctuations in arterial blood pressure are buffered by oscillations in heart rate (HR). Simultaneous recordings of beat-to-beat mean arterial pressure (MAP), systolic pressure (SP), left cardiac stroke volume (SV, pulsed ultrasound Doppler), and HR were obtained in 10 healthy young adults during spontaneous breathing. Recordings were obtained in the supine and tilted positions both before and after pharmacological autonomic blockade. Mid-frequency variations in the recorded variables were quantified by spectral analysis, and cross-spectra analysis provided phase angles between the cardiovascular variables.
Fluctuations in SV, cardiac output (CO), total peripheral resistance (TPR), MAP and SP at the frequencies 0.07 to 0.15 Hz decreased after removal of HR variations in the supine position ($P < 0.05$). In 30 degree tilted position, MAP and CO variations were unchanged, while SV, TPR and SP oscillations decreased ($P < 0.05$) after atropine administration. In the supine position, variations in HR and in SV are in inverse phase, and variations in CO and in TPR are in inverse phase. We conclude that HR variations contribute to variations in CO at ~0.1 Hz. Fluctuations in CO buffer oscillations in MAP caused by variations in TPR. Hence, HR variations buffer oscillations in MAP at ~0.1 Hz.

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OC20-5

STRETCH DEPENDENT VESICAL MOTOR AND ELECTRICAL REACTIONS ON IONS: INFLUENCE OF Ca, Ba AND Sr
Neu E., Wirth M., Matis U., Michailov M.Ch., Seidenbusch W., Staehler G., Welscher U.

Divalent ions (Ca, Ba, Sr; n=47) influenced the stretch dependent motor reactions [1-3] of urinary bladder (3 to 50 mN). $CaCl_2$ (2.1 mM = 1x = also for Ba and Sr) had no essential effect on amplitudes (A) of spontaneous phasic contractions of detrusor (SPC; 1-5/min) and tonic ones of trigone (STC; 0.1-0.5/min), but an inhibitory - on frequency (F), stronger after stretch (84.6 and 42.6% resp.). Contrary to this, addition of $BaCl_2$ (0.5-1.5x) had a stimulatory effect: A of SPC increased, at 3 mN 296.8 for SPC and 203.1% for STC (for 0.5x); at 50 mN - 186.8 and 191.0 resp. F (for 1x) was for STC 150.1 and 74.4% (after stretch). (Equimolar) replacement of $CaCl_2$ by $BaCl_2$ (0.5 to 0.75x) had also an augmentory effect on A of SPC and increased basal tone. After total replacement the motor activity was nearly blocked. After replacement of $CaCl_2$ by $SrCl_2$ (0.5 to 0.75x) appeared an increase of SPC, after 1x - a decrease. Electrical activity: Detrusor myocytes generate spikes (S), bursts (B) and burst-plateaus (BP) with more than 10 subtypes (intracellular rec.). After stretch (>50 mN) and $BaCl_2$ (>0.5x) S were transformed into BP, whereby after higher concentrations the plateau duration extremely increased. Rate of rise and of fall of S increased over 2-times. The stimulatory motor effect of Ba and Sr (also K, Rb) is probably in correlation with the BP. Experiments on dielectric and magnetic properties of detrusor and trigone preparations concerning water and mesomorphic state of biomolecules (nematic, smectic, cholesteric) in dependence of stretch and ionic influences could open new perspectives for vesical cellular biophysics, physiology and therapy. Lit: [1] Michailov et al: Physiol Res S 48, S96, 1999. [2] Neu et al: Eur J Physiol, S 420, R99, 1992 [a]. Biomed Tech 39, 312-313, 1994 [b]. Biophys Mol Biol S 65, 170, 1996 [c]. Proc IUPS Helsinki 17, 529, 1989 [d1], Christchurch (20), ID:291, 2001 [d2]. [3] Welscher et al: Physiol Res 48, S138, 1999.

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S20-3

BAROREFLEX CONTRIBUTION TO LINEAR AND NONLINEAR COMPONENTS OF BLOOD PRESSURE-HEART RATE COUPLING
Di Rienzo M., Castiglioni P., Parati G., Zwiener U., Pompe B., Hoyer D.

It's well known that the coupling existing between arterial blood pressure and heart rate variability is characterized by the presence of both linear and non linear links. The magnitude of this coupling, however, has never been fully explored. Indeed till now only selected components of the coupling between blood pressure and heart rate have been considered, and for these selected components only the linear dependencies have been evaluated.
In this study we further explored this issue by 1) investigating the overall coupling existing between blood pressure and heart rate variability in daily life and 2) identifying the contribution of the baroreflex to such a coupling.
For this purpose we developed a new signal processing procedure based on the estimation of the Cross-Mutual Information (CMI) between systolic blood pressure (SBP) and RR-Interval (RRI) beat-to-beat values. CMI has been selected because of its capability to quantify both linear and nonlinear components of the coupling between variables over time scales in the order of minutes. The new procedure has been used to analyze data recorded in spontaneously behaving cats before and seven days after the surgical opening of the baroreflex loop as obtained by a sinoaortic denervation.
We observed that in intact animals the cumulative physiological level of coupling between SBP and RRI corresponds to about 40% of the theoretical maximal coupling. After removal of the baroreflex influence CMI values drastically dropped with respect to baseline levels (-70% on average).
Thus use of CMI indicates that over a time scale in the order of minutes the arterial baroreflex is the major determinants of the SBI-RRI link, accounting for about 2/3 of the total measured coupling existing between these variables.

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S20-4

OSCILLATIONS AND VASCULAR SMOOTH MUSCLE CELLS
Persson P. B., Stauss H. M.

Low frequency blood pressure fluctuations, also referred to as Mayer waves, can reflect alterations in sympathetic modulation of vascular tone associated with a variety of diseases. However, it is unknown which mechanisms determine the time course of the vascular response to sympathetic inputs. Candidates are functions related to vascular receptors and post-receptor processes involved in the contractile response.
Contractions of aortic rings were elicited by adrenergic and purinergic receptor stimulation and potassium-induced membrane depolarization. Development of force was faster with depolarization and purinergic receptor stimulation than with adrenergic receptor stimulation. The slow adrenergic response was confirmed in vascular smooth muscle cells (VSMC). In addition, VSMC were periodically stimulated with KCl and phenylephrine at 6 different stimulation frequencies. The corner frequency of the low-pass filter characteristic of the response to periodic stimulation was lower for phenylephrine (0.08±0.01 Hz) than for KCl (0.16 ±0.04 Hz, $p<0.05$).
In conclusion, the time course of sympathetic-mediated vasoconstriction depends on a slow α1-adrenergic and fast P2X-purinergic pathway. Different distributions of α1-adrenergic and P2X-purinergic receptors may contribute to species differences in the frequency of Mayer waves.

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POSTER SESSION

P20-01

PARAMETERS OF VASOMOTION IN FROG'S SKIN MICROCIRCULATION

Aivars J., Svikis I., Veliks V., Marcinkevics Z., Uljanovs A.

The aim of this study was to examine the key parameters of vasomotion (the period and amplitude) in frog's (Rana temporaria L.) skin arterioles in the period of hibernation. Experiments were carried out on two groups of denervated frog males in weight of 40±2 g. Observations were spent during the period of a hibernation: in December and in the beginning of March. The object of observations was arterioles (diameter 100-150 mm) of a. cutanea r. lateralis. Experiments were carried out through 30 min after a denervation at temperature 19 0C. Intravital video microscopy was used. Video recording was processed by image analysis using a "frame by frame" method. For revealing periodic changes of diameter, the received time series were processed by a method of the spectral analysis. Results: vasomotion might have been quasiperiodic during some time then might have become irregular. An oscillation amplitude also might have been stationary enough, and then it was sharply or gradually decreased. Skin arteriolar vasomotions of denervated frogs in the beginning and at the end of a winter season have similar structure and periods. Oscillations with period 9,70±2,02 s are more frequently observed. Amplitude of oscillations was 1,2±2,2% from effective diameter. Therefore, amplitude of vasomotion of skin arterioles of denervated frogs, in the winter period, are below than in mammals. Conclusion: the basic parameters of arteriolar vasomotion in frog's skin don't change significantly during the period of hibernation.

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P20-02

CAPTOPRIL BLOCKS THE CENTRAL EFFECTS OF ANGIOTENSIN I ON HEART RATE AND HEART RATE VARIABILITY

Lancien F., Mimassi N., Mabin D., Le Mével J.C.

The present study was performed in order to get new insights about the existence of a brain renin-angiotensin system (RAS) in teleost fish. For this purpose, we investigated, in the unanesthetized trout, the effects of centrally administered angiotensin (ANG) I ([Ans1, Val5, Asn9] ANG I) and ANG II ([Asn1, Val5] ANGII) on heart rate (HR) and heart rate variability (HRV) prior to or after pretreatment of the animals with captopril, an angiotensin-converting enzyme (ACE) - inhibitor. This ACE - inhibitor is known to block the cleavage of the decapeptide ANG I to the octapeptide ANG II. The trout were equipped with two ECG electrodes and an ICV canula was inserted within the third ventricle of the brain. The short-time Fourier transform was used to analyse the time-course actions of the angiotensins on HRV. The intracerebroventricular (ICV) injection of vehicle (0.5 µl) had no effect on the recorded parameters. The ICV injections of ANG I or ANG II at a dose of 50 pmol produced a progressive and significant increase in HR (+35 % and + 45 % respectively) but elicited a profound decrease in HRV (- 82 % and - 90 %, respectively). ICV injection of captopril (10 µg) had no effect on HR or HRV. However, this ACE-inhibitor prevented tachycardia and abolished the decrease in HRV mediated by ANG I. By contrast, captopril had no effect upon the cardiac actions of ANG II. These results give the first support for a functional implication of an ACE-like activity in the brain of a teleost fish and suggest that the brain RAS in this species of vertebrates may be implicated in the control of cardiac chronotropic activity.

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P20-03

AUTONOMOUS REGULATION OF CIRCULATION IN HYPERTENSIVE ADOLESCENTS

*Zavodna E., *Hrstkova H., Lokaj P., Novakova Z., *Paderova J., *Vaclavkova P., Fiser B., Honzikova N.*

Introduction: The aim of the present study was to determine differences in baroreflex regulation of circulation between hypertensive and healthy adolescents.
Methods: We examined 102 adolescents (16-19 years): 86 healthy controls (C) and 16 adolescents with hypertension (H) who had repeatedly high causal blood pressure. We recorded systolic blood pressure (SBP) and pulse intervals (PI) for 5 min (Finapres, metronome controlled breathing at a frequency of 0.33 Hz). Index of baroreflex sensitivity BRS [ms/mmHg] was determined by spectral method. The SBP and PI variability were determined as spectral power in the range of the 10-second rhythm (varSBP, varPI). Adolescents were divided into groups according to the BRS: low 1-5.79 ms/mmHg (lBRS), mean 5.8-12.79 ms/mmHg (mBRS), and higher than 12.8 ms/mmHg (hBRS).
Results: Besides higher SBP ($p<0.01$), H had lower BRS ($p<0.01$), higher varSBP ($p<0.01$), weight ($p<0.01$) and BMI ($p<0.01$) than C. In group lBRS, H had higher weight ($p<0.01$) and BMI ($p<0.01$) than C. In group mBRS, H had higher varSBP ($p<0.01$) and varPI ($p<0.01$) than C. There were 24 controls and no hypertnsive adolescent in group hBRS.
Conclusions: An increased SBP in adolescents is associated with overweight. BRS is decreased in Hy adolescents. In group with low BRS, dampening effect of BRS on varSBP is insufficient in both Hy and Co. In the group with medium level of BRS, Hy had significantly higher SBP variability. It could be a sign of a primarily increased sympathetic vasomotoric activity.
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P20-04

AUTONOMOUS REGULATION OF CIRCULATION IN CHILDREN AND ADOLESCENTS AFTER ANTITUMOUR THERAPY

*Zavodna E., Honzikova N., Novakova Z., *Hrstkova H.*

Introduction: The aim of the study was the comparison of body and circulatory parameters in healthy subjects (C), and subjects who were previously treated for a malignant tumour (T) with respect to plasmatic lipids in T group.
Methods: We examined 206 subjects (15.2±2.8 years) in C and 97 subjects (15.7±3.2 years) in T with a long lasting remission. We recorded pulse intervals (PI), systolic (SBP) and diastolic (DBP) blood pressures for 5 min. We calculated the baroreflex sensitivity (BRS in ms/mmHg). We used standard deviation for a variability estimation of PI (PIsd), SBP (SBPsd) and DBP (DBPsd). We followed cholesterol, HDL, LDL and triglycerides (TG). Subjects T were divided according to the level of cholesterol into two groups: 84 subjects (TL) with cholesterol <5 mmol/l and 13 subjects (TH) with cholesterol >5 mmol/l.
Results: T had higher BMI ($p<0.01$), prolongation of PI ($p<0.01$), increased TIsd ($p<0.05$), decreased SBP ($p<0.01$), DBP ($p<0.01$) and DBPsd ($p<0.05$), and lower BRS ($p<0.01$) than C. The TH subjects had shorter PI than TL ($p<0.05$). BMI correlated negatively with HDL ($p<0.01$), and positively with TG ($p<0.05$). Cholesterol and LDL correlated negatively with PI ($p<0.05$). HDL correlated negatively with weight ($p<0.01$), and height ($p<0.01$). TG correlated positively with age ($p<0.05$), and weight ($p<0.05$).
Conclusions: The T had increased parasympathetic and decreased sympathetic tonic activity, and decreased baroreflex sensitivity than C. The increased variability of PI could be explained by an increase of parasympathetic tonic control of the heart. The circulatory changes did not bear on obesity, but they were rather specific effect of antitumour treatment.
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P20-05

ELECTROCUTANEUS CONDUCTANCE OF ACUPOINTS

Musienko V.D.

Material base, physiology and therapeutic mechanisms of acupunture today remain unclear. The aim of the research was to study the electrocutaneus conductance (EC) as index of the functional state of the system of Chinese acupoints and meridians. The clinical study of EC-index on representative acupoints of meridians by computer version of Nakatani method (more than 1000 examines) showed the high variability of these parameters and its correlation with “energetic” condition of meridians and functional state of human organs and systems. It was established that irritation of the acupoints led to increase of index of EC. Certain interrelations of alteration of these parameters with disease and pathological conditions were discovered. Positive dynamics of the acupoints EC-indexes under the influence of acupuncture microwave resonance therapy (54-75 GHz) was noted. Some findings confirmed functional ties between meridians were found. As hypothesis it suggest that meridians and acupoints are important part of the

whole regulatory system which probably phylogenic more ancient than nervous or humoral system. Apparently acupunture induce genetically fixed mechanisms of compensation, adaptation and repair for realisation of its therapeutic effect and it is necessary to preserve these mechanisms during surgery. The results indicate that EC-index of acupoint may have clinical implications for diagnosis and control of acupunture treatment.

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P20-06

HEART RATE VARIABILITY IN SEDENTARY AND ACTIVE ELDERLY : RELATIONSHIP WITH DAILY PHYSICAL ACTIVITY
Buchheit M., Viola A.U., Simon C., Doutreleau S., Piquard F., Brandenberger G.

Objectives : To evaluate the influence of life-style on heart rate variability (HRV) in very old aldults with regards to their daily physical activity. Methods : Subjects were divided into two groups according to their sport score evaluated by the Modified Baecke Questionnaire for Older Adults. Sedentary subjects (SED) were then compared to active elderly involved in sport activities (ACT). Five minutes lying heart rate recordings followed by 3 minutes active stand-up recordings were used to determine HRV indexes as the standard deviation of all normal intervals (SDNN), the root-mean-square differences of successive normal R-R intervals (RMSSD), and the high and low frequency power (HF and LF). Postural adaptation was estimated by the ratio of the 30th on the 15th beat following standing-up (30/15 index). Physical activity was evaluated during one week by triaxial accelerometry device (RT3, Stayhealthy), and then analyzed according to intensity and duration of activity periods. Results : ACT showed significant lower resting HR (61.2 ± 1.7 vs 69.1 ± 1.3 bpm; $p<0.05$) and higher HRV indexes : SDNN (35.3 ± 3.1 vs 24.8 ± 1.5; $p<0.05$), RMSSD (32.3 ± 4.9 vs 19.9 ± 1.7; $p<0.05$), LF (379.0 ± 66.9 vs 171.2 ± 29.9 Hz/msec²) and HF (568.9 ± 197.7 vs 142.5 ± 24.4 Hz/msec²) than SED, whereas LF/HF were similar between both groups. 30/15 index was higher in ACT. Daily physical activity energy expenditure was higher in ACT than in SED (583.7 ± 54.1 vs 426.1 ± 52.5 Kcal/day; $p<0.05$). ACT spent longer time per week in activity of intensity > 3 METs (3 times basal rest metabolism) ((8.9 ± 1.0 vs 4.1 ± 0.8 hrs; $p<0.05$), but total activity time was higher for SED than ACT (71.3 ± 5.1 hrs vs 62.5 ± 4.0; $p<0.05$). Conclusions : These results indicate that sport better than all-day activities may counteract the decline in HRV in very old subjects. This may be linked to longer time spent in higher intensity activities, and not to total activity time.

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P20-07

STRETCH DEPENDENT VESICAL MOTOR REACTIONS ON IONS: INFLUENCE OF K, Li, Rb AND Cs
Michailov M.Ch., Seidenbusch W., Gornik E., Martin D., Neu E.

Motor activity of urinary bladder of various species depends on topographic regions: In guinea pig detrusor appeared spontaneous fast phasic (SPC; 1-5/min) and in trigone - periodic slow tonic contractions (STC; 0.1-0.5/min) [1-2]. New results [method: 1a, 2] inform about non-uniform effects of Li^+, Rb^+, Cs^+ compared to K^+. Not only SPC, but also KCl-excitatory effects (more than normal 5.6 mM = 1x = also for Li, Rb, Cs) increased after stretch (3 to 50 mN; n=53). Addition of LiCl (>1x) had some inhibitory effects on amplitudes (A) of SPC and STC; the basal tone was unchanged. Frequency (F) of STC decreased stronger at 3 than at 50 mN (for 1x 42.4 and 84.5% resp.). After (equimolar) replacement of KCl by LiCl (0.25 up to 1x) the effect was similar, but a relaxation appeared. Addition of RbCl (1-2x) stimulated SPC, but at >4x only F and basal tone increased. The effect of RbCl was essentially weaker (of KCl stronger) in stretched prepar. (for 1x: 182.7 and 114.3% resp.). Replacement of KCl and RbCl (0.25 up to 1x) induced a progressive increase of A, but not of F and basal tone. Effects of KCl and RbCl (3x): A of trigone decreased; F increased 2-3-times stronger after stretch by RbCl (for 1x: 227.1 and 469.2% resp.). CaCl (>4-9x) had an augmentory effect on F. Contrary to this, electrostimulation was also changed by K, Li, Rb, Cs. It is concluded that the non-uniform effects of K as well as Li, Rb, Cs (also of Ca as well as Ba and Sr; see contrib. Neu et al.) cannot be explained by simple ionic mechanisms, but probably by differences in atomic structures, i.e. their interaction with water and (chromo-, lipo-, glyco-) proteids. Lit: [1] Michailov et al: Eur J Physiol, S 419, R98, 1991 [a], Gyn-Geb Rdsch S 33, 333-334, 1993 [b]. J Biosci 24, S142, 1999 [c]. Proc IUPS Vancouver 16, 117, 1896 [d1], Glasgow (18) 216, 1993 [d2], St. Petersburg (19) P036.03, 1997 [d3]. [2] Neu et al: Biophys Mol Biol S 65, 170, 1996.

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P20-08

MECHANISM OF INDUCED RHYTHMIC ACTIVITY IN RAT AORTIC SMOOTH MUSCLE CELLS (SMCS)
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Many types of blood vessels exhibit rhythmic activity that can be either spontaneous (vasomotion in small arteries) or induced by vasoactive agents (large arteries). The precise mechanism of oscillatory contraction, which may differ in different vascular beds, is not clear. Therefore, the mechanism of rhythmic activity induced by phenylephrine (PE) and KCl in rat thoracic aorta has been investigated using tension measurements in endothelium-denuded rings and the whole-cell patch clamp recording in single SMCs. Two types of rhythmic activity were observed. 15-20 mM KCl induced sustained contraction with superimposed oscillatory contractions (OCs). OCs had the mean amplitude of 176±16 mg (mean±S.E.M.), duration of 5.2±0.2 s and frequency of 0.08±0.01 Hz (n=23). 20-40 nM PE induced a tonic contraction with superimposed rhythmic contractions of 99±7 s (n=34) in duration, termed oscillatory waves (OWs). OWs had the amplitude of 377±22 mg and frequency of 0.36±0.02 OWs/min (n=34). Both OCs and OWs, but not PE-induced sustained contraction, where inhibited by 0.2-1 μM diltiazem, 1-3 μM ryanodine and 5-10 μM cyclopiazonic acid. OWs were transformed into OCs in the presence of caffeine (0.5-1 mM). The duration and amplitude of OWs increased progressively with increasing doses of TEA (1-5 mM), but not in the presence of selective inhibitors of BKCa channels iberiotoxin (IBTx, 50-100 nM) or paxilline (1-2 μM). Whole-cell K^+ currents recorded in SMCs perfused with 200 nM Ca^{2+} revealed that the major K^+ current activated between -40 and 0 mV is a TEA-sensitive (IC50 = 3.1±0.6 mM, n=5) and paxilline- and IBTx- insensitive voltage-gated (Kv) current. We propose that both OWs (representing a summation of OCs) and OCs are triggered by Ca^{2+} entry via L-type Ca^{2+} channels, followed by Ca^{2+}-induced Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores. Activation of Kv channels provides a negative feedback mechanism, hyperpolarising the SMC membrane and closing L-type Ca^{2+} channels.

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S21 FROM GENE TO MUSCLE FUNCTION

ORAL SESSION

S21-1

THE CONTRIBUTIONS OF ENDOGENOUS AND EXOGENOUS PRECURSOR CELLS TO REGENERATION OF SKELETAL MUSCLE

Partridge T., Beauchamp J., Morgan J., Heslop L., Zammit P., Tajbasksh S., Kelly R., Buckingham M.

Skeletal Muscle regenerates rapidly after injury, although the muscle fibres themselves are 'end cells' in which the nuclei are post-mitotic. The progenitor of the myogenic cells that divide rapidly and fuse together to replace and repair damaged fibres is generally considered to be the satellite cell. However, recent studies have shown that cells of non-muscle origin, notably from the bone marrow, can contribute to muscle regeneration. Our observations on irradiated muscle argue against any major contribution from non-local sources.
At the same time, studies of isolated muscle fibres show that satellite cells are not a homogeneous population in terms either of function or of identifying markers. Immunostaining and transgenic marker studies, point to a rare cell type in the satellite cell position but devoid of all of the markers present on the majority cell type. Similarly, only a minority of satellite cells seems capable of proliferation either when put into tissue culture or when transplanted into a donor muscle.
Both types of investigation suggest that a minority of satellite cells is of an early progenitor or 'stem cell' type, and that the proportion of such cells in the satellite cell compartment diminishes with age.

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S21-2

DISSECTING SIGNALING PATHWAYS INVOLVED IN ACTIVITY-DEPENDENT MUSCLE GENE REGULATION

Schiaffino S., McCullagh K., Ciciliot S., Calabria E., PallafacchinaG., Serrano A L., Argentini C.

We are interested in identifying the transduction pathways that mediate the effect of nerve activity on the muscle phenotype with a focus on fiber size and fast/slow fiber type specification. Using pharmacological approaches and in vivo transfection with plasmid DNAs coding for constitutively active or inhibitory factors, we have previously shown that calcineurin controls nerve activity-dependent fiber type specification but not muscle growth. The transcription factor NFAT is well known target of calcineurin in different cell systems. However, the role of NFAT as a calcineurin downstream effector in skeletal muscle is controversial. We have found that transfection in vivo with a plasmid coding for the NFAT peptide inhibitor VIVIT linked to GFP blocks the expression of slow myosin heavy chain (MyHC) induced by slow motor neuron activity in regenerating rat soleus muscle. In contrast, the increase in fiber size in regenerating muscle is not impaired by VIVIT-GFP. In the adult soleus muscle, VIVIT-GFP also inhibits the expression of the MyHC-slow transcripts and the activity of a MyHC-slow but not a MyHC-fast 2B promoter. Surprisingly, VIVIT-GFP inhibits the activity of MyHC-slow and MLC2-slow promoters lacking NFAT binding sites. Two NFAT-dependent reporters were used to monitor NFAT transcriptional activity in vivo. NFAT activity is higher in muscles expressing a slow phenotype compared to muscles with a fast phenotype and is blocked by VIVIT-GFP and by the calcineurin inhibitor cain/cabin1. NFAT activity is decreased by denervation in adult slow muscles and is increased by electrostimulation of denervated muscles with a low frequency impulse pattern, mimicking the firing pattern of slow motor neurons, but not with a high frequency pattern typical of fast motor neurons. The results demonstrate that NFAT is a nerve activity sensor in skeletal muscle in vivo and controls activity-dependent fiber type specification.

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OC21-1

MAPK SIGNALLING AND MUSCLE CELL DIFFERENTIATION

Dérijard B., Salles J., Coldéfy A.S., Cabane C., Ragno M.

SAPK have so far been involved in a lot of inflammation- and stress-induced transcriptional activation. More recently, several differentiation processes such as neuron, lymphocytes, keratinocytes and vascular smooth muscle cell differentiation have been shown to depend on SAPK signaling. Since most components of these cascades are potently expressed in skeletal muscle cell, we investigated whether they might be involved in the differentiation and/or proliferation of this cell type.
Indeed we showed that the terminal differentiation of C2C12 skeletal muscle cells is inhibited in stable clones expressing a dominant negative form of MKK3, a p38 activator. In addition we showed, using this cell line together with inhibitors, that Akt/PKB is controlled by p38 during differentiation at the transcriptional and post-traductional levels.
We showed recently that both ERK and p38 are potently and transiently activated in an in vitro wound/healing model using physical wounding of a confluent monolayer of C2C12 muscle cells. We also showed that the metaloproteinase MMP-9 gene is regulated during differentiation in a p38/Akt dependant manner.
Finally we are currently trying to monitor and further analyse the main signaling pathways potentially affected in one of the most important adult muscular dystrophy, the Steinert's myotonic dystrophy (DM1). The genetic disorder involved affects the expression of Myotonic Dystrophy Protein Kinase 1, DMPK, known to interact with upstream activators of the SAPK. The study relies on the analysis of the activated signaling kinases from i) muscle cells transfected with the DMPK cDNA ii) DM1 patients skeletal muscle biopsies or primary cultures and iii) muscle samples from the DM1 mouse models. Our preliminary results show that both the ERK and p38 pathways are affected.
Altogether, these experiments should lead to a better understanding of the precise role of SAPK and MAPK in the proliferation versus differentiation balance in muscle cells.

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OC21-2

MOTOR AND C-FOS IMMMUNOREACTIVE CHANGES INDUCED BY ACUTE MUSCLE INFLAMMATION IN FELINE SPINAL CORD

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The contribution of group III and IV muscle afferents from free nerve endings to pathological motor phenomena resulting in chronic muscle pain is still under debate. Therefore, we now investigated the effect of an acute carrageenan-induced myositis of the gastrocnemius-soleus (GS) on c-fos immunoreactivity and reflex activity (monosynaptic reflexes of flexors and extensors and reflex pathways from group III and group IV muscle afferents, activated by injection of KCl or bradykinin into the muscle artery of GS) in the lumbar spinal cord. Experiments were performed in anaemically decapitated, high spinal, paralyzed cats. After infiltration of GS with carrageenan (1%) the monosynaptic reflexes of flexors and extensors showed a distinct increase and there was a slight increase of the facilitation of the flexors by chemically activated group III and IV afferents. The inhibition evoked from these afferents in extensors reacted less uniform. The reflex effects of carrageenan started within one hour and reached their maximum after about 11/2 hours. On the ipsilateral side of the spinal cord of cats, which received a carrageenan infiltration (sacrificed in the experiment 41/2 hours later), the number of c-fos immunoreactive cells was significantly increased in the laminae I-II, V-VI and VII of the segments L6 and L7 and in the laminae V-VI and VII of segment S1.
The results show that the input from acutely inflamed muscles may induce an increase of the reflex responsiveness of flexors and extensors which is not mediated via the gamma-spindle-loop and which coincides with increased c-fos immunoreactivity. The increased c-fos immunoreactivity was concentrated on main projection areas of afferent fiber terminals from the GS muscle.
This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG SCHO 37/12-1) and from INTAS (INTAS-01-2130).

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OC21-3

IDENTIFICATION OF A NEW GUANINE NUCLEOTIDE EXCHANGE FACTOR FOR THE SMALL G-PROTEIN RHOB.

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The small G proteins of the Rho family including RhoA, RhoB, Rac1 and Cdc42 function as tightly regulated molecular switches that govern cellular functions such as actin cytoskeleton organization, adhesion and motility, oxidant generation, apoptosis, membrane trafficking, cell cycle control and gene expression. Rho proteins are activated by Guanine nucleotide Exchange Factors (GEFs) that possess tandem Dbl homology (DH) and pleckstrin homology domains and catalyze the replacement of bound GDP for GTP on Rho proteins. The aim of the present work was to identify and characterize new human GEFS for Rho proteins.
By screening protein data banks with the DH domain, we identified four new sequences. Among them, the FLJ00018 has been cloned and functional analyses have been performed. Using yeast three-hybrid assay, we found that FLJ00018 selectively activates RhoB. Transfection of FLJ00018 in fibroblasts stimulates c-fos promoter-driven luciferase activity indicating that FLJ00018 activates the SRE-dependent transcription. This effect is inhibited by TAT-C3, which inactivates Rho protein, and by expression of dominant negative mutant of RhoB whereas dominant negative forms of RhoA, Rac and Cdc42 have no effect. These results suggest that FLJ00018 is a selective GEF for the small G protein Rho protein RhoB. Functional role of this GEF is therefore expected in tissues where both proteins are co-expressed. Analysis of RhoB and FLJ00018 transcript expression in a broad range of human tissues reveals co-expression of both transcripts in various tissues, particularly in the heart and vessels. RhoB is an atypical member of the Rho family that controls survival and proliferation, and is one of the targets responsible for the inhibition of cell proliferation induced by farnesyltransferase inhibitors. As RhoB-mediated functions are involved in a wide range of vascular disease, FLJ00018 could represent a new therapeutical target in vessels where both the GEF and RhoB are expressed.

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S21-3

INFLUENCE OF LONG-TERM SPACEFLIGHT ON NEUROMECHANICAL PROPERTIES OF MUSCLES IN HUMANS

Goubel F.

A number of studies have documented that the microgravity environment encountered during spaceflight or simulated by using models of weighlessness induces alterations in skeletal muscle function. The majority of knowledge, however, comes from animal studies. In humans, loss of muscle mass and force has been reported after spaceflight or prolonged bed rest whereas the velocity characteristics measured in muscle groups led to discrepant results. Thus the first aim of the present study was to investigate changes in force/velocity characteristics in human postural muscle group as a result of long-term spaceflight (90-180 days). Stiffness properties of muscle group and joint were also investigated with the view in mind to link their changes with changes in reflex excitability. Such an experiment relies on a specific mechanical device, the ankle ergometer. This apparatus offers the possibility to test during the same experiment neuromechanical properties of the human ankle plantarflexors. In 14 cosmonauts spaceflight was found to induce a decrease in maximal isometric torque (17%), whereas an index of maximal shortening velocity was found to increase (31%). Musculotendinous stiffness was found to be increased by 25%. Whole joint stiffness decreased under passive conditions (21%), whereas it remained unchanged under active conditions. Reflex activities were increased when mechanically induced (T reflex : 57% ; stretch reflex : 31%). Otherwise no changes in H reflex were found. These neuromechanical changes are interpreted in terms of central adaptive processes (mainly increase in synaptic efficacy) and peripheral mechanisms (muscle fibre type transition, tendon adaptation and muscle spindle responsiveness). Finally, in active conditions, the invariance in joint stiffness suggests an adaptative mechanism to counterbalance the decrease in stiffness of passive structures by an increased active stiffness. Changes in neural drive could participate to this equilibrium.

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OC21-4

ROLE OF INFLAMMATION IN THE EXERCISE-INDUCED IMPAIRMENT OF MUSCLE FUNCTION IN DYSTROPHIC MDX MOUSE

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Dystrophin, absent in Duchenne muscular dystrophy, mechanically reinforces sarcolemma. Accordingly, an increase in work load worsens the mild dystrophic condition of the mdx mouse. Four week-old mdx mice chronically exercised on treadmill developed, after 1 month, a significant weakness, the age-related increase in normalized fore-limb strength (strength/body weight) being 0.26+/-0.2 (n mice=8) vs. 0.68+/-0.4 (n=6) of sedentary counterparts. Ex vivo current clamp recordings showed that exercise hampers the regeneration-induced increase in chloride conductance (gCl) typical of extensor digitorum longus (EDL) muscle, gCl being 1633+/-66 microS/cm2 (n fiber=46) vs 2493+/-88 microS/cm2 (n=22) of sedentary mice. A further lowering of gCl by overload was observed in the mdx diaphragm. Exercised mdx hind limb muscles also showed 1) a 70% increase in mononuclear inflammatory cells detected histologically; 2) a 20% increase in the already high cytosolic calcium by fura-2 imaging. Also, in vitro application of 10 microg/ml of TNF-alpha, a pro-inflammatory cytokine, significantly reduced by 20% the gCl of mouse EDL muscle fibers. We evaluated the occurrence of an exercise-induced inflammatory reaction by in vivo treatments with either 1mg/kg alpha-methylprednisolone (PDN) or 10 mg/kg cyclosporine A (CSA). Thus, 4 week-old mdx mice were daily treated per os for up to 4-8 weeks during the period of exercise. Both treatments counteracted the decrease in muscle force (CSA>PDN). PDN fully restored gCl in both EDL and DIA muscle fibers. CSA counteracted by 30% the decrease in gCl in diaphragm, while it fully countered the exercise-induced decrease in gCl of EDL muscle (2230+/-63 microS/cm2; n=66). PDN, in contrast to CSA, significantly ameliorated the calcium homeostasis. The results support the hypothesis that exercise enhances an inflammatory and immunological reaction which likely contributes to the progressive degeneration of dystrophic muscle (Telethon-Italy # 1150).

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S21-4

ADAPTIVE CHANGES IN THE MUSCLE TRANSCRIPTOME

Billeter R., Wittwer M., Hoppeler H., Friedmann B., Flück M.

Skeletal muscle responds to a wide variety of different conditions with specifically tailored adaptations which involve changes in the levels of mRNAs coding for proteins of the adapting structures. Using cDNA arrays that probe for mRNAs involved in many different cellular functions, we found 105 of 395 mRNAs significantly changed in a study on atrophied rat soleus muscles (>50% atrophy), pointing to changes in most systems probed this way. Most of these changes were less than two fold.
A similar study comparing biopsies from m. vastus lateralis of professional cyclists with untrained subjects (vO2 max. 71 vs. 39 ml/kg.min) revealed distinctly greater inter-individual variability in the expression patterns of human compared to rat muscles. 9 of 144 safely detected transcripts were significantly changed, several coding for proteins involved in DNA repair and transcription. Interestingly, the expression patterns of the cyclists muscles correlated better with each other than the patterns of the untrained.
The larger scatter in the human expression patterns makes the detection of small, but functionally important differences difficult. It also could be a decisive factor behind the considerable inter-individual differences in the functional response to standardised exercise, as illustrated in the HERITAGE study. In a recent study on the effect of hypoxia on low intensity – high repetition strength training, we could not find any significant differences in strength parameters or the contents of a number of mRNAs (determined with real time RT-PCR) between a group training in hypoxia and another in normoxia. Significant correlations, however, were found for all the measured hypoxia marker mRNAs (PFK, myoglobin, VEGF and LDH B) in the hypoxia but not in the normoxia group, which indicates systematic hypoxia adaptations on an individually variable scale.

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S21-5

CARDIOCHIPS AND MOLECULAR PORTRAIT OF HEART FAILURE

Steenman M., Lamirault G., Le Meur N., Le Cunff M., Escande D., Léger J.J.

The multifactorial nature of heart failure implies a diversity of molecular phenotypes based on the accumulation of different molecular events. The identification of this diversity may represent a tool to classify patients into sub-groups with diagnostic and/or prognostic significance, independent of the classical clinical classification. Our aim was to determine whether we could define a molecular portrait of heart failure. We constructed cDNA microarrays containing ~1000 cardiac-relevant clones (CardioChip). We obtained these clones from subtraction experiments performed between failing and non-failing human heart samples. Using the CardioChip, we analyzed gene expression levels in left ventricle tissue from 15 explanted failing hearts and from 2 explanted non-failing hearts. All experiments were performed in triplicate and stringent analytical tools were applied. A total of 159 genes displayed significant differential expression among the 17 patients and these genes were therefore used to perform hierarchical clustering analysis. This resulted in a classification of the 17 patients into 2 major sub-groups of 8 and 9 patients, based on a difference in the level of expression of the atrial (ANF) and brain (BNP) natriuretic factors. The sub-group of 9 patients with a relatively high level of expression of ANF and BNP was further divided into 2 sub-groups of 3 and 6 patients, based on differential expression of genes coding for structural proteins (e.g. titin) and genes coding for metabolic proteins (e.g. cytochrome c oxidase II). Within this sub-classification, a relatively high level of expression of the structural genes coincided with a relatively low level of expression of the metabolic genes. In conclusion, we identified the existence of molecular portraits of heart failure that enable a novel classification of patients. The next step will be to determine the diagnostic and prognostic significance of these molecular portraits.

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POSTER SESSION

P21-01

VALIDATION OF A SIMPLE CALIBRATION METHOD FOR HUMAN MUSCLE MICRODIALYSIS

Desvigne N., Barthélémy J.C., Bertholon F., Gay-Montchamp J.P., Freyssenet D., Costes F.

Microdialysis gives a unique opportunity to identify substrates and measure their concentration changes in the human muscle interstitial fluid. A major methodological limitation lies in the difficulty to calibrate the microdialysis probes in order to know the interstitial substance actual concentrations. Indeed, the concentration of a substance in the dialysate is only influenced by its interstitial concentration as well as by the probe recovery of the substance which vary greatly in vivo with changing environmental conditions. The aim of our study was, to validate in vitro a new calibration method, the slope method, based on the determination in individual probe of probe recovery of a substance from that of ethanol, in various dialysis conditions reproduced by changes in substance concentration and recovery. The method feasability and accuracy were confirmed in vivo in a muscle resting study as compared to a reference method, the NO Net Flux method (NNF). Determination of the linear relationship, in a steady state, between recovery of ethanol and that of the substance of interest (lactate (Lac), glucose (Glu)) allows to calculate the concentration of the latter in any conditions. In vitro, we obtained very strong relationships between recovery of ethanol and recovery of Lac or Glu, with a r ranging from 0.986 to 1.000 (all $p<0.001$); the error of estimation of Lac or Glu concentrations in th dialysis bath was limited to -0.6±5.8% and 0.7±6.2% for, respectively, Lac and Glu, in a large range of probe recovery. In human muscle, the slope method was as accurate as NNF to evaluate interstitial Lac concentration (1.82±0.58 vs 1.83±0.47 mM, respectively). The improved precision of the slope method results from the in situ determination of the individual relationship for each microdialysis catheter. We conclude that, after an initial calibration step, the slope method allows accurate interstitial muscle metabolites measurement and could monitor rapid metabolic changes.

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P21-02

QUANTITATIVE POINCARE PLOT ANALYSIS OF HEART RATE VARIABILITY: EFFECT OF ENDURANCE TRAINING

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RHeart rate variability (HRV) provides non-invasive assessment of the autonomic control of heart rate. HRV is expected to decrease from supine rest to upright position and further during exercise. Conversely, endurance training seems to increase HRV. But discrepant results were obtained with linear time- and frequency-domain methods that require stationary R-R intervals time series. The non-linear Poincaré plot of HRV depicts trend changes in heart dynamics independent of R-R stationarity. This study was aimed at showing the usefulness of the Poincaré method to assess the training-induced changes in HRV. Four 10 min manoeuvres were performed (supine lying, standing, steady state exercising and subsequent recovery) by 8 control subjects before and after short-term endurance training and by 8 subjects trained for at least 3 years. HRV was assessed by time- and frequency-domain indexes, in parallel with the Poincaré plot analysis. In the latter each R-R interval is plotted as a function of the previous one, and the standard deviations of the instantaneous and long-term R-R interval variability are calculated. In our subjects, the Poincaré scattergrams became gradually narrower from supine to exercising, with progressive parasympathetic withdrawal. Short- and long-term endurance training led to higher aerobic power and ventilatory threshold shifted towards higher power output. All HRV evaluation methods showed that HRV values were higher after training both when supine lying and standing. The Poincaré scattergrams were wider in the trained state. Standard deviations of the Poincaré plot were significantly correlated with the main parameters of the time- and frequency-domain analyses, especially concerning the parasympathetic indicators. These results suggested that Poincaré plot parameters as well as the "width" of the scattergram could be considered as surrogates of time- and frequency-domain analysis to assess training-induced changes in HRV.

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P21-03

COLLOID OSMOTIC PRESSURE AND FLUID DISTRIBUTION VOLUMES IN THE DENTAL PULP

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Interstitial fluid colloid osmotic pressure (COP) is a basic transcapillary fluid balance parameter. In the dental pulp COP and the fluid distribution volumes are unknown. We decided to measure intravascular and interstitial fluid volumes and to test if pulp interstitial fluid could be isolated by centrifugation. Studies were performed in anaesthetized Wistar rats. Incisors pulps were removed in a humidity chamber and sealed in airtight vials. Total extracellular fluid volume measured as the 120 min distribution volume of [51Cr]-labeled EDTA after nephrectomy, averaged 0.631 ± 0.071 (SD) ml/g wet weight (ww). The corresponding intravascular volume, determined as the five min distribution volume of [125I]-labeled human serum albumin, averaged 0.03 ± 0.018 ml/g ww, resulting in an interstitial fluid volume of 0.601 ± 0.077 ml/g ww. To isolate interstitial fluid, pulps were transferred to tubes provided with a basket made from nylon mesh. To examine if fluid derived from the intracellular and intravascular compartment, the ratio for extracellular fluid equilibrated [51Cr]- EDTA and plasma volume equilibrated [125I]- human serum albumin between the fluid and plasma was measured. At 239 G, the centrifugate fluid to plasma ratio of [51Cr]-labeled EDTA and [125I]-labeled human serum albumin averaged 0.864 ± 0.053 and 0.03 ± 0.027, respectively. HPLC of pulp centrifugate showed a pattern resembling that of plasma except for a peak eluting in the ~40 kD MW range. COP was measured in pulp fluid isolated from rats not pretreated with isotopes by use of a colloid osmometer. COP in the incisor pulps interstitial fluid averaged 19.7 ± 3.7 mmHg that was 82 ± 12 % of COP measured in the corresponding plasma. We conclude that dental pulp interstitial fluid can be isolated by centrifugation, and that its interstitial fluid COP is relatively high. The pulp has a high content of extracellular fluid (63%), mainly located outside the vascular compartment (60%).

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P21-04

AGE-RELATED CHANGES IN MICROVASCULAR FILTRATION CAPACITY

Charles M., Pichot V., Desvignes N., Denis C., Costes F.

The microvascular filtration capacity is an important determinant of local muscle exchange during exercise. It is determined by the product of the surface area available for exchange and the microvascular permeability per unit surface area. A non invasive determination of the microvascular filtration capacity (Kf) in muscle has been proposed by Gamble et al. (J Physiol 1993, 464:407-22). Briefly, mercury-in-silastic strain gauge plethysmography was used to measure volume changes of the limb in response to small cumulative pressure steps applied to produce venous congestion via a cuff inflated proximally from the strain gauge. Each pressure step of 8 mmHg was maintained for 5-7 min to ensure completion of the vascular filling response and to obtain slopes representing fluid filtration only. With elderly, a decrease in capillary density has been demonstrated but its importance is still debated, especially in upper limbs where it could be preserved. In order to test whether microvascular filtration capacity could be altered in older subjects, we performed comparative measurements of Kf of the forearm and the calf in old and young healthy volunteers. We recruited seven old healthy subjects aged 73 ± 4 years and 7 healthy young volunteers aged 29 ± 5 years. All subjects were relatively fit. In the forearm, Kf were not significantly different, 9.01 ± 2.91 and 6.49 ± 1.08 ml/(100ml).min-1.mmHg-1.10-3 in old and young subjects respectively (p=0.064). By contrast, in the calf, Kf was significantly lower in old than in young subjects (1.99 ±0.53 vs 4.78 ±1.42 ml/(100ml).min-1.mmHg-1.10-3; p=0.015). We conclude that, with elderly, microvascular filtration capacity was differently decreased in the upper and the lower limbs. It remains to be determined whether this corresponds to a larger decreased in the capillary density of the calf and whether this is related to the limb exercise capacity of these subjects.

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P21-05

DETERMINATION OF ISOKINETIC MUSCLE STRENGTH IN CHF PATIENTS AND IN COPD PATIENTS

Degache F., Costes F., Calmels P., Garet M., Barthelemy J.C., Roche F.

Objective : To evaluate the relationship between isokinetic strength, and aerobic aptitude in patients with chronic heart failure (CHF) and with chronic obstructive pulmonary disease (COPD).
Methods : Seventeen stable CHF (n=11) and COPD (n=6) patients aged 51.12±9.95 ys old (15 men, 2 women, CHF patients : NYHA class II: n=8 and III: n=3) underwent a dynamic isokinetic force evaluation of the quadriceps (IsoK) at an angular velocity of 180°s-1, a symptom-limited cardiopulmonary exercise test with simultaneous monitoring of respiratory gases, and a 6-min walking test. Body composition was assessed by electrical bioimpedance. The relationships between muscle strength, maximal aerobic performance, results of endurance tests and anthropometric datas were assessed by simple regression analysis.
Results : The isokinetic evaluation has been successfully performed in all patients. No cardiac arrhythmic event nor abnormal haemodynamic response was observed in neither group of patients. In CHF patients, individual quadricipital muscle strength was significantly related to fat-free mass ($r=0.862$; $p<0.01$). However, neither peak O_2 (global: $r=0.562$, p=NS or weight-adjusted: $r=0.083$ p=NS) nor endurance capacity ($r=0.068$, p=NS) were significantly related of the peripheral muscle strength.
In COPD patients, quadricipital muscle strength was not significantly related to fat-free mass ($r=0.242$; p=NS) nor any other measured factor.
Conclusion : Physiopathological mechanisms of exercise limitation in CHF and COPD patients are complex. Isokinetic strength and O2 peak are not related to each other and are therefore independent. Therefore, a sysrematic evaluation of isokinetic muscle strength may be of great value.
Early degradations in aerobic capacity are probably followed by an alteration in muscle strength.
Keywords : Chronic Heart Failure, Chronic Obstructive Pulmonary Disease, isokinetic, squeletal muscle strength, aerobic capacity.

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P21-06

DNA-CHIPS REVEAL COORDINATED CHANGE IN EXTRACELLULAR MATRIX-RELATED GENE EXPRESSION IN VARICOSE VEIN

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Vascular wall remodeling, resulting from global and coordinated change in gene expression, is a common feature of many vascular diseases affecting arteries or veins such as chronic venous insufficiency (CVI). CVI leads to the development of varicose veins, characterized by extracellular matrix (EM) disorganization and loss in smooth muscle reactivity. However, the molecular mechanisms underlying these processes are unknown. This work was aimed to identify the global pattern of differentially expressed genes in human varicose saphenous vein smooth muscle (SV) in order to reveal genes involved in vein wall dysfunction and remodeling. We used suppression subtractive hybridization to identify differentially expressed genes in healthy SV, obtained from patients undergoing coronary bypass (n=27) and surgically removed varicose SV (n=25). Two cDNA libraries were generated containing up- and down regulated clones in varicose SV. Microarray analysis of these cDNA libraries (n=5) revealed that 16% of clones showed at least a 1.5-fold difference in expression in varicose SV. Half of these clones corresponded to gene coding for EM proteins including Matrix Gla protein (MGP), a known vascular calcification inhibitor, which was up-regulated in varicose SVSM. Using real-time RT-PCR, we confirmed and quantitated the differential expression of these genes in varicose ($p<0.05$, n=8) compared to healthy SV (n=8). Von Kossa staining which allows detection of calcification areas has been used to assess the functional consequences associated with change in MGP expression. Ectopic calcification process was observed in 80% of varicose SV wall tested. This result suggests that a new phenotypic transition associated with mineralization occured in varicose SV. Furthermore, this work provides the first characterization of a coordinate up-regulation of a set of genes related to the EM in varicose SV that could be directly involved in the alteration of the vein wall functions and organization.

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P21-07

ROLE OF THE MAP KINASE SIGNALLING PATHWAYS IN SKELETAL MUSCLE DEGENERATION AND REGENERATION

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The MAP kinase pathways play important roles in all stages of the cell life cycle: proliferation, differentiation, and death. Dysregulation of these signalling pathways result in a variety of disorders, including cancer and degeneration. The aim of our research is to clarify the role played by the MAP kinase pathways in muscle cell regeneration upon physical wounding and cytokine treatment. Injury to a muscle induces the necrosis of affected myofibers. Subsequently, regeneration occurs as a result of the activation, proliferation, and fusion of myogenic stem cells into myotubes that mature into myofibers. However, little is known about the involvement of the MAP kinase pathways during muscle wounding and recovery.
We have analysed the changes in the levels of p38, JNK, and ERK after muscle wounding. Our results show a rapid increase in the level of phosphorylated p38 and ERK after wounding in C2C12 cells. Treatments of the C2C12 cells with TNFα and IL-1 also result in an increase in activated ERK and p38 levels. In addition, we observed that conditionned medium from wounded cells is able to increase phosphorylated p38 levels but not ERK in unwounded cells. In light of these results, it seems that injured C2C12 cells release certain factor(s) which can act on unwounded cells to trigger p38 (but not ERK) activation. Degradation and synthesis of several macromolecular components of extracellular matrix have also been demonstrated after injury to adult myofibers. We compared the levels of matrix metalloproteinase-2 (MMP-2) and -9 (MMP-9) by zymography, as physiological markers of muscle cell wounding and recovery. We observed an MMP-9 overexpression in wounded C2C12 cells against intact cells. Furthermore, using inhibitors of the different MAPK pathways, we demonstrated that this MMP-9 overexpression is p38 pathway-dependent.
In conclusion, our results indicate that the p38 pathway could play an essential role in the muscle skeletal regeneration process.

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P21-08

ENDOTHELIN RESPONSES TO ENDURANCE EXERCISE AND TO SIX WEEKS TRAINING AFTER HEART TRANSPLANTATION
Doutreleau S., Piquard F., Lonsdorfer E., Lampert E., Mettauer B., Richard R., Epailly E., Lonsdorfer J., Geny B.

Objectives: Short-term survival is no longer the pivotal issue after heart transplantation but most heart-transplant patients (Htx) still present with reduced exercise capacity. Increased endothelin (ET), a potent vasoconstrictive peptide, might mediate important complications and participate in Htx's exercise capacity limitation. The two objectives of this study are to investigate, for the first time after heart transplantation: 1) whether prolonged exercise increases circulating endothelin and 2) whether training-induced exercise capacity improvement is associated with reduced baseline and exercise ET in Htx.
Methods: After comparing their maximal exercise capacity to that of matched controls, 9 male Htx performed a 45 minutes endurance exercise test (EE). We determined their ET response at rest, at the end of EE and at 30 minutes of recovery. Five Htx also completed a 6 weeks training program, consisting in 3 exercise session per week.
Results: Resting heart rate, systemic blood pressure, creatinine and endotheline (4.8 ± 1.1 vs 1.6 ± 0.2 pmol.l-1) were increased in Htx. Maximal tolerated power was decreased (132 ± 11 vs 205 ± 14 watts) and the the anaerobic threshold occured early in Htx (at 49.5 ± 7.5 % of VO2 max.). EE did not modify ET, even at 30 minutes recovery. Training improved significantly Htx's submaximal and maximal exercise capacity (12 ± 2 % and 15 ± 6 %, P =0.01), decreased heart rate but only tended to decrease ET.
Conclusion: This study demonstrates that long duration submaximal exercise fails to increase circulating ET after heart transplantation. Further supporting the usefulness of training, it also suggests that training-induced improvement in exercise capacity is not obtained mainly through a decreased ET levels in Htx.

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P21-09

EFFICIENCY OF WORK AT 100% OF MAV : SHORT INTERMITTENT EXERCISE VERSUS CONTINUOUS EXERCISE
Tardieu M., Thevenet D., Prioux J.

Introduction : The aim of this study was to compare the time spent at maximal oxygen uptake (tVO2max) during a 30s-30s realized at 100% of maximal aerobic velocity (MAV) (active recovery : 50% of MAV) with the one measured during maximal continuous run (100% of MAV). Method. 11 endurance-trained runners (16.5 years ± 0.3, VO2max : 62.5 ml.min-1.kg-1 ± 1.3 and MAV : 17.6 km.h-1 ± 0.25) performed 3 field-tests (400-m outdoor tartan track) until exhaustion : 1) an incremental test to determine their VO2max and MAV. 2) an intermittent exercise (IE) alternating 30s at 100% of MAV and 30s at 50% of MAV. 3) a continuous run (CR) at 100% of MAV. Tests IE and CR were randomized. During all tests, respiratory gas exchanges were measured breath-by-breath using a portable system (Cosmed K4b2, Italy). Total exercise duration (tlim), total exercise time at 100% of MAV (tMAV), time spent at VO2max (tVO2max) and blood lactate concentration at the end of exercise ([La]) were measured for IE and CR. Results. tlim and tMAV are significantly ($p < 0.001$) longer during IE (tlim = 1412.7s ± 147.9 and tMAV = 706.4s ± 74) than during CR (tlim = tMAV = 362.2s ± 93.8). During IE, mean tVO2max, expressed in absolute value (second) is not significantly higher than the one obtain during CR (300s ± 145.2 vs 222.3s ± 32.4). On the other hand, if we express tVO2max in relative value (related to tlim, %tlim) we note that during IE, subjects spend 23.7% (± 35.3) of their total exercise time at VO2max against 60% (± 27) during CR ($p < 0.05$). [La] obtain at the end of IE and CR are not significantly different (respectively 8.7 mmol.L-1 ± 0.9 vs 10.3 mmol.L-1 ± 0.7). Our results seem to demonstrate that tVO2max during IE is not significantly ifferent from CR. Our hypothesis is not verified. It even seem that continuous run at 100% of MAV is more efficient : related to total exercise time, time spent at VO2max during CR is significantly higher than during IE.

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P21-10

EARLY ALTERATIONS OF CARDIAC MITOCHONDRIAL FUNCTION AFTER CORONARY ARTERY LIGATION IN PIGS
Zoll J., Ponsot E., Doutreleau S., Mettauer B., Lampert E., Mazzucotelli JP., Diemunsch P., Piquard F., Geny B.

Objectives: To investigate the characteristics of possible early mitochondrial alterations in ischemic myocardium after coronary artery ligation in vivo in experimental pigs.
Methods : Left descending coronary artery was performed after sternotomy in six White pigs. 45 minutes after ligation the heart was quickly removed. In-situ maximal O2 uptake (Vmax, µmol O_2.min-1.g-1 dry weight) of saponin-skinned myofibers were then measured from both ischemic (I) and normal (N) part of the ventricular myocardium, using three different substrates. Increasing amounts of ADP concentration allowed to measure a Km for ADP in the presence (Km+) or absence of creatine (Km-).
Results: Vmax decreased by 20% in ischemic myocardium with both glutamate-malate (18.1±1.3 vs 22.1±1.7 in N, P=0.04) and pyruvate substrates (19.3±1.0 vs 23.3±2.0 in N, P=0.05). No difference was observed with palmityl carnitine (15.6±1.8 in I vs 16.6±0.9 in N). The Km- for ADP decreased in I by 24% (679±79 vs 899±84µM of ADP in N, P= 0.04), reducing the stimulatory effect of creatine (Km-/Km+ = 11.6±2.5 in I vs 18.0±2.2 in N, P=0.04).
Conclusions: Thus, ischemia lasting 45 minutes is sufficient to reduce significantly the maximal oxidative capacity of ventricular myocardium and to alter the mitochondrial respiration control, through a decrease in the functional role of mitochondrial creatine kinase. Interestingly, palmityl carnitine utilization was not affected, supporting that cardiac ischemia results in specific alterations of the mitochondrial function related to the level of energy demand.

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P21-11

BTBD1, A NOVEL TOPO1 INHIBITOR, IS IN VITRO INVOLVED IN SKELETAL MUSCLE CELL DIFFERENTIATION.
Pisani D.F., Cabane C., Yeow K., Piétu G., Derijard B., Dechesne C.

ABTBD1 is a recently cloned novel protein homologous to the topoisomerase 1 (TOPO1) inhibitor BTBD2. BTBD1 is able to bind to TOPO1 and is expressed in many tissues with a preferential expression in skeletal muscle. Our objectives were to determine BTBD1 involvement in proliferation and differentiation of skeletal muscle cell and its interaction type with TOPO1.
C2C12 murine myoblasts were used for this study. These cells are able to differentiate in myotubes and have a BTBD1 basal expression.
First we studied BTBD1 gene expression and we showed that : 1/ The BTBD1 gene was highly up-regulated during C2C12 differentiation. 2/ The

BTBD1 gene was down-regulated in cell wounding experiments, where confluent scratched cells proliferated again to regenerate. 3/ A small BTBD1 up-regulation was found in modified C2C12 cells blocked at confluence without differentiation. These results suggest that BTBD1 is involved in the proliferation arrest step that precedes the cellular differentiation process, and showed that BTBD1 gene expression is inversely correlated with TOPO1 activity.
Secondly, we studied BTBD1 interaction with TOPO1. In C2C12 cells we stably expressed a non-functional BTBD1 dominant (nfBTBD1) able to bind to TOPO1 and localised in both nucleus and cytoplasm. nfBTBD1-C2C12 cell proliferation was largely affected and no differentiation was observed. These alterations are characteristic of TOPO1 up-activation. This hypothesis was confirmed by nuclear TOPO1 (= active TOPO1) western blot analysis that showed a nuclear TOPO1 decrease during wild type but not nfBTBD1-C2C12 cell differentiation. Moreover, we showed by immunofluorescence analysis that TOPO1 is sequestered in nfBTBD1-C2C12 nucleus.
In conclusion we have determined that BTBD1 is a negative regulator of TOPO1 and that BTBD1 activity is essential for skeletal muscle cell differentiation.

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P21-12

NANDROLONE DECANOATE AFFECTS Ca^{2+} AND Sr^{2+} SENSITIVITY OF CONTRACTILE PROTEINS IN RAT MUSCLES
Bouhlel A., Joumaa W., Léoty C.

It is generally accepted that anabolic-androgenic steroids treatment improve skeletal muscle performance. However, a few works have been devoted to the analysis at the cellular level of the change on the skeletal muscle excitation-contraction coupling mechanism. Therefore, the effects of nandrolone decanoate (ND) treatment on the properties of contractile proteins were investigated in Triton-skinned fibres isolated from rat edl and soleus muscles.
One group of male rats received weekly (for 8 weeks) an intramuscular injection of ND (15 mg Kg-1) and the other group received weekly the similar doses of vehicle (sterile peanut oil). Small bundles of two to five fibres were dissected and after skinning mounted in an experimental chamber as already described by Joumaa and Léoty (2002). All procedures accord with the Declaration of Helsinki.
Following ND treatment, the maximal-activated tension recorded in pCa 4.5 and pSr 4.5 solution was increased in edl by 34% and 29%, respectively and in soleus muscles by 28% and 23%, respectively. The tension-pCa or -pSr relationship was significantly shifted leftwards in both types of muscle. In control edl, the pCa50 and the pSr50 were 6.14 ± 0.04 and 5.17 ± 0.03, respectively and in treated edl were 6.26 ± 0.04 and 5.45 ± 0.02, respectively. Furthermore, in control soleus, the pCa50 and the pSr50 were 6.28 ± 0.03 and 5.86 ± 0.01, respectively and in treated soleus 6.45 ± 0.02 and 6.22 ± 0.03, respectively. The hill coefficient for Ca^{2+} and Sr^{2+} sensitivity of the contractile apparatus was decreased in edl by 34% and 24%, respectively and in soleus fibres by 26% and 25%, respectively.
The present results show that the contractile apparatus of skeletal muscles were similarly affected by ND treatment and that the sensitivity of contractile proteins in treated edl was similar to that found in untreated soleus muscle.
Joumaa WH, Serrurier B, Bigard X, Léoty C (2002). Acta Physiol Scand 175(3):189-199

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P21-13

EXPIRATORY FLOW LIMITATION UNDER NEGATIVE PRESSURE DURING EXERCISE: ROLE OF EXPIRATORY MUSCLES
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Introduction: Expiratory flow limitation (EFL) promotes dynamic hyperinflation and limits exercise tolerance both in healthy subjects and particularly in chronic obstructive pulmonary disease patients. Negative pressure at the mouth during tidal expiration (NEP) allows the detection of EFL. However, patients are differently susceptible to this technique.
Objectives: To assess why subjects respond differently to NEP, electromyographic activity of expiratory muscles (EMG) was measured.
Hypotheses: (1) During exercise, NEP has no effect on EFL below a critical pressure (Pc) which varies among subjects. (2) High NEP is needed for subjects who don't use expiratory muscles during exercise, whereas low Pc results in the use of these muscles.

Methods: Eight healthy subjects (24-58 years old) were tested under different NEP (3, 5, 7 and 9hPa) at rest and during an exercise performed on a cycle ergometer at 30% and at 60% of maximal aerobic power. EFL and average rectified value (ARV) of surface EMG activities of transversus abdominal muscle were evaluated.
Results: Subjects were classified according to 3 profiles: no limitation during exercise (P0), limitation up to a 3-5 hPa Pc (P1) and limitation up to a 7 hPa Pc (P2). For P0 and P1 subjects (n=3), ARV with NEP was higher than without NEP at rest and during exercise. On the other hand, for P2 subjects (n=5), ARV with NEP was lower than without during exercise. NEP did not increased muscular activity in a given exercise condition and even decreased in some subjects.
Conclusion: For P2 subjects, NEP must be sufficiently high to remove EFL, because the muscle was less active than in P0 and P1 subjects. These results suggest that, by an unknown mechanism, P0 and P1 subjects increase their muscular activity to reduce the effect of NEP. The difference observed among subjects in response to NEP during exercise warrants further investigations.

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P21-14

MITOGEN ACTIVATED PROTEIN KINASE SIGNALLING IN DM1 SKELETAL MUSCLE
Coldefy A.S., Pisani D., Ragno M., Desnuelle C., Dechesne C., Dérijard B.

Myotonic dystrophy (DM1) is an autosomal dominant disorder caused by an instable CTG repeat sequence in the 3'-untranslated region of a protein kinase gene (dmpk).
DM1 is the most common adult onset neuromuscular disease. It involves myotonia, progressive weakness and wasting of skeletal muscle. The congenital form of DM1 is also associated with evidence of delayed or arrested muscle maturation. Furthermore, DMPK is a serine / threonine kinase but its cellular function is still unknown.
Research in our group focuses on the function of Mitogen Activated Protein Kinase (MAPK) signalling pathways in skeletal muscle growth and differentiation. There are three MAPK pathways : the ERK (ERK1 / ERK2) pathway acts primarily to positively regulate the cell cycle, while the p38 and JNK pathways may oppose these function and elicit stress-induced cell cycle arrest and differentiation. It has been recently shown that ERK and p38 pathways play important roles in the process of myogenesis.
Therefore we wanted to know if ERK, p38 or JNK might be involved in the DM1 muscular deficiency. In collaboration with several different groups, we studied MAPK activation in patients skeletal muscle biopsies, in muscles from a myotonic dystrophy mouse model, in human DM1 satellite cells and in C2C12 myoblasts clones.
Considering the Ser / Thr kinase nature of DMPK, cell signalling and regulation of gene expression are very likely to be affected in this disease. Our preliminary results show a variation in the activation of the three MAPK in all our DM1 "studying models", although they are still confusing regarding JNK. Nonetheless, our overall conclusion is that the activation of the MAPK signalling pathways is likely to be impaired in DM1 disease.

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P21-15

EFFECTS OF CLENBUTEROL TREATMENT AND EXERCISE ON MUSCLE METABOLISM IN RATS
Cavalié H., Mounier R., Clottes E., Bricout V., Lac G.

The aim of this study was to test the effects of a selective beta-2 adrenergic receptor agonist : clenbuterol, on muscle performance and metabolism in growing 3-month old male Wistar rats treated over 8 weeks.
Méthods - Thirty-two Wistar rats weighing 234 ± 2 g were assigned for 8 weeks, five days each week, to a progressive isometric strength training exercise program plus oral clenbuterol (2 mg/kg body weight/day, Exercised + Clenbuterol group : ECL), exercise program without clenbuterol (Exercised group : E), no exercise program plus oral clenbuterol (2 mg/kg/day, Untrained + Clenbuterol : UCL), or no exercise without clenbuterol (Untrained : U). At the end of the 8 weeks, body composition was measured by Hologic QDR 4500A DEXA technique. On day 58,

plasma, soleus (Sol) and plantaris (Plan) were harvested after sacrifice until analyses.
Results - We found that fat mass was decreased in E, and decreased further in ECL. Lean mass was increased in UCL and ECL. Insulin-like growth factor-1 (IGF-1) was increased in E, and decreased in UCL. Sol and Plan phenotype, based on myosin heavy chain (MHC) composition, was both affected by treatment or/and training with a shift to a faster phenotype in both Sol and Plan. Strength endurance capacity was decreased in ECL. Muscular metabolism was also altered by clenbuterol administration and/or training in Sol and Plan with a decrease in citrate synthase (CS) and lactate deshydrogenase (LDH) activity in both muscle and an increase in creatine kinase (CK) activity.
Conclusion - This study suggests that clenbuterol-induced muscular hypertrophy treatment is independent of plasma IGF-1 and that the decrease of muscular strength endurance capacity is mainly caused by the decrease of oxydative and glycolytic metabolism in muscle.

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P21-16

X-RAY INTERFERENCE MEASURES THE WORKING STROKE OF MUSCLE MYOSIN WITH SUBNANOMETRE PRECISION.
Reconditi M., Linari M., Lucii L., Sun Y.-B., Boesecke P., Narayanan T., Stewart A., Irving T., Fischetti R. F., Piazzesi G., Irving M., Lombardi V.

Force and shortening in muscle are generated by ATP driven cyclic interactions of the myosin head domains, extending from the myosin filament, with the actin filament. According to crystallographic models, in each interaction an interdomain tilting of the myosin head accounts for ~10 nm filament sliding. However, the function of the myosin motor depends on the interaction between conformational changes and external forces, and this cannot be reproduced in crystallographic studies. In the skeletal muscle, due to the quasi crystalline arrangement of the motor proteins in the three-dimensional lattice, small-angle X-ray scattering (SAXS) can be used to record the conformational changes of the myosin motor in the native structure. The improved brightness and focusing of synchrotron X-ray beamlines like ID2 at ESRF (Grenoble, France) and Bio-CAT at APS (Argonne, IL, USA) led to the development of a new technique that can measure the motions of myosin heads in muscle fibres with Å-scale sensitivity. The method depends on X-ray interference between the two arrays of myosin heads in each bipolar myosin filament, which superimposes a finely spaced fringe pattern onto the ~14.5 nm reflection originating from the axial repeat of the myosin heads in each array. We used this method to study the motions of myosin heads associated to the early length transient (1-10 ms duration) that follows a 0.1 ms step in force superimposed on the isometric contraction of single fibres from frog skeletal muscle at 4 °C and 2.1 um sarcomere length. The results provide evidence for a mechanism of force generation based on a working stroke in the myosin heads attached to actin and show that under isotonic contraction the amount of filament sliding accounted for by the elementary step increases from 6 to 10 nm as the force reduces from 75% to 25% the isometric force.

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P21-17

THE P38 PATHWAY REGULATES AKT AT THE PROTEIN AND TRANSCRIPTIONAL ACTIVATION LEVELS DURING MYOGENESIS
Cabane C., Yeow K., Ragno M., Dérijard B.

Muscle cell differentiation is controlled by extracellular growth factors whose signals are transduced into the nucleus. Stress Activated Protein Kinase cascades are involved in this process and members of these pathways are potently expressed in skeletal muscle.
We use the mouse skeletal muscle C2C12 cell line as a model system. This cell line is able to differentiate in vitro such that the major steps in myotube formation are identifiable. Several stable clones expressing various members of the stress-induced MAPK cascades have been isolated, including a C2C12 clone expressing a dominant negative form of the MAPKK MKK3 (p38 pathway).
This stable clone was unable to undergo myotube formation. These cells have been used side by side with control cells in a cDNA grid array screening in order to study gene regulation involving the p38 signalling pathway during differentiation. One of these genes, c-Akt (PKBalpha), is down-regulated during differentiation in the MKK3 dominant negative stable clone.
In addition to the MKK3 dominant negative stable clone, we also show that C2C12 cells stably transfected with a dominant negative form of Akt do not differentiate. Since both the MKK3/p38 and PI3K/c-Akt pathways are required for myogenesis, we decided to study the interaction between these two pathways in C2C12 cells. Our results suggest that Akt acts downstream of p38 in myogenic cell differentiation. Activating the p38 pathway results in the concurrent activation of Akt ; conversely, inhibiting the p38 pathway prevents Akt activation. C2C12 cells stably expressing dominant negative MKK3 express reduced levels of Akt messenger RNA and total protein. Interestingly, levels of phosphorylated Akt are less regulated in these cells. Our results show for the first time that p38 can directly affect Akt at the transcriptional level as well as at the protein activation level during myogenic differentiation.

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P21-18

CHOLINERGIC DRUGS CHANGE THE SYNAPTIC DELAYS OF QUANTAL RELEASE AT THE FROG ENDPLATE
Nikolsky E.E., Samigullin D., Bukharaeva E.A., Vyskocil F.

Acetylcholine (Ach) can hinder the neuromuscular transmission by lowering end-plate current (EPC) amplitude. This lowering is caused by decreasing the number of quanta forming EPC and by postsynaptic receptor desnsitization. However, the decrease of the EPC might also be caused by greater dispersion in synaptic delays of individual quanta1,2. The delay distribution of quanta release differs in the proximal and distal parts of the 100-200 mm long frog endplate nerve terminal and there also exists progressive slowing down of nerve spike conduction velocity in the proximal-distal direction. The minimal synaptic delays are longest (0.50 ms) in proximal part and shortest in distal part (0.31 ms), but the delay dispersion is highest in the former parts and decreases in the distal parts3. Frog endplate therefore provides a unique possibility of studying the sections of the same synapse with more compact or more dispersed quanta release. In the distal parts, the uni-quantal EPCs with long delays increased in number when 5x10-4 M Ach was applied. P90 (an interval at which 90 % of all uni-quantal EPCs had occurred), was reversibly increased by 66% from 0.51 ms to 0.85 ms. In the distal parts of the endplate, stimulation-evoked EPCs with long release latencies increased in number also when non-hydrolysable carbacholine (CB) was applied. This increased asynchrony leads to a substantial drop in the size of reconstructed multiquantal EPCs which was lower by 28% than control one. The presynaptic action of Ach, CB (and also other cholinergic drugs) includes the modulation of the quantal secretion time course and by this the efficacy of the synaptic transmission. Grants: MSMT 113100003, GAÈR 305021333, 202021213, RFBR 000448901.
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P21-19

STRUCTURAL ASPECTS OF FORCE GENERATION BY MYOSIN HEADS PROBED BY X-RAY INTERFERENCE IN FROG MUSCLE
Piazzesi G., Brunello E., Linari M., Reconditi M., Sun Y.-B., Narayanan T., Panine P., Irving M., Lombardi V.

Force generation in muscle is thought to be due to transition between states with different degree of tilting of the light chain domain of the myosin head. We tested this idea by using X-ray diffraction interference to measure the changes in axial position of the heads associated to changes in isometric force with temperature, a factor that is known to change the force per myosin head. During the isometric contraction of single muscle fibres of Rana temporaria, X-ray interference between the two arrays of myosin heads in each myosin filament splits the M3 reflection from the 14.5 nm axial repeat of the heads into two main peaks. In the experiments reported here patterns were collected at ID2 (European Synchrotron Radiation Facility, ESRF, France) on an image intensified FReLoN CCD detector placed at either 10 m (to collect intensity and fine structure of the low order meridional reflections) or 3 m (to collect intensity of the higher order meridional reflections and of the actin layer lines). Increasing the temperature from 0 °C to 17 °C increased the isometric tetanic force by 44%, decreased the relative

intensity between the high angle peak and the low angle peak of the M3 reflection from 0.9 ± 0.02 (mean ± SD, n= 12 fibres) to 0.75 ± 0.01 and increased the spacing of the M3 reflection (14.568 nm at 0 °C) in proportion to the isometric force with a slope about twice that estimated during the elastic response to rapid length changes. The intensity of the first actin layer line, measured in the same range of temperatures, increased by 56 ± 18% (6 fibres). Changes in the fine structure of M3 reflection that accompany temperature dependent changes in the isometric force are interpreted using the tilting lever arm model of the myosin head and a structural model of the sarcomere with distributed filament compliance.

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P21-20

THE FORCE RESPONSE TO STRETCH IN HUMAN SKELETAL MUSCLE FIBRES WITH DIFFERENT MYOSIN ISOFORMS

Linari M., Bottinelli R., Pellegrino M.A., Reggiani C., Lombardi V.

The functional heterogeneity of skeletal muscle in mammalians is based mainly on the existence of multiple isoforms of the myosin heavy chain subunit (MHC) of the molecular motor myosin II: in fibres containing fast MHC isoforms, the unloaded shortening velocity, the maximum power output and the ATPase activity are three-to tenfold larger than in fibres containing slow MHC isoforms. Force enhancement during lengthening of an active muscle, a condition that normally occurs during locomotion in vivo, is accompanied by decrease in ATP consumption below the isometric level, while the rate of cross-bridge detachment/attachment increases with lengthening velocity: the myosin head under strain is prevented for completing the conventional cycle and detach from actin through a step that does not imply ATP splitting. We investigated the kinetics and mechanical features of this cycle in Ca^{2+} activated single skinned fibres from human skeletal muscle containing different MHC isoforms, identified with single fibre gel electrophoresis. Fibre were activated by using a new set-up that allows development of most of tension following a jump from 0-1°C to test temperature (~12°C). In this way we could prevent the development of sarcomere non-uniformities and record sarcomere length changes with a striation follower in any phase of the mechanical protocol. We found that (i) at any pCa fibres with fast MHC isoforms develop larger isometric forces (~60%) than those with slow isoforms, due to both increased fraction of force generating myosin heads and, to a lesser extent, increased force per head; (ii) independent of the MHC isoform lengthening of maximally activated fibres elicits similar steady forces indicating that mechanical and kinetic properties of the actin-myosin interaction under stretch are similar; in both fibre types force enhancement by stretch is due to recruitment of myosin attachments, without increase in strain per head above the value generated by isometric heads.

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P21-21

Ca^{2+} INFLUX AND RESTING Ca^{2+} DECREASE DURING SLOW-TO-FAST TRANSITION OF UNLOADED RAT SOLEUS MUSCLE

Fraysse B., Desaphy J-F., Pierno S., De Luca A., Liantonio A., Rolland J-F., Conte Camerino D.

The present study was designed to determine the muscle-type specificity of passive Ca^{2+} entry. Using the fura-2 Ca^{2+} probe and the Mn^{2+} quenching technique, we observed in control rats that the slow-twitch soleus muscle (SOL) presented a higher $[Ca^{2+}]i$ and a higher Ca^{2+} influx at rest with respect to the fast-twitch extensor digitorum longus muscle (EDL). By comparing effects of nifedipine and gadolinium on the quench rate, we concluded that the muscle-type specificity of the passive Ca^{2+} entry was related to difference in expression and/or activity of Ca^{2+} permeable stretch-activated channels (SAC) and that leak Ca^{2+} channels activity was similar in both muscle types. During hindlimb unloading (HU), it is well known that rat SOL undergoes a slow-to-fast phenotype transition. In line with this, the resting $[Ca^{2+}]i$ and the passive Ca^{2+} entry were drastically decreased in rat SOL after 14 days of HU, becoming, together with the response to caffeine, similar to that of control EDL. For a shorter period of HU, 3 days, the caffeine-induced Ca^{2+} release assessed in SOL was still typical of a slow-twitch muscle. In contrast, the resting $[Ca^{2+}]i$ and the passive Ca^{2+} influx were already significantly decreased in SOL fibres as compared to control, due to a reduced SAC activity. On the other hand, no correlation between HU-induced muscle wasting and resting $[Ca^{2+}]i$ changes was found in SOL. These results clearly show that SOL resting $[Ca^{2+}]i$ decrease is an early event during suspension, which precedes most of the slow-to-fast functional changes, but could rely on an early reduced Ca^{2+} entry trough SAC. Accordingly to the crucial role of $[Ca^{2+}]i$ in the regulation of muscle phenotype, our results suggest that the slow-to-fast transition taking place in rat SOL during unloading is a Ca^{2+}-dependent process in contrast to the HU-induced muscle atrophy (grants ASI I/R/040/01, ASI I/R/305/02).

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P21-22

FATIGUE-RELATED CHANGES OF TRICEPS SURAE MOTONEURONE ACTIVITY INDUCED BY STRETCH IN DECEREBRATE CATS

Ljubisavljevic M., Bugaychenko L., Kalezic I., Kostyukov A., Pilyavskii A., Windhorst U., Johansson H.

We investigated fatigue-related changes of the triceps sure muscle (TS) motoneurones activity in decerebrate cats in response to muscle stretch of the homonumous muscle. Long-lasting (1.5–2 hours) intrasomatic recordings were produced from 11 motoneurones. The animals were subjected to bilateral pneumothorax and artificially ventilated while additional rigid fixation of the spinal cord enabled lasting intrasomatic recording during strong long-lasting muscle contractions. Right hindlimb was completely denervated except for the TS. Fatiguing muscle contractions of TS were evoked by electrical stimulation of the otherwise cut S1 ventral root (current strength 1.4 threshold, rate 40 s -1 and duration ranging from 5 to 15 min). Microelectrodes were filled with 2 M solution of potassium acetate and 0.6 M potassium chloride with resistance 1.8 – 2.3 MOhms. Standard slow bell-wise muscle stretches of different amplitude and velocity were applied; maximal amplitude of the stretches did not exceed 8 mm. The stretches evoked very stable and reproducible waves of membrane depolarization reflecting summation of a great number of low-amplitude individual EPSPs. The depolarization in many cases was accompanied by generation of a steady spike discharge with instantaneous rate related closely to temporal course of the muscle stretches. After cessation of the fatiguing stimulation both the Ia EPSPs evoked by TS nerve stimulation and the stretch-evoked depolarization were noticeable depressed reaching 20%. The depolarization decline could increase with duration of the fatiguing stimulation and amplitude of the fatiguing muscle contractions. The stretch-related spike activity was also suppressed; the post-fatigue decrease reaching 5-7 s-1 with following restoration to a pre-fatigue level during 5-10 min.
The results further delineate the central processes controlling effectiveness of afferent inflow regulation of motoneurone activity during muscle fatigue.

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P21-23

EFFECTS OF EXERCISE AND DIETARY INTERVENTION ON HUMAN SKELETAL MUSCLE METABOLISM IN THE IGT PERSONS

Venojärvi M., Puhke R., Hämäläinen H., Marniemi J., Rastas M., Atalay M., Hänninen O., Rusko H., Aunola S.

Aims: The purpose of this study is to discover if middle-aged obese people with IGT are able to improve their muscular performance capacity, improve glucose metabolism and decrease oxidative stress in skeletal muscle by means of a 2-year exercise and dietary intervention.
Methods: The study subjects were 22 persons from the Finnish Diabetes Prevention Study with IGT who volunteered to give samples of biopsies from the vastus lateralis muscle. Changes in glucose tolerance, the activities of muscle fibre glycolytic/oxidative enzymes, MHC-phenotype (myosin heavy chain) and markers of oxidative stress were studied. The intervention consisted of (1) dietary advice and (2) strength & power and/or aerobic exercise training.
Results: Exercise training increased LDH (lactate dehydrogenase), LDH-1 (LDH isotype, heart) and CS (citrate synthase) activities in the vastus lateralis muscle. This, together with dietary advice decreased the amount of HO-1 (heme oxygenase) and 4-HNE (4-hydroxy-2-nonenal) proteins in muscle tissue as well as up-regulated amount of thioredoxin protein in muscle tissue. Strength & power type exercise training induced shifts myofibres phenotype to IIX-IIA-I but not aerobic exercise training.
Conclusions: Exercise training improves both glycolytic and oxidative enzyme activities in the vastus lateralis muscle and may enhance protection

against oxidative stress together with dietary advice in middle-aged persons with IGT.

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S22 OXYGEN SIGNALLING

ORAL SESSION

S22-1

COMING TO GRIPS WITH OXYGEN SENSING

Bauer C.

The physiological transfer of oxygen involves: a) convectional transport of O_2 in the lung and in the circulation; b) diffusional transport from the lung alveoli into the blood and out of the tissue capillaries to the mitochondria. A shortage of oxygen (hypoxia) can occur at all levels of this oxygen transport chain. However, a number of physiological processes are geared to maintain a constant flow of oxygen to our organs. In other words, biological systems have a number of protective devices that actually prevent the occurrence of harmful levels of hypoxia e.g. via VEGF, Erythropoietin, enhanced glycolysis and the heat shock proteins that rescue mis-folded proteins. All of these diverse biological activities are "guided" by the well-known transcription factor HIF1α that is exponentially up-regulated by hypoxia.
Nonetheless, it was far from being clear what biochemical entity represents the biological "O_2 electrode" that regulates the accumulation of HIF1α under hypoxia. In this Symposium, Peter Ratcliffe will tell us about an enzyme that functions as a biological "O_2 electrode". The name of the game is "prolyl hydroxylase" that acts in concert with the von Hippel-Lindau tumor suppressor protein to enhance the half-life of HIF1α. His presentation will be followed by considerations regarding the in vivo function of such "oxygen sensors", and we are interested to learn what Jacques Pouysségur has to report in this regard. As a "follow-up" to these in vivo considerations Roland Wenger will tell us about a fundamental system, the spermatogenesis which physiologically takes place under severe hypoxia and requires thereby a special set of "effector molecules". Finally we will enjoy the latest news from the carotid body: in this journey Jose Lopez Barneo will take us from "oxygen sensing" to "glucose sensing". Particularly in the brain, the combination of oxygen and glucose deprivation requires a particularly forceful line of defense.

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S22-2

OXYGEN SENSING BY HIF HYDROXYLASES

Ratcliffe P.J.

Analysis of HIF-a subunits has demonstrated that activity is regulated by a series of oxygen dependent enzymatic hydroxylations at specific prolyl and asparaginyl residues. Combined structural/genetics approaches have identified the relevant enzymes as members of the 2-oxoglutarate dependent dioxygenase super-family. HIF prolyl hydroxylation is performed by a closely related set of isoenzymes (PHD1-3) that differ in abundance and sub-cellular localization. Hydroxylation of either HIF-1a Pro402 or Pro564 promotes interaction with the von Hippel-Lindau tumour suppressor protein (pVHL) via a hydrogen bonding network within a well defined hydroxyproline binding pocket in the pVHL b-domain. In oxygenated cells this process targets HIF-a for rapid proteasomal destruction. HIF asparaginyl hydroxylation is performed by a protein termed FIH. In oxygenated cells hydroxylation of HIF-1a Asn 803 prevents interaction with p300 providing a second mechanism by which HIF mediated transcription is inactivated. Genetic studies demonstrate a critical function for both types of enzyme in regulating the HIF transcriptional cascade. Limitation of activity in hypoxia supports a central role of these hydroxylases in cellular oxygen sensing. Supply of other co-substrates and co-factors particularly the cellular availability of iron may also play an important regulatory role. Crystallographic analysis of FIH has demonstrated the anticipated b-barrel 'jelly-roll' conformation, but also unusual features at the binding sites for substrate and co-substrate. Conservation of key structural features strongly suggests that FIH belongs to a more extensive group of 2-OG dependent dioxygenases that are predicted to function in protein hydroxylation. The wider role of protein hydroxylation in directing cellular responses to oxygen availability will be considered.

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OC22-1

OXYGEN REGULATION OF THE WILMS' TUMOR GENE WT1 INVOLVES HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1)

Wagner K.D., Wagner N., Wellmann S., Bondke A., Schley G., Theres H., Scholz H.

Experimental evidence suggests that the availability of sufficient amounts of oxygen is critical for normal tissue formation. The product of the Wilms' tumor gene Wt1 is among the transcription factors, which are required for the development of certain organs including the kidneys and heart. In this study, we tested the hypothesis that hypoxia is a physiological stimulus for Wt1 expression. Furthermore, we aimed at analysing the mechanism(s) of hypoxic induction of Wt1. We show here that Wt1 mRNA and protein is up-regulated 5-fold in the heart and kidneys of rats that were exposed to normobaric hypoxia (8% O_2) and 0.1% carbon monoxide, respectively. Wt1 immunoreactivity was detected in the renal tubules and the coronary vasculature of hypoxic rats, both structures, which normally do not contain Wt1. De novo expression of Wt1 was also observed in the myocardial vessels of the ischemic heart after ligation of the left coronary artery. Wt1 mRNA and protein was enhanced approximately 6-fold in the human osteosarcoma cell line U-2OS and in Reh lymphoblast cells that were grown either at 1% O_2 or at 20% O_2 in the presence of $CoCl_2$ and desferrioxamine (DFX), respectively. The promoter of the Wt1 gene enhanced the activity of a luciferase reporter in response to hypoxia, $CoCl_2$ and DFX more than 6-fold. We identified a hypoxia-responsive element in the Wt1 promoter sequence that bound to hypoxia-inducible factor-1 (HIF-1) and was required for activation of the Wt1 promoter by $CoCl_2$ and HIF-1. These findings demonstrate that Wt1 expression can be stimulated by hypoxia and this involves activation of the Wt1 promoter by HIF-1. It is suggested that hypoxic induction of Wt1 has a role in the adaptive response of the coronary vasculature and the renal tubules to low oxygen tension.

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OC22-2

HYPERCAPNIC CHEMOTRANSDUCTION IN THE RAT ADRENAL MEDULLA

Muñoz-Cabello A.M., Toledo-Aral J.J., Echevarría M., López-Barneo J.

In this work we have studied the participation of adrenal medullary (AM) cells in hypercapnic chemotransduction. Rat neonatal AM cells are known to be O_2-sensitive during a period (from P1 to ~P15) at which the carotid body is still immature. Therefore, we investigated whether AM cells are also physiologic CO_2 chemoreceptors. Here we show results indicating that AM cells are able to sense hypercapnia. Using amperometric recordings we have measured catecholamine secretion from cells in AM slices from neonatal (1-8 days old) and adult (>= 1 month old) rats, perfused with solutions bubbled with different concentrations of CO_2 at constant pH (7.4) and O_2 tension (150 mm Hg). The secretory activity (in fC/min, mean ±SE, n=7) increased from 3532 ± 904 with 5% CO_2 to 7063 ± 2094 and 18110 ± 6877 with 10% and 20% CO_2, respectively. Responsiveness to hypercapnia was more frequent in neonatal slices (64 % of cells tested, n=61) than in the adult (35 %, n=17). The secretory response to hypercapnia required extracellular Ca^{++} influx, as it was abolished by extracellular Cd^{++} (0.5 mM). Supporting the role of AM in CO_2 sensing we have shown the presence of carbonic anhydrase (CA) in AM cells by RT-PCR and in situ hybridization. Interestingly, the time course of CA expression in the AM showed a peak around 1-8 days after birth, consistent with the increase in the secretory response to CO_2 at that age. Quantification of CA expression levels by real time PCR have shown an increase of ~ 20 folds in neonatal medulla compare to adult. Thus, our data show that AM cells are CO_2 sensors, particularly during neonatal life, acting as a main peripherical chemoreceptor when carotid body is not completely mature.

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OC22-3

FUNCTIONAL ASPECT OF OXYGEN DELIVERY AND OXIDATIVE STRESS DURING ISCHEMIA AND REPERFUSION

Bertuglia S., Giusti A.

Increased formation of ROS on reperfusion after ischemia underlies ischemia reperfusion (I/R) damage. We measured in real time oxygen tension in

microvessels and tissue and oxidant stress during postischemic reperfusion in hamster cheek pouch microcirculation. We measured PO_2 by using posphorescence quenching microscopy and oxygen radical species (ROS) production in the systemic blood. We evaluate the effects of a NOS inhibitor (L-NMMA) and superoxide dismutase (SOD) on the oxidative stress during reperfusion. Microvascular injury was assessed by measurement of diameter changes, perfused capillary length (PCL) and leukocyte adhesion.
During early reperfusion arteriolar PO_2 was significantly lower than baseline while capillary PO_2 varied between 7-0 mm Hg. After 30-min reperfusion PO_2 had returned to baseline only in arterioles and tissue. During 5- and 15-min reperfusion ROS increased by 72 and 89% vs. baseline, respectively and declined to baseline after 30-min reperfusion. Pretreatment with SOD reduced ROS levels, increased arteriolar diameter and PCL, and decreased leukocyte adhesion ($p < 0.05$). L-NMMA decreased ROS only within 5-min reperfusion, that increased significantly by 72% during later reperfusion. L-NMMA worsened PCL and leukocyte adhesion ($p < 0.05$).
Our results demonstrate how PO_2 significantly decreased in arterioles and tissue at early reperfusion while capillaries were characterized by low PO_2 throughout reperfusion. Scavenging ROS by NO inhibition or SOD, ROS production decreased on early reperfusion, while only SOD decreased the ROS production during later reperfusion. It is suggested that low oxygenation might be related to increased ROS production and the observed protection on capillary perfusion by SOD may be due to scavenging ROS thus increasing oxygen supply. This work was supported by the NATO SCIENCE PROGRAM, Collaborative Linkage Grant LST CLG 977837.

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OC22-4

ASCORBATE DEFICIENCY DOES NOT AFFECT CARDIOVASCULAR PARAMETERS AND POSTISCHEMIC RECOVERY IN RATS

Vergely C., Goirand F., Perrin-Sarrado C., Moreau D., Guilland J.-C., Dumas M., Rochette L.

Vitamin C is considered as a very efficient water-soluble antioxidant, whose ability to fight against the deleterious effects of oxidative stress might be useful for the treatment of myocardial reperfusion injury. Moreover, several new cardiovascular properties of this molecule have been described recently. The aim of this study was to determine the in vivo effects of a severe depletion of vitamin C on cardiac and vascular parameters and reperfusion arrhythmias.
For this purpose, we used a mutant strain of Wistar rats, Osteogenic Disorder Shionogi (ODS), in which the key enzyme for vitamin C synthesis from glucose is lacking. After a 15 days vitamin C-deficient diet, ODS rats showed a 90% decrease of plasma and tissue levels of ascorbate, as compared to ODS rats fed with a vitamin C-supplemented diet, or to normal Wistar rats. Several biochemical parameters were investigated, such as plasma antioxidant capacity, proteins, tocopherol, urate, catecholamines, lipids, and nitrate. None of them were influenced by the vitamin C deficiency in ODS rats. Moreover, heart rate and arterial pressure were not different between ODS vitamin C-deficient and -supplemented rats. After 5 min of a regional myocardial ischemia, realized in vivo through the occlusion of the left anterior descending coronary artery, various severe arrhythmias were observed, such as ventricular premature beats, tachycardia and fibrillation. Their duration and occurrence were not modified in vitamin C-deficient rats. In another set of experiments, the vascular reactivity measured in vitro on thoracic arteries was not altered by ascorbate deficiency in ODS rats. Decreasing plasma and heart tissue vitamin C status (90%) was associated neither with a lower recovery of cardiovascular parameters nor by a higher incidence of reperfusion arrhythmias.
In conclusion, the results of this study suggest that compensatory mechanisms play a role in maintaining normal cardiac function in vitamin C-deficient rats.

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S22-3

IN VIVO INTERPLAY BETWEEN OXYGEN SENSORS

Pouyssegur J.

A remarkable signaling system devised for rapid adaptation to and survival in a low oxygen environment (hypoxia) has been conserved throughout evolution. The hypoxia inducible transcription factor HIF, found in worms, flies and vertebrates is central to this adaptation and as such, hif-1 represents a 'master' gene in oxygen homeostasis. HIF-1 is a transcriptional complex that plays a pivotal role in cellular adaptation to low oxygen availability. In the presence of oxygen, the HIF-a subunits are targeted for destruction by proline hydroxylation, a specific modification that provides recognition for the E3 ubiquitin ligase complex containing the von Hippel-Lindau tumour suppressor protein (pVHL). Three mammalian HIF prolyl-hydroxylases (PHD1, 2, 3), homologous to the HIF prolyl-hydroxylase of C. elegans (EGL-9), were recently identified and shown, at least in vitro, to down regulate HIF-a subunits. In this presentation we will show that specific 'silencing' of HIF prolyl-hydroxylase 2 (PHD2) with short interfering RNAs (siRNA) is sufficient to stabilize and activate HIF-1a in normoxia in all the human cells investigated. Surprisingly, 'silencing' of the other two HIF prolyl-hydroxylases, PHD1 and PHD3, had no effect on the stability of HIF-1a either in normoxia or upon re-oxygenation of hypoxic cells. However, the stabilisation of HIF-1a in normoxic cells, following PHD2 ablation is progressively "desensitized" within 4 to 5 days in culture uncovering a more complex mechanism than originally thought where interplay between oxygen sensors takes place. Central to this action appears to be a HIF-dependent activation process, a model of which will be presented. We conclude that PHD2, a cytoplasmic enzyme capable of shuttling between cytoplasm and nucleus, is the critical oxygen sensor targeting HIF-1a to proteasomal degradation. Interestingly, PHD2 is up-regulated by HIF-1 providing an auto-regulatory mechanism driven by the oxygen tension.

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S22-4

PHYSIOLOGICALLY HYPOXIC CELL DIFFERENTIATION: TESTIS-SPECIFIC EXPRESSION OF PASKIN AND HIF-1A

Wenger R.H.

The testis represents a unique environment where profound differentiation processes occur at a high rate within a small compartment. Spermatogenesis appears in synchronized waves of layers of differentiating germ cells which sequentially migrate from the basal toward the luminal regions of the seminiferous tubuli. Within this region, oxygen partial pressures as low as 2 mmHg have been reported, which are among the lowest values found in the body and otherwise occur only in the vicinity of mitochondria. We recently discovered two PAS proteins specifically expressed during spermatogenesis which might be required for adaptation to these unusual environmental conditions: PASKIN and HIF-1aI.1. The PAS domain is a versatile protein fold, capable of sensing environmental changes in light intensity, oxygen concentration and redox potentials, which can be found in many archaeal, bacterial and plant proteins. PASKIN is a putative mammalian PAS-sensor protein kinase related to the oxygen sensor FixL from Rhizobium species. The two yeast homologues of PASKIN coordinate sugar flux and protein translation via phosphorylation-dependent control of two enzymes involved in glycogen synthesis and three translation factors. To elucidate the function of mammalian PASKIN, we cloned and inactivated the mouse Paskin gene. The targeted integration of a lacZ reporter gene allowed the identification of the cell types expressing mouse PASKIN. HIF is a oxygen-labile transcription factor consisting of two PAS protein subunits. mHIF-1aI.1 and hHIF-1aTe are two testis-specific isoforms of HIF-1a found in mouse and human spermatozoae, respectively. Due to the lack of the DNA-binding domain, at least hHIF-1aTe might serve as a dominant-negative inhibitor. To elucidate the function of HIF-1 during spermatogenesis, we are currently establishing mouse models deficient for HIF-1a specifically in the testis.

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S22-5

CAROTID BODY GLOMUS CELLS ARE COMBINED OXYGEN AND GLUCOSE SENSORS

López-Barneo J.

The main O_2 sensor mediating the acute responses to systemic hypoxia in mammals is the carotid body. Glomus cells, the O_2-sensitive elements in the carotid body, are electrically excitable and have O_2-regulated K^+ channels. Hypoxia signaling in glomus cells involves inhibition of K^+ channels leading to cell depolarization, external Ca^{2+} influx, and neurotransmitter release, which, in turn, stimulates the afferent sensory fibers. These fibers form synapses with brainstem respiratory neurons to produce hyperventilation and sympathetic activation. We have recently developed a carotid body slice preparation that has allowed us to study by patch clamping and single-cell

amperometry the properties of glomus cells in optimal physiologic conditions. We have further confirmed that K^+ channels are the major effector molecules in the acute response of glomus cells to hypoxia. We have also shown that responsiveness to hypoxia is independent of the mitochondrial electron transport, although it is selectively occluded by rotenone. Our studies have demonstrated that, as suggested by the old literature, carotid body cells are highly sensitive low-glucose detectors. Like hypoxia, low-glucose activates glomus cells by inhibition of K^+ channels. The mechanisms and channel types involved in oxygen and glucose sensing appear to be different, although the two stimuli (hypoxia and hypoglycemia) converge to raise cytosolic $[Ca^{2+}]$ and to release transmitters in glomus cells. The strategically located carotid bodies may be of special importance for brain homeostasis as neurons are particularly vulnerable to the simultaneous lack of glucose and oxygen. The function of glomus cells as combined O_2 and glucose sensors, in which the two stimuli potentiate each other, is surely advantageous to facilitate activation of the counterregulatory measures in response to small reductions of any of the regulated variables.

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POSTER SESSION

P22-01

THE EXTRACORPOREAL DIALYSIS AND LITHOTRIPSY INDUCE THE PRODUCTION OF REACTIVE OXYGEN SPECIES

Micle L., Muresan M., Micle O., Burta L., Bumbu G., Maghiar T., Szilagy L., Kovari P., Dorofteiu M.

The chronic renal failure is associated with an oxidative stress. Such patients survive only by being submitted repeatedly to the extra corporeal haemodialysis. A question was raised: Is the dialysis able to remove the oxidative stress of the patients? Our study assessed the oxidant/antioxidant balance by testing the level of malondialdehyde (MDA), carbonylated proteins and ceruloplasmin on a group of patients with chronic renal failure, before and after dialysis. The results were compared with that obtained on a control group. Instead of the removal of the oxidative stress, the haemodialysis worsened it.
In practical medicine, the renal stones have a high frequency. The modern urological treatment of the renal stones consists in crushing them with ultrasounds. Even the stone migration through the excretory extra renal pathway causes slight haemorrhages and urinary infections responsible for an oxidative stress. In our research on a group of patients with renal stones, the extra corporeal shock wave lithotripsy was proved to be a strong generator of reactive oxygen species.

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P22-02

THE EFFECT OF THERMAL WATER UPON THE OXIDATIVE STRESS IN AUTOIMMUNE DISORDERS AND POST TRAUMATIC LESIONS

Muresan M., Micle L., Burta L., Micle O., Porumb V., Muresan I., Dorofteiu M.

A first group of patients with sero negative and sero positive rheumatoid arthritis were tested. For certifying the presence of oxidative stress, the level of MDA (malondialdehyde) and carbonylated proteins and of ceruloplasmin (the principal antioxidant factor of the plasma) were assessed, before and after 14 days of balneotherapy. The thermal water of Felix (resort situated in the western part of Romania) has a temperature of 36-37 degrees and a slight radioactivity of 0.37 milimicrocurie/l. After the treatment the concentration of MDA and carbonylated proteins returned to normal. (The normal value was established on a control group of healthy persons. These results were associated with the reduction of erythrocytes sedimentation rate and the removal of the main symptoms. Similar results were obtained after the treatment of patients with Dupuytren's contracture. In the post traumatic lesions the balneotherapy has no effect on the oxidative aggression.

University of Oradea – ROMANIA

P22-03

PATHOLOGIC HYPOXIC HYPOXIA AND ANEMIC HYPOXIA GENERATE REACTIVE OXYGEN SPECIES (ROS)

Muresan I., Muresan M., Burta L., Micle L., Antal L., Porumb V., Dorofteiu M.

In experimental conditions of hyperbaric hyperoxia or hypobaric hypoxia the presence of an oxidative stress were proved. Our study assessed the production of ROS in pathologic hypoxic hypoxia like: the brochopneumonia of children, in patients in the period of asthma attacks and immediately after the birth (in the ombilical blood). The existence of an oxidative stress in these conditions was acknowledged by the increased blood level of MDA malondialdehyde), carbonylated proteins and the variation of ceruloplasmin (one of the main antioxidants in the plasma).Under a specifically treatment of the infection with antibiotics, or after the suppression of the bronchospasm, the oxidants concentration returns to normal. The parturiton was associated with a high level of MDA and carbonilated proteins in the ombilical blood and a very low concentration of ceruloplasmin.In the group of patients with post hemoragic anemia, in spite of the reduced ability of the blood to transport oxygen, the oxidative stress was obvious. All the results were compared with the results established on a control group.

Individual praxis – Oradea – Romania

P22-04

THE OXIDATIVE STRESS IN THE STORED BLOOD

Burta L., Muresan M., Micle L., Micle O., Burta O., Evanics T., Dorofteiu M.

We tested the MDA (malondialdehyde), CP (Carbonylated Proteins) and ceruloplasmin levels in preservation blood bags, in certain moments: immediately after blood collection, 5th, 10th, and 15th day of storing. The elevation of the MDA, CP and ceruloplasmin decrease, indicated the presence of oxidative stress (OS) in stored blood. The presence of the OS was associated with an increased activity of SOD (Superoxiddismutase) and GSH-Px (Glutathione-peroxidase). An interesting result was the evidence of higher concentration of oxidative substances in the blood collected from smoker donors. We were able to prove that the main source of ROS (Reactive Oxygen Species) were the white blood cells (mako\ing a comparison between different blood products), in which probable the NADPH-oxidase was stimulated. In other experiments we were able to prevent the oxidative stress in the stored blood. The suppression of OS in the stored blood was possible by the administration (in vivo) of 2 vials of Vitamine C (i.v.) to the donors before blood collection and by introduction in the preservation bags (in vitro) of 1 ml a-lipoic acid.

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P22-05

EFFECT OF PRENATAL HYPOXIA ON MYOCARDIAL ISCHEMIA REPERFUSION INJURY IN ADULT RAT

Li G., Zhang L.

Epidemiological studies indicate a significant correlation between an adverse intrauterine environment with a consequent low birth weight and an increased risk of death from ischemic heart disease in the adult. We tested the hypothesis that prenatal hypoxia inhibited the heat stress-mediated protection of myocardial ischemia reperfusion injury in adult rat hearts. Time-dated pregnant rats were divided between normoxic control and hypoxic (10.5% O_2 from day 15 to 21 of gestational age) groups. Prenatal hypoxia resulted in a decrease in body weight at birth. The male progeny were studied at 2 months of age, at which no differences in body weight between normoxic control and prenatally hypoxic rats were found. Rats were treated in the presence or absence of heat stress (42°C for 15 min). After 24 hr, hearts were excised and subjected to 30 min of global ischemia and 1 hr of reperfusion in the Langendorff preparation. Prenatal hypoxia did not change baseline left ventricular function. Heat stress significantly increased the expression of heat shock protein 70 (HSP70) in the hearts from normoxic control rats, but not in those from prenatally hypoxic rats. In normoxic control rats, heat stress significantly improved the postischemic recovery of left ventricular function, and decreased ischemia/reperfusion (I/R)-induced myocardial infarct size and apoptosis, as compared with the control group. In contrast, there were no significant differences in postischemic recovery of left ventricular function and I/R-induced myocardial infarct size and apoptosis between the heat stress and control groups in prenatally hypoxic rats. We conclude that prenatal hypoxia increases the susceptibility of adult heart to I/R injury in part by inhibiting its endogenous protection mechanism of HSP70. These findings support the hypothesis of fetal origins of ischemic heart disease in later adult life.

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P22-06

POSSIBLE ROLE OF REACTIVE OXYGEN SPECIES IN THE EARLY ANTIARRHYTHMIC EFFECTS OF PRECONDITIONING

Végh Á., Hajnal Á., Papp J.Gy., Parratt J.R.

There is evidence that the generation of reactive oxygen species (ROS) may play a role in the early and the delayed infarct size limiting and anti-stunning effects of preconditioning (PC). However, relatively few studies have examined the involvement of ROS in the protective effects of PC against arrhythmias.
The present study was designed to assess the contribution of ROS to the early antiarrhythmic effect of PC in chloralose-urethane anaesthetised dogs. In 21 dogs PC was induced by two 5 min occlusions of the left anterior descending coronary artery (LAD) with a 20 min reperfusion interval in between. Twenty min later, the LAD was re-occluded for 25 min, and then rapidly reperfused. In 9 out of these PC dogs, the ROS scavanger N-2-mercaptopropionyl-glycine(MPG) was infused locally into a small side branch of the LAD in a dose of 0.15 mg.kg-1.min-1 commencing 10 min prior to, and then throughout the entire PC procedure. Eleven dogs, subjected to a 25 min occlusion/reperfusion insult, served as controls. Another 9 control dogs were also given MPG infusion over a period of 60 min prior to occlusion. Compared to the controls, PC markedly reduced the numbers of premature ventricular beats (VBPs, 377 ± 83 v 109 ± 44), episodes of ventricular tachycardia (VT, 9.7 ± 3.2 v 2.5 ± 1.1) and the incidences of VT (91% v. 60%) and ventricular fibrillation (VF, 92% v 0%) during prolonged occlusion. Survival in PC dogs was significantly increased (40% v 0 %). MPG given before and during the PC procedure failed to modify the protective effects of PC. Thus, the number of VPBs (115 ± 49) and of VT episodes (1.5 ± 1.3) as well as the incidences of VT (20%) and VF (0%) during occlusion were similar to that in the untreated PC dogs and 30 % of the dogs survived. MPG did not modify arrhythmia severity in dogs not subjected to PC.
Thus, we conclude that the generation of ROS is not necessary for the antiarrhythmic protection in this species.

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P22-07

THE EVOLUTION OF ROS AFTER STEADY PHYSICAL EFFORT WITH PERFORMANCE SPORTSMEN

Orasan R., Muresan A., Mitrea D.

The aim of our study was to investigate the appearance of the reactive oxygen species in performance athlets aged 15-19, which suggests a direct connection between effort and the intensity of oxydative processes.
The forming of the reactive oxygen species in physical effort has been described mainly by "in vitro" research in the last five years. This is one of the reasons for which our investigation has been performed "in vivo", with a significant sample of performance sportsmen.
20 sportsmen have been investigated - 14 males and 6 females. The subjects had daily training according to a rigorous programme and under strict surveillance. After 30-35 minutes of warming up, the main training consisted of running a 3000m distance in 60 minutes. Before and right after running the distance in abour 8 minutes, blood samples were taken and ceruloplasmine and lipid peroxides were determined.
The results showed that lipoperoxides grew exponentially with the intensity of the physical effort, their concentration being higher in the trained sportsmen.
The ceruloplasmine values decreased probably because of its consumption by the excessive presence of the reactive oxygen species.
The modifications in the dynamics of the serum lipid peroxides and that of the serum ceruloplasmine during physical effort behaved like divergent pressure values.
Effective physical training should reduce the discrepancy by lowering the lipid peroxides during effort and by reducing the lowering of the ceruloplasmine.
Future supervision of physical effort should take into consideration ROS investigation
as antioxidant supply could lead to new sports performance.

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P22-08

EFFECTS OF ET-1 AND NO ANTAGONISM ON LPS-INDUCED CORONARY VASOCONSTRICTION AND MYOCARDIAL DEPRESSION

Tu J., Shan QX., Jin HF., Xia Q.

Aim of the study: A wide array of inflammatory mediators have been suggested to be involved in the cardiovascular alterations in septic shock. We proposed that endothelin-1 (ET-1) and nitric oxide (NO) might contribute to lipopolysaccharide (LPS)-induced vasoconstriction and myocardial depression. In the present study, the selective ET-1 type-A receptor antagonist and NO synthase inhibitor were used to determine this hypothesis.
Materials and methods: Rats were treated with LPS (10mg/kg, i.p.) and 4 hours later the hearts were excised for perfusion at a constant perfusion flow (8ml/min) by the Langendorff technique. Either BQ-123 (0.8micromol/kg, i.p.), a selective ET-1 type-A receptor antagonist, aminoguanidine (AMG, 100 mg/kg, i.p.) or both was given 15 min before LPS challenge. Coronary perfusion pressure (CPP) and the indexes of the cardiac contractile function were recorded.

Results: (1) In hearts taken from rats treated with LPS, there was a marked increase in CPP. (2) Pretreatment with BQ-123 significantly reduced the increase of coronary perfusion pressure induced by LPS, while AMG had no effect. (3) LPS induced a significant decrease in left ventricular developed pressure and heart rate (LVDP x HR), as well as +/-dP/dtmax. Pretreatment with either BQ-123 or AMG partially reversed this effect of LPS on the isolated perfused hearts. However, administration of these two drugs prevented myocardial depression induced by LPS.
Conclusion: LPS induced a coronary vasoconstriction, which was mediated by ET-1. This increased coronary resistance and the subsequent maldistribution of coronary flow was not a main cause of LPS-induced myocardial depression. Both NO and ET-1 contribute to myocardial depression in the perfused hearts isolated from LPS-treated rats.
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P22-09

HYPOXIA-INDUCED METHANE FORMATION IN RAT LIVER MITOCHONDRIA
Boros M., Torday C., Ghyczy M.

Introduction: An abnormal increase in intracellular reducing power may occur through an interruption in the flow of electrons down the mitochondrial electron transport chain. We have recently proposed that under such conditions biomolecules with electrophilic methyl groups may act as electron acceptors, and that this mechanism may entail the generation of methane gas (Br J Nutr 85:409-414, 2001). Since methane (CH4) generation by mammalian cells and organelles has never been described, we designed experiments with rat liver mitochondria in which the formation of CH4 was envisaged.
Methods: Freshly isolated mitochondria were incubated in gas-tight vials. 0.1 mM $FeCl_3$, 0.1 mM EDTA and 5 mM ascorbate (ASC) or other substances (NADH, NADPH, dithiothreitol, reduced glutathione, etc.) were added as reducing agents. A 20-min exposure to nitrogen was used to induce hypoxia and the reaction was started by the addition of 10 mM H_2O_2. CH4 formation was determined by gas chromatography with flame-ionisation detectors (Carlo Erba Instruments HRGC 5300 Megaseries) and a Chrompack capillary column.
Results: Increasingly high amount of CH4 (up to 2 nmol/60min/mg protein) was reproducibly generated after the addition of ASC and 1-100 mM H_2O_2. CH4 formation was linearly related on the amount of mitochondria incubated, the amount of H_2O_2 added (between 0 and 20 mM), and the pH of the reaction. Acidic pH increased CH4 formation, but there was a significant CH4 evolution even at pH 7.0. Catalase (300 U/ml) abolished the increase in CH4 production indicating that mitochondrial H_2O_2 is required for the hypoxic activation of the CH4-generating reaction. DMTU, pyruvate and mannitol was less effective (approx. 80% inhibition was observed).
Conclusion: We report for the first time the generation of methane by rat liver mitochondria The mechanistic details of this pathway suggest a new concept to explain the loss of methyl groups in pathologies with redox imbalance.

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P22-10

THE EFFECTS OF ACUTE STRENUOUS EXERCISE ON PLASMA ANTIOXIDANT STATUS AND PLATELET RESPONSE IN RATS
Ficicilar H., Ersoz G., Zergeroglu A.M., Tekin D.

The reports regarding the effect of acute physical exercise on antioxidants and platelet functions are very contradictive. The purpose of present study was to investigate the effect of an acute bout of strenuous exercise on in vitro platelet functions and its possible relationship with exercise-associated changes of plasma antioxidant capacity. It was also planned to evaluate the effect of aspirin in order to see the role of platelet TxA2 formation in the exercise-induced changes.
Male Sprague-Dawley rats (14-16 weeks old) were randomly assigned to one of four experimental groups: 1)control (n=24), 2)exercise (n=19), 3)aspirin-control (n=10), and 4)aspirin-exercise (n=15). The aspirin groups were administered 100 mg/kg oral aspirin 24 hours before the blood sampling. The exercise group ran on a motorized treadmill designed for small animals for 60 min at about 65% of maximal O_2 uptake. Plasma total antioxidant status (TAS) was measured spectrophotometrically by using a specific Randox kit. Collagen (2 μg/ml) and ADP (10 μM) induced platelet aggregation and platelet ATP release were evaluated in whole blood by using electrical impedance and bioluminescence techniques, respectively.
Exercise enhanced collagen-induced platelet ATP release and decreased plasma TAS ($p<0.05$). Plasma TAS was higher in the aspirin-exercise group than that of the exercise group ($p<0.05$) while there weren't any differences in the aspirin-exercise and aspirin-control groups with respect to the control group. Moreover significant negative correlation ($r=-0.27$, $p<0.05$) was found between TAS and ADP-induced platelet aggregation.
In conclusion, our results show that acute exercise at 65% maximal oxygen uptake leads to platelet hyperreactivity which could be related to oxidative stress induced by acute strenuous exercise, and aspirin may be able to display an antioxidant activity during exercise.

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P22-11

CARDIOPROTECTIVE ROLE OF NITRIC OXIDE
Yuzkiv M., Tumanovska L., Moybenko A.

The role of NO in cardiac function is complex, considering paracrine interaction between endotheliocytes and cardiomyocytes. The aim of our study was to elucidate the influence of L-arginine on haemodinamic indices, infarct size and changes of biochemical parameters in different zones of myocardium during ischemia reperfusion in closed-chest dogs.
Coronary artery was occluded with controllable probe. Ischemia–90 min, reperfusion-180 min. NOS and arginase activity, content of NO_2, urea, leukotrien C4 and tromboxan B2 (TXB2) were assessed in the necrotic, bordering and non-ischemic myocardium.
Inracoronary L-arginine (1 mg/kg/min) led to decrease of MAP, LVP, dP/dt, compare to vehicle group ($P<0.05$) but a decrease of stroke volume and an increase of total vascular resistance and coronary vascular resistance were less prominent in L-arg group ($P<0.05$). Reperfusion arrythmia were minimal in L-arg group.
Infarct size significantly decreased in L-arg group and was 13,79±1,38% vs 35,1±3,6% vehicle ($P<0.05$).
L-arginine inhibited NOS activity in all zones of myocardium compare to vehicle group, although there was no difference in NO_2 content in non-ischemic zone between both groups.
Arginase activity was reduced in all zones of L-arg group, albeit enzyme activity decreased in necrotic zone vs non-ischemic zone, whereas in vehicle group arginase activity was 5-fold higher in necrotic zone compare to the non-ischemic zone. Similar changes are incident to urea content in the different zones of both groups.
LTC4 content in the necrotic zone was 3 times lower ($P<0.05$) in L-arg group vs vehicle group, as well as TXB2 content in necrotic zone ($P<0.05$).
L-arginine improved haemodynamic indices, exerted antiarrythmogenic action. Smaller infarct size in L-arg group correlated with diminished LTC4 and TBX2 content in necrotic and bordering zone of myocardium.

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P22-12

EFFECT OF LYCOPENE ADMINISTRATION ON OXIDATIVE STRESS IN EXPERIMENTAL HYPERTHYROIDISM ON RATS
Joanta A., Andrei S., Filip A., Clichici S., Suciu S., Dorofteiu M.

In recent years, the concept about possible relationship between thyroid function disorders and reactive oxygen species has increasing importance.
Lycopene, the main carotenoid in tomato, has been shown to be a potent antioxidant in vitro and in vivo.
The effects of altered thyroid states and lycopene administration on oxidative stress markers(lipid peroxides, oxidised proteins) and on antioxidant systems(enzymes, ceruloplasmine) were examined. Experiments were applied on white, male, Wistar rats. Hypothyroidism was induced by administering carbimazole in drinking water for 15 days. Hyperthyroidism was elicited by a 10 days treatment of hypothyroid rats with thyroxine, intraperitoneal administered.
Catalase activity was determined using a classic permanganometric method, glutathion peroxidase through a spectrophotometric assay, total peroxidase activity using the Chance method with guaiacol, superoxiddismutase through the Matkovics method with adrenaline, lipid peroxides level by thiobarbituric reaction substances(TBARS), protein oxidation through the carbonyl groups

estimation using a photometric method with dinitrophenylhidrazine and ceruloplasmine with the colorimetric method Ravin.
The antioxidant enzymes activity, in blood and in target tissues(thyroid gland, liver),was increased in rats treated with thyroxine and decreased in rats treated with diet rich in lycopene comparatively with control group. Also, we observed an increase of: serum ceruloplasmine, plasmatic and tissues levels of lipid peroxides and oxidised proteins in rats treated with thyroxine and a decrease of the same parameters in rats treated with lycopene.
All these findings show that hyperthyroidism is associated with a pro-oxidant state which will be reflected as an oxidative stress in plasma and in target tissues.Lycopene supplementation in hyperthyroid rats led to reduction of oxidative stress markers and susceptibility to oxidative stress and to increasment of antioxidants level.

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P22-13

THE EFFECTS OF COENZYME Q 10 AND GINGKO BILOBA 761 ADMINISTRATION ON THE AEROBIC PHYSICAL EXERCISE
Tache S., Bidian C.

Our observation on the favourable effect of certain antioxidant preparations-Coenzyme Q 10(CoQ10) and Gingko biloba(EGB 761), in moderate doses, on the physical performances of trained animals determined us to study their influence on short-term and long-term aerobic physical exercise.
Researches were made on 6 groups, each containing 10 white Wistar race rats, trained by running them on treadmil for 4 weeks:groups I and II-control, groups III and IV received CoQ10(10 mg/kg/day), groups V and VI received EGB 761(20 mg/kg/day). The exercise capacity was estimated based on the running distance, time and speed. Groups I, II and V performed short time aerobic physical exercise(maximum 30 minutes) and groups II, IV, VI performed long-time aerobic physical exercise(30-90 minutes). Venous blood samples were collected before and after training. The following serum indicators were determined:lipoperoxydes(through acid thiobarbituric method), ceruloplasmin(Ravin method) and uric acid(spectrophotometrical method).
Running speed increased during the period of training for all the groups. The increases were very significant in groups III- VI, comparing with groups I and II. After 28 days of training the results shown a very significant increasment in lipoperoxydes values and a very significant decreasement of ceruloplasmin and uric acid values in groups I and II, a significant increasement of lipoperoxydes values and a decreasment of ceruloplasmin and uric acid values in groups III- VI.
Moderate doses of CoQ10 and EGB 761 induce an increasement of the aerobic physical training capacity, the increasement being higher after long-term aerobic physical training and stimulate antioxidative capacity in trained animals. Higher effects appeared after long -term aerobic physical training.

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P22-14

THE STUDY OF ERYTHROCYTE DEFORMABILITY IN ONCOLOGIC AND DIABETIC PATIENTS
Beder I., Mataseje A., Kittova M., Zahorec R., Carsky J., Babinska K.

The aim of the study was the erythrocyte deformability monitoring as one of the important factors influencing the appropriate oxygen tissue perfusion. Erythrocyte deformability was studied in 7 patients with carcinoma of the colon before and after operation and in 11 patients with diabetes mellitus type I. In addition, the influence of selective membrane active substances on erythrocyte filtrability in diabetes mellitus was observed. The obtained variables were compared with the group of 12 normal subjects.
Erythrocyte deformability was determined by filtration method with centrifugation and calculated as percentage of filtered erythrocytes from the total erytrocyte count. The erythrocytes of diabetic patients were in vitro incubated with aminoguanidine (AG), newly synthetized substance pyridoxylidenaminoguanidine (PAG) and pyridoxal (PA). In all examinated groups the basic haematological variables were determinated.
In the group of 12 normal subjects the value of erythrocyte deformability was 72,9±5,4%, in oncologic patients the preoperative value was 65,1±3,4%. After surgery this value decreased by 4,2%, in the postoperative days erythrocyte deformability was increasing to the initial values. In diabetic patients the erythrocyte deformability value was 69,1±4,4%. In this group AG improved the filtration ability of erythrocytes by 5,4%, PAG by 6,2% and PA by 12,2%.
In oncologic patients the erythrocyte deformability value significantly decreased in comparison to the control group of normal subjects ($p<0,01$). In diabetic patients antioxidative membrane active substances AG and PAG demonstrated a mild improvement of erythrocyte deformability, a significant improvement was obtained by the effect of pyridoxal ($p<0,05$).
The recommendation for editor: Prof.J.Slezak, Slovak Academy of Sciences, Stefanikova 49, 81433 Bratislava,Slovakia

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P22-15

THE EFFECT OF NITRIC OXIDE ON EXERCISE PROTEINURIA IN RATS
Kuru O., Gunduz F., Senturk U.K.

Temporary proteinuria occurring after exercise is a common finding, and is explained predominantly by alterations in renal hemodynamics. In this study, it was investigated whether nitric oxide (NO), which is known to be effective on renal hemodynamics and to increase during exercise, has a role in post-exercise proteinuria.
We investigated the status of proteinuria induced by acute exhaustive exercise in rats and the effects of single dose pretreatment with a non-selective NO synthase enzyme inhibitor (N-nitro-L-arginine methyl ester, L-NAME), NO donor (Isosorbide mononitrate, ISMN) or vasodilator Diltiazem (calcium channel blocker). Along with the control and exhaustive exercise groups three groups of rats were used for evaluation of separate drug effect without exercise and additional three groups, receiving the agents separately, performed a single bout of exhaustive exercise.
The urinary protein levels in animals, which performed acute exhaustive exercise, were considerably elevated compared to the control animals. Significantly elevated urinary protein levels were observed in animals, which received L-NAME (2 hours prior to exercise, 10 mg/kg i.p.) before exhaustion, compared to both the control and exhausted groups, and mixed type proteinuria was detected, as in all exhausted animals. Mixed type proteinuria and the elevation in urinary protein levels that occur as a consequence of exhaustive exercise were prevented by ISMN treatment (1 hour before exercise, 2 mg/kg p.o.). Urinary protein levels were not different in exhausted rats with or without calcium channel blocker treatment (1 hour before exercise, 0.3 mg/kg, i.p.), and were also higher than those in control group. The blood pressure lowering range was similar for both ISMN and Diltiazem.
These results suggest that, endogenous NO might prevent the post-exercise proteinuria to grow more severe by affecting hemodynamic changes that occur during exercise.

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P22-16

ISCHEMIA-REPERFUSION-INDUCED APOPTOSIS IS REDUCED BY TACROLIMUS, AN IMMUNOSUPPRESIVE DRUG
Laurens M., Gugenheim J., Heurteaux C., Schmid-Alliana A., Crenesse D.

In hepatic surgery, ischemia-reperfusion phases, ussed to avoid excessive blood loss, lead to hepatocyte damage which induce post-surgical liver dysfunction. The ischemia-reperfusion injury results in apoptosis and necrosis, which may occur in parallel, both contributing in cell death. A Stress Activated Protein Kinase, JNK1/SAPK1, is activated by ischemia-reperfusion and leads to caspase 3 activation which is involved in the triggering of apoptosis. The aim of these study was to determine if hepatoprotective effect of Tacrolimus (a current-used immunosuppressive drug) could result in reduced ischemia-reperfusion-induced JNK1/SAPK1 activation and consequently, in decreased apoptosis process.
It was performed in rat, in classic ischemia-reperfusion model and on primary cultured hepatocytes, subjected to hypoxia-reoxygenation phases mimiking surgical conditions. JNK1/SAPK1 activation was evaluated by immunoprecipitation or Western-blotting experiments.
Apoptosis was assessed by measuring caspase 3 activation and by TUNEL reaction.
After Tacrolimus pretreatment before ischemia-reperfusion, JNK1/SAPK1 activation and apoptosis were significantly decreased and survival rates were improved. likewise, in hepatocytes subjected to hypoxia-reoxygenation phases, Tacrolimus reduced JNK1L/SAPK1 and caspase 3 activation.

In conclusion, Tacrolimus conferred prevention of ischemia-reperfusion-induced apoptosis.

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P22-17

THE INFLUENCE OF ANTIOXIDANTS UPON THE EXPRESSION OF ENDOTHELIAL ADHESION MOLECULES

Siska I.R., Crisnic D., Nistor D., Plesa A., Tanasie G., Bunu C., Paunescu V.

The aims of our study were to analyze the influence of anoxia/reoxygenation upon the expression of endothelial cell adhesion molecules (ECAM) CD54, CD106, CD62E and CD62P, to determine if the changes of ECAM expression can be mimicked by physiologically relevant concentrations of H2O2, and to assess the effects of the treatment with several antioxidants upon ECAM expression induced by anoxia/reoxygenation. In order to mimic ischemia/reperfusion-induced vascular changes in vivo we used a monolayer of cultivated human umbilical vein endothelial cells (HUVEC) exposed to anoxia/reoxygenation. ECAM expression was assessed by flow citometry - double staining technique, in different conditions: normoxia, after short anoxia (1 hour), and also after periods of anoxia/reoxygenation (1 hour/30 and 60 minutes, 2 and 4 hours). In all cases, experiments were performed with and without previous 12 hours incubation with TNFalpha (1ng/ml). The ECAM expression was also studied under normoxic conditions, but after cells exposure to H2O2 (0.1-1mmol/l) for 2-4 hours, and after anoxia/reoxygenation with previous addition of free radical scavengers (vitamin C, vitamin E, N-acetyl-cysteine).
Only CD54/ICAM-1 were expressed under basal conditions; after a 12 hours exposure to TNFalpha all ECAMs significantly increased. ECAM expression increased after 1 hour of anoxia followed by reperfusion only when TNFalpha was added. After anoxia/reoxygenation, an increase in CD54 expression was noted, its level remaining elevated for 4 hours. Unlike CD54, CD106, CD62E and CD62P increased after 0.5 hours of reoxygenation, than gradually decreased and returned close to baseline values. The addition of H2O2 led to an increase in ECAM expression similar to the rise achieved by reoxygenation. Oxygen-induced expression of VCAM-1 but not of ICAM-1 was inhibited by addition some free radical scavengers, such as N-acetyl-cysteine; a combination of vitamins C and E reduced CD54, CD106, and CD62E expression.

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P22-18

THE EFFECT OF NO INHIBITORS ON THE REACTIVITY OF RAT AND HUMAN TRACHEO-BRONCHIAL PREPARATIONS

Siska I.R., Tanasie G., Noveanu L., Bunu C., Mihalas G.

The aim of our study was to compare in vitro respiratory smooth muscle reactivity in rats and humans after addition of an inhibitor of NO synthesis, N-nitro-L-arginine (L-NA).
We used human bronchi from 6 patients undergoing resection for pulmonary carcinoma and tracheal spirals from 10 healthy rats (Sprague Dowley) and 10 ovalbumin sensitised rats. The preparations were mounted in aerated organ-bath, filled with Krebs Henseleit solution at 37 degrees C. The isometric contraction was registered using an isometric force transducer connected to a data aquisition computerised system.
For human bronchi. acetylcoline-induced contractions were significantly increased ($p<0.05$) when 10-6M L-NA was added (from 0.19±0.05 g to 0.28±0.12 g). For normal rat tracheal spirals contraction at 10-5 M acetylcholine was 0.66±0.4 g without L-NA and 0.77±0.4 g after L-NA addition ($p<0.05$). In case of ovalbumin sensitized rats. we noticed an increased effect of L-NA which modified the amplitude of acetylcholine contraction from 0.29±0.12 g to 0.36±0.16 g ($p<0.01$).
We concluded that local NO production is involved in maintaining in vitro tracheo-bronchial tone in both rats and humans. Despite differences between species, rat models may be reliable for the study of bronchial reactivity in both normal and hyperreactive airways.

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P22-19

ANTIOXIDANT SUPPLEMENTATION PREVENTS THE SULFITE INDUCED INCREASE IN MACROPHAGES FUNCTIONS

Bulbul M., Tan R., Izgut-Uysal VN., Kucukatay V.

The purpose of this study is investigate the effects of sulfite and antioxidant treatment on peritoneal macrophages functions from male wistar rats. Sulfite was administered by adding into drinking water at a dose of 25 mg/kg for 6 weeks. The animals were divided into four groups; control, sulfite, antioxidant and sulfite+antioxidant. During this period, antioxidant mixture (Vit-E 100 mg/kg + Vit-C 100 mg/kg) (in antioxidant and sulfite+antioxidant groups) and vehicle(in control group) were administered orally. Chemotactic and phagocytic activities were evaluated in macrophages obtained by peritoneal lavage. In sulfite group, the phagocytic and chemotactic activities increased in comparison with control group. In conclusion, antioxidant administration prevents the increments in chemotactic and phagocytic activities.

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P22-20

ANTIOXIDANTS IMPROVE THE LOW AFFINITY STATE BETA1-ADRENOCEPTOR-INDUCED VASODILATION IN HYPERTENSION

Mallem Y., Desfontis J.-C., Gautier F., Bucas V., Gogny M.

We investigated whether the low affinity state b1-adrenoceptors (b-AR)-induced relaxation was improved after antioxidant treatment in spontaneously hypertensive rats (SHR) aorta at 12 weeks old.
Aortic rings isolated from Wistar Kyoto (WKY) and SHR rats were constricted with phenylephrine (a1-AR agonist), then, cumulative concentration-relaxation curves (CCRC) to the low affinity state b1-AR agonists were constructed in endothelium-denuded aorta.
In WKY aorta, CGP 12177 (CGP) or cyanopindolol (low affinity state b1-AR agonists) produced concentration-dependent relaxation (pD2=5.3±0.1; 6.7±0.1 and Emax=97.7±3%; 97.9±2%; n=5-6 respectively). This relaxation was significantly altered in SHR aorta (pD2=4.02±0.1; 5.8±0.1 and Emax=87.4±5.4%; 85.7±5%; n=5 respectively, $P < 0.05$ vs control). To explore the role of oxygen-derived free radicals in this impairment, CCRC were established in the presence of two oxygen free radicals scavengers, superoxide dismutase (SOD) and N-acetylcysteine (NAC). The free radicals scavenging effect was checked by the attenuation of the inhibited relaxation to acetylcholine induced by xanthine (500 mM)/xanthine oxidase (0.01 U/ml), a superoxide anions generating system. The SOD (200 U/ml) or NAC (100 mM) treatment significantly improved the CGP or cyanopindolol-induced relaxation in aorta from SHR, but have no effect in aorta from WKY rats. As it has been described that oxygen free radicals may exert their effect through the activation of a tyrosine kinase pathway, we tested the CGP-induced response in the presence of a tyrosine kinase inhibitor (genistein, 30 mM). The CCRC to CGP was shifted to the left in both strains. However, the inhibitory effect of genistein was more potent in SHR than in WKY aorta.
We conclude that the impairment of the low affinity state b1-AR-induced relaxation in SHR aorta could be linked to a stimulatory effect of the overproduction of oxygen free radicals on a hypersensitive tyrosine kinase pathway.

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P22-21

MITOCHONDRIAL INVOLVEMENT IN KV3.1B CHANNEL MODULATION BY HYPOXIA

Wang J., Gurney A.M.

Kv3.1b potassium channels are inhibited by hypoxia, through a mechanism that requires functional mitochondria. Complex III of the electron transport chain has been proposed as the O_2 sensor in pulmonary artery myocytes. This study investigated the potential role of this mechanism in the hypoxic inhibition of mKv3.1b channels expressed in HEK293 cells.
Cells were superfused with a physiological solution. Hypoxia was induced by bubbling with 100% N2. Whole-cell patch clamp was used to record Kv3.1b current, activated by steps to 20mV from a holding potential of –80mV. Results are given as mean ± S.E.M. and analysed by two-tailed Student's t-test, $p<0.05$ considered significant.
Exposure to hypoxia for 10 min reduced current amplitude by 24 ± 2% (n=17) from 157 ± 12 pA/pF. The effect was abolished in the presence of 1μM rotenone (n=7) or the protonophore carbonyl cyanide p-(trifluomethoxy) phenylhydrazone (FCCP,1μM, n=7). Hypoxia also failed to

inhibit the current when tested in the presence of 1μM rotenone and 5mM succinate (n=4), suggesting O_2 sensing was upstream of complex III. Since electron transport is coupled to NADH oxidation, we investigated the effect of NADH (2mM) on the response to hypoxia by adding it to the pipette solution. This reduced Kv3.1b current amplitude from 213 ± 25 pA/pF (n=14) to 112 ± 16 pA/pF (n=8) and abolished the response to hypoxia (n=8). In contrast, NAD^+ (2mM) increased current amplitude to 306 ± 30 pA/pF (n=6), but did not affect the response to hypoxia (n=6). The redox agent, glutathione (oxidised, n=6 or reduced, n=8), had no effect on the response to hypoxia. It is concluded that mitochondrial inhibition prevents hypoxia from inhibiting Kv3.1 channels by causing the accumulation of NADH, which acts through a mechanism independent of redox modulation.

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S23 K^+ CHANNELS

ORAL SESSION

S23-1

TWO-PORE DOMAIN POTASSIUM CHANNELS AND THEIR ROLE IN THE REGULATION OF NEURONAL EXCITABILITY

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Background potassium (K) channels control both the resting membrane potential and the excitability of many mammalian neurons. The two-pore domain K (2-PK) channel superfamily has been proposed to underlie these background K channels. As their name implies, the individual subunits of this superfamily have two pore regions in the amino acid sequence which both contribute to the single pore of the functional channel. There are at least 14 different genes encoding for 2-PK channels in mammals and they are widely distributed throughout the nervous system. 2-PK channels are modulated by a range of factors, such as neurotransmitters, temperature, mechanical stretch and a variety of intracellular mediators. Important therapeutic agents such as certain neuroprotective drugs and general anaesthetics also modulate the activity of these channels.
We are studying the functional properties of five of these 2-PK channels, TASK-1, TASK-2, TASK-3, TREK-1 & TRAAK. Our aim is to establish a detailed functional fingerprint for each of these channels to help us to determine which of them are the most important contributors to the background currents recorded from particular native neurons. Interestingly, although these channels are open at all potentials, only TASK-1 and TRAAK channels show no voltage dependence of activation or inactivation.
TASK-1 and TASK-3 channels are often co-expressed in the same neurons, however it has proven particularly difficult to distinguish functionally between these two channels. Recently, we have found that zinc is a relatively selective blocker of hTASK-3 channels. Our data suggest that residues H98, predicted to lie at the mouth of the first pore region, and E70, within the large M1-P1 loop, are involved in the selective block by zinc of hTASK-3 over hTASK-1 channels. This selective action of zinc should aid future identification of currents through TASK-3 channels in native neurons.

Supported by the MRC and the BBSRC.

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S23-2

THE PHYSIOLOGY OF ERG POTASSIUM CHANNELS

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The ether-à-go-go-related gene (erg) K^+ channels belong to the EAG family of voltage-gated K^+ channels. Three erg family members are known which differ in their voltage dependence of activation, their gating kinetics and their steady state inactivation. At least in heterologous expression systems, erg subunits are able to form functional heteromultimers with either intermediate or dominant properties. All erg channels show a peculiar gating behaviour transferring inward-rectifying characteristics to the channels. This is brought about by inactivation kinetics being much faster than activation kinetics and recovery from inactivation being faster than deactivation.
All erg channels exhibit a high sensitivity to methanesulfonanilides such as E-4031. These substances have therefore been used as tools to isolate native erg-mediated currents and to investigate their physiological function. The best known example is the erg1 current in the heart which contributes to the cardiac action potential repolarization.
All three erg channels are expressed in the brain - where their functional role still has to be explored - and also in the pituitary. Beside our recent finding that erg channels determine the resting potential of gonadotroph cells, the role of the erg current in lactotroph cells is well documented. Lactotroph cells can express all three erg channels. The erg current is involved in the setting of the resting potential and in mediating the TRH-induced depolarization resulting in increased prolactin secretion. The TRH effects on erg channels include a shift in the voltage dependence of channel activation to more depolarized potentials, an acceleration of deactivation kinetics and a decrease in the maximal available erg current. Although a number of second messenger systems like different protein kinases, arachidonic acid and nitric oxide can affect the properties of erg channels, the mechanisms of the TRH-induced modulation of erg currents still have to be elucidated.

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OC23-1

POLARIZED TRAFFICKING AND SURFACE EXPRESSION OF KCNQ1 CHANNELS.

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Voltage-regulated KCNQ1 (KvLQT1) channels are partly responsible for the repolarisation after an action potential in the heart and are, in addition, of crucial importance for transepithelial transport in a number of organs, such as kidney, glands and the intestinal tract. In all cases, KCNQ1 channels seem to be modulated by one of the 5 members (KCNE1-5) of a one-transmembrane-segment family of β-subunits. It has been suggested that KCNQ1 channels may be located in the basolateral membrane in some epithelia and in the apical menbrane in others. In the present study we have examined the targeting of KCNQ1 channels after heterologous expression in the polarized epithelial MDCK cell line. When expressed alone, confocal microscopy showed that the channels were exclusively targeted to the basolateral membrane of the MDCK cells. To determine if the KCNE β-subunits affected the targeting of the channels, MDCK cells were co-transfected with KCNQ1 and each of the β-subunits (KCNE1-5). Assembly of KCNQ1 and the KCNE subunits was confirmed electrophysiologically, and subsequent immunolabeling showed, in all cases, clear basolateral localization. In addition, we examined the targeting of a N-terminal deletion (Δ 1-95). In this case, the KCNQ1 channel expression was arrested in intracellular perinuclear compartments. The motif required for surface expression was narrowed down to 20 amino acids in the N-terminus. In conclusion, our studies show that KCNQ1 channels are targeted to the basolateral membrane of polarized MDCK epithelial cells independently of co-expressed β-subunits. Furthermore a 20 amino acid stretch of the N-terminal is required for surface expression of the channel.

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OC23-2

SK4-LIKE CHANNELS ARE THE DOMINANT KCa CHANNELS IN AIRWAY CELLS AND CONTROL Cl^- SECRETION

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cAMP-or calcium-activated Cl^- secretion in secretory epithelia necessitate the presence of luminal Cl^- channels and basolateral K^+ channels. Ca^{2+}-and cAMP-dependent K^+ conductances have been reported to participate to Cl^- secretion by favoring the driving force for Cl^- exit. The aim of that study was to identify the K^+ channels involved in ion transport in airway cells. The 16HBE14o- cell line, derived from human bronchial epithelia, and primary culture of human bronchial cells (NHBE) constitute models of Cl^- secretion. The NCI-H292 cell line, derived from a human pulmonary mucoepidermoid carcinoma, is a model of mucin secretion. Characterization of Cl^- and K^+ conductances was assessed by a pharmacological approach combined to measurement of the short-circuit current and 86Rb effluxes. The presence of SK and KvLQT1 channels and associated KCNE ß-subunits, candidates to Ca^{2+}-and cAMP-dependent K^+ conductances were investigated by RT-PCR.
In all airway cells, 1-EBIO stimulated while clotrimazole, charybdotoxin or W7 inhibited the K^+ permeability, indicating the presence of SK4-like K^+ channels. Furthermore, blockade of SK4 channels by SK4 blockers resulted in a Cl^- secretion inhibition. These channels are essentially expressed in basolateral membranes in 16HBE14o- cells and NHBE while mainly expressed in apical membranes in NCI-H292 cells, a finding linked to the cell phenotype and to its associated function. Conversely, we failed to stimulate the K^+ permeability in these two cell types by increasing intracellular cAMP, suggesting the absence of functional KvLQT1 channels. RT-PCR experiments confirmed the presence of SK4 mRNA in the three cell types, but KvLQT1 mRNA was also detected. Thus, the lack of cAMP-dependent K^+ conductance in 16HBE14o-, NCI-H292 and NHBE cells may be due to the absence of KCNE subunit associated with the KvLQT1 channel. In conclusion, our results underly a key role in Cl^- secretion for SK4 channels in human bronchial cells.

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OC23-3

CHARACTERISATION AND FUNCTION OF AN H-CURRENT PRESENTED BY THE COCHLEAR PYRAMIDAL CELLS OF THE RAT

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Pyramidal cells are one of the prominent types of neurones in the dorsal cochlear nucleus (DCN), whose axons project to the contralateral inferior colliculus. In the present study we investigated the contribution of a hyperpolarization-activated conductance to the membrane properties and to the intrinsic activity of the pyramidal cells.
200 µm thick DCN brain slices were prepared from 6-14-day-old Wistar rats for whole-cell patch-clamp recording. When hyperpolarizing stimuli were applied, a slowly activating current could be recorded showing no inactivation tendency. The reversal potential of this component was -32 ± 3 mV (mean ± SEM, n = 6), while the half-activation voltage of this current was -94 ± 5 mV with a slope factor of 12.3 ± 0.6 mV (n = 24). This current was highly sensitive to the extracellular application of both 1 mM Cs^+ (blocking effect 77 ± 6%, n = 14) and 100 µM ZD7288 (blocking effect 78 ± 6%, n = 9). The electrophysiological properties and the pharmacological sensitivity of this current indicated that it corresponded to a hyperpolarization-activated non-specific cationic current (Ih).
It has been described earlier that the pyramidal cells have an ability to fire spontaneous action potentials, and in this work we could also detect intrinsic activity. Moreover, the inhibition of the h-type current effectively reduced the firing frequency, regardless whether CsCl (the firing activity was reduced to 16 ± 1% of the control, n = 5) or the more specific ZD7288 (25 ± 9%, n = 5) was applied.
The steady-state activation and some kinetic parameters of the h-current presented here suggested that HCN2 channel subunits possibly contributed to the assembly of these non-specific cationic channels. Immunohistochemical experiments were conducted to prove this possibility and to assess the precise subunit composition of the h-type ion channels expressed by the pyramidal cells of the DCN.

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OC23-4

TASK DEPENDENT NEURONAL APOPTOSIS

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Rat mature cerebellar granule, unlike hippocampal neurons, die by apoptosis when cultured in a medium containing a physiological concentration of K^+, but survive in high external K^+. In this work we studied the role of TASK-1 and TASK-3, encoding the pH-sensitive standing outward K^+ current IKso, in granule neuron apoptosis. We show that cell death in physiological K^+ parallels the developmental expression of TASK-1 and TASK-3 in cerebellar granule neuron cultures. Apoptosis is prevented by conditions that specifically reduce K^+ efflux through the TASK-3 channels, i.e. lowering of external pH or pharmacological inhibition by ruthenium red or muscarine m3 agonists. Moreover, we have used the Semliki Forest virus to transfer the TASK subunits into neuronal cells. Genetic transfer of the TASK subunits in hippocampal neurons, lacking IKso, induces apoptosis, while their genetic inactivation protects cerebellar granule neurons. These findings show that the TASK channels are responsible for K^+ deprivation induced apoptosis in granule neurons. Most likely, cell apoptosis occurs because of an increased cellular loss of K^+ leading to caspase activation and/or osmolytic water loss with following cell shrinkage. TASK channels may play an important role in the apoptotic machinery leading to cell elimination during cerebellar development.

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S23-3

EPITHELIAL K^+ CHANNELS IN TRANSGENIC MICE

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Potassium channels are found in virtually all mammalian cells. They form the most diverse group of ion channels (about 80 pore-forming subunit genes) that can be divided into three main structural classes comprising 2, 4 or 6 trans-membrane segments. In addition to the pore-forming subunits themselves, K^+ channels comprise in their structure associated modulatory subunits designed as ?-subunits. They are usually not essential for the formation of the ionic pore, but they determine the stability of the channel complex in the membrane and modulate biophysical, regulatory and pharmacological properties. We have produce knockout mice for KCNE1, an auxiliary subunit that associate with KCNQ1 to underlie the IKs channel, and for TWIK1 and TASK2, two background channels from the class of the 2P-domain K^+ channels. These three K^+ are found in several epithelial tissues, including the kidney where they are mainly found in proximal tubules. Clearance, micropuncture and electrophysiological comparative studies on wild type and knockout animals have allowed understand their respective roles in this nephron segment. In addition, we found that KCNE1 and KCNQ1 mRNAs are highly expressed in the zona glomerulosa of adrenal gland. In order to evaluate whether KCNE1 is modulating K^+ homeostasis and mineralocorticoid secretion, balance studies have been performed on kcne1 knockout mice and their WT counterparts Our results demonstrate that IKs participates in the direct control of aldosterone production by the plasma K^+ concentration. Were these results relevant for human genetic defects of IKs this would be capital for patients with congenital Long QT syndrome (LQT1) since hypokalemia is a known risk factor for the occurrence of torsades de pointes ventricular arrhythmias

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S23-4

PROPERTIES OF NEURAL KCNQ POTASSIUM CHANNELS

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Five members of the KCNQ gene family are known (KCNQ1-5). Four (KCNQ2-5) are present in the nervous system: Q4 is restricted to the cochlea and central auditory / vestibular pathways, but Q2,3 and 5 are widely distributed in the CNS, sympathetic and sensory ganglia. These form the subunits that make up neural M channels. However, when expressed homomerically in mammalian cell lines or oocytes, all members of this family generate 'M currents' as defined biophysically (low threshold, slow activation, lack of inactivation) and pharmacologically (inhibited by linopirdine and by Gq-linked muscarinic receptors). Q3 forms heteromers with Q2,4 or 5. Individual sympathetic ganglion cells express Q2, Q3 and Q5 mRNA and protein. It has been proposed that native M channels in sympathetic neurons are composed of Q2/3 heteromers. We have used the differential sensitivity of these two subunits to TEA to test this, by comparing inhibition curves for native M ganglionic M currents with inhibition curves for currents generated by co-expressed and concatameric Q2/3 subunits in CHO cells (Hadley et al., J.Neurosci., in press). In adult (P45) ganglion cells, inhibition curves accorded with a single population of heteromeric channels with a 1:1 Q2:Q3 stoichiometry, whereas in young (P17) neurons results suggested the additional presence of a small population of homomeric Q2 channels. No evidence for a substantial proportion of functional Q3/Q5 or Q5 channels could be obtained. Formation of Q2/3 heteromers may be favoured by increasing expression of Q3 mRNA during development. Hippocampal pyramidal cells also express Q2,3 and 5 mRNA and protein. Similar experiments with TEA (Shah et al., 2002: J.Physiol., 544,29) indicated that, in most cells tested, native M channels are also composed of Q2/3 heteromers, but that, in a minority, some of the channels are probably composed of Q3/5 heteromers or Q5 homomers.
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POSTER SESSION

P23-01

Na^+ AND Ca^{2+} CHANNEL BLOCKADE IMPROVES EFFECTIVENESS OF EGIS-7229, A CLASS III ANTI-ARRHYTHMIC DRUG

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EGIS-7229 is a class III anti-arrhythmic agent displaying also class Ib and IV effects. The class III action of EGIS-7229 is due to blockade of the rapid component of the delayed rectifier K^+ current (K0.5: 1.1±0.1 μM, in dog ventricular myocytes) and it appears at lower concentration than the class Ib and IV effects. According to the results of in vitro and in vivo studies, EGIS-7229 was advantageous compared to pure class III drugs from therapeutical point of view. EGIS-7229 prolonged APD/effective refractory period (ERP) in a reverse frequency-dependent manner at low concentration in various myocardial tissues. However, this feature obtained at 3 μM in rabbit papillary muscle (changes in ERP at 0.5, 1 and 2 Hz: 56, 53 and 24 %, respectively) diminished at 10 μM (54, 46 and 36%), while the effect of dofetilide was reverse frequency-dependent at high concentration (100 nM: 66, 47 and 22 %). Another disadvantage of most class III anti-arrhythmic drugs is their reduced efficacy during sympathetic activation. In fact, the effect of dofetilide (100 nM) on ERP was completely eliminated by isoproterenol (10 nM), while EGIS-7229 at 10 μM was able to keep its ERP lengthening effect under the same condition. In addition, EGIS-7229 demonstrated low pro-arrhythmic activity at 100 μM, as the drug produced no early after depolarisations (EAD) in rabbit papillary muscle, while sotalol induced EAD in 10 out of 12 preparations and EAD occurred even in 3 out of 11 control tissues bathed in hypokalemic organ bathes containing Cs^+. The torsadogenic effect of EGIS-7229 and GLG-V-13, a safe non-selective class III compound, was similar in conscious rabbits. In conclusion, the anti-arrhythmic effects of EGIS-7229 were maintained at elevated sympathetic tone and were not frequency dependent at high concentration, moreover the compound had low pro-arrhythmic potential. EGIS-7229 is an effective class III anti-arrhythmic drug with advantageous features probably due to its multi-channel activity.

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P23-02

EFFECT OF HYPOTONIC SHOCK ON AN ATP-SENSITIVE K^+ CHANNEL IN FROG PROXIMAL TUBULE CELLS

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A previous study has demonstrated that the K^+ channel inhibitor barium (Ba^{2+}) blocks volume regulation in response to a hypotonic shock in single proximal tubule cells isolated from frog kidney. Electrophysiological studies have identified an ATP-sensitive K^+ channel in these cells that is also inhibited by Ba^{2+}. The aim of the following study was to examine whether this K^+ channel plays a role in volume regulation.
Single proximal tubule cells were isolated by enzyme digestion. Standard patch clamp techniques were utilised to examine K^+ channel activity. Cell attached patches of the basolateral membrane were obtained with high K^+ in the pipette. Clamp potential was held at 0 mV. Cells were initially superfused with high Na^+ Ringer, which contained 84 mM mannitol. The absence of cation non-selective stretch-activated channels was confirmed by application of pressure across the patch. Cells were exposed to a hypotonic shock by the removal of 40 mM mannitol and after 10 minutes 5 mM Ba^{2+} was added to the bath.
Initially, the single channel current was -1.53 ± 0.28 pA and open probability (Po) was 0.28 ± 0.07 (n=5). On application of pressure across the patch both single channel current and Po were unchanged, -1.46 ± 0.32 pA and 0.24 ± 0.08 (n=5), respectively. On removal of pressure single channel current was -1.67 ± 0.42 pA and Po was 0.33 ± 0.06 (n=5). Exposure to a hypotonic shock did not alter these values, -1.41 ± 0.27 pA and 0.33 ± 0.07 (n=5), respectively. However, addition of 5 mM Ba^{2+} to the bath decreased Po to 0.22 ± 0.09 (n=5).
In conclusion, these data demonstrate that the ATP-sensitive K^+ channel located on the basolateral membrane of single frog proximal tubule cells is not regulated by an increase in cell volume initiated by hypotonic shock. This suggests that an additional K^+ channel plays a role in volume regulation in the renal proximal tubule.

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P23-03

TOWARDS TISSUE-SELECTIVE ATP-SENSITIVE K^+ CHANNEL OPENERS.

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In the search for new ATP-sensitive K^+ (KATP) channel modulators, we recently developed original diazoxide analogues. Particular attention was paid to the identification of structural requirements leading to improvement of drug potency and selectivity.
3-(Alkylamino)-7-halogeno-4H-1,2,4-benzothiadiazine 1,1-dioxides were synthesized and their activity on rat insulin-secreting cells and rat aorta rings were compared to that of the KATP channel activators diazoxide and pinacidil. Structure-activity relationships indicated that an improved potency and selectivity for the pancreatic tissue was obtained by introducing a fluorine atom in the 7-position and a short linear or cyclic hydrocarbon chain on the nitrogen atom in the 3-position. By contrast, strong myorelaxant activity was gained by the introduction of a halogen atom different from the fluorine atom in the 7-position and a bulky branched alkylamino chain in the 3-position.
Thus, 3-ethylamino-7-fluoro-4H-1,2,4-benzothiadiazine 1,1-dioxide expressed a marked inhibitory activity on pancreatic B-cells (IC50 = 1 μM) associated with a weak vasorelaxant effect (ED50 > 300 μM), whereas 7-chloro-3-(1,1-dimethylpropyl)amino-4H-1,2,4-benzothiadiazine 1,1-dioxide, which was only slightly active on insulin-secreting cells (IC50 > 10 μM), was found to be very potent on vascular smooth muscle cells (ED50 = 0.29 μM).
Radioisotopic, fluorimetric (insulin-secreting cells) and electrophysiological investigations (inside-out patches from Xenopus oocytes expressing KATP channel subtypes) performed with 7-chlorinated, 7-iodinated and 7-fluorinated 3-alkylamino-4H-1,2,4-benzothiadiazine 1,1-dioxides indicated that these drugs activated Kir6.2/SUR1 KATP channels.

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P23-04

THE ROLE OF DIAZOXIDE AND NO DEFICIENCY IN PROTECTIVE EFFECTS ON ISCHEMIA/REPERFUSION INJURY IN RATS

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One of the way of adaptation of the heart to ischemia similar to the ischemic preconditioning (IP) can be pharmacological treatment. Pretreatment with diazoxide (D), mitochondrial K[ATP] channel opener, triggers protection the heart against ischemia/reperfusion (I/R) injury. The influence of NO deficiency (NOD) on the mechanisms of I/R and IP has not been sufficiently elucidated so far. This study has been trying to characterize the effects of pharmacological interventions aimed at the modulation of ischemic tolerance on the damage caused by myocardial ischemia, on the subcellular level of alterations of myocardial proteins.
Isolated Langendorff-perfused hearts from control rats or rats with chronic NOD were subjected to 25 min global ischemia followed by 35 min reperfusion (II). In additional group, diazoxide was infused 15 min before II. The activities of matrix metalloproteinases were determined by zymography. The levels and activation of mitogen-activated protein kinases (MAPK) were determined by Western blot analysis.
D and chronic NOD are capable attenuating I/R injury of isolated perfused hearts. II induced changes in levels of some proteins and this was reversed in NOD rats and after D-pretreatment. Tissue mettaloproteinases-2 (MMP-2) activities were decreased after II. NOD and D pretreatment did not influence these activities. The levels of extracellular signal regulated kinases (ERK) were without changes but the content of phosphorylated ERK was decreased after II. Important was the finding that NOD and D treatment improved the ERK activation. D pretreatment revealed also significant effects on p38-MAPK pathway.
In conclusion, cardioprotection mediated by NOD a D in rats is associated with modulation of protein expression and/or activation of MAPK signalling cascades. The decreased tissue MMP-2 activities after II could reflect also their release into coronary effluent and implicates their involvement in I/R injury.

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P23-05

K^+ TRANSPORT IN AN INSECT EPITHELIUM: IS A KATP CHANNEL INVOLVED?

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The presence of ATP-regulated (KATP) K^+ channels in Tenebrio molitor L. Malpighian tubules (Mt) was investigated by studying effects on fluid secretion and basolateral membrane potentials (Vbl). In insect Mt the electrochemical gradient for K^+ is often cell inward. Closing of K^+ channels, for instance by Ba^{2+}, may therefore cause a hyperpolarization. Glibenclamide (0.1 or 0.5 mM), a KATP channel blocker, slowed fluid secretion. In low bath K^+ (5 mM), glibenclamide (0.5 mM) either hyperpolarized or depolarized Vbl, resembling the effect seen with Ba^{2+}. Subsequent addition of Ba^{2+} (6 mM) caused a further hyper- or depolarization of Vbl. In control Ringer (50 mM K^+, 90 mM Na^+) glibenclamide had no visible effect on Vbl. One mM ouabain reduced fluid secretion. In low bath K^+ (high Na^+) the Na^+/K^+-ATPase is expected to function at a high rate. Blocking it with ouabain is expected to raise the local ATP concentration at the basolateral membrane. In low bath K^+ in the presence of Ba^{2+} Vbl responded by a small but significant hyperpolarization from –51 ± 4 mV to –56 ± 4 mV (n = 16; $p<0.001$) to 1 mM ouabain. Repeating the experiments in the presence of both glibenclamide and Ba^{2+} resulted in a depolarisation of Vbl when ouabain was added. Increasing the K^+ concentration, replacing Na^+ by K^+, stops Na^+ entry and therefore slows down the Na^+/K^+-ATPase. In the presence of Ba^{2+} Vbl rapidly depolarized, but this was followed by a re-polarization. Repeating the experiments in the presence of glibenclamide markedly reduced the depolarizing effect and abolished the re-polarization, with a gradual decrease in the sensitivity of Vbl to $[K^+]$. These results suggest the presence of KATP channels in the basolateral membrane. Glibenclamide had no visible effect on Vbl in high K^+ or in the absence of Ba^{2+}, indicating that other highly conductive K^+ channels may mask the presence of KATP channels. This is the first demonstration of the presence of KATP channels in an insect epithelium.

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P23-06

HCN1, HCN2 AND HCN4 SUBUNITS IN THE PYRAMIDAL NEURONES OF THE DORSAL COCHLEAR NUCLEUS OF THE RAT

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The pyramidal cells of the dorsal cochlear nucleus receive direct synaptic inputs from the type I acoustic nerve fibres and their axons project to the contralateral inferior colliculus. They are regarded more than mere relay-stations in the signal processing, as they are capable of firing three different activity patterns in response to the same stimulus (chopper, build-up and pauser firing). We have shown that these neurones possess an h-current, which contributes to their spontaneous firing ability. It is known that the channels, responsible for the genesis of the h-current form as the result of an interaction between four possible types of channel subunits, termed HCN1-4. We found that the activation time constants of the h-current expressed by the pyramidal cells in thin brain slices are 150-400 ms ($\tau1$) and 800-1600 ($\tau2$). These taus suggest a relatively slow gating process, rendering it unlikely that the channels are HCN1 homomers. To clarify their possible subunit composition, immunocytochemical experiments were conducted, and the presence of three possible channel forming subunits (HCN1, HCN2, and HCN4) was tested.
For the immuncytochemical experiments pyramidal neurones have been isolated from the dorsal cochlear nuclei of 3-8-day-old rats with the application of a combined enzyme (collagenase and pronase) treatment followed by gentle mechanical trituration. The unambigous identification of the nerve cells was achieved by neuron specific immunostaining (neurone specific enolase, NSE). The presence of the appropriate HCN subunits was detected by applying anti-HCN primary (1:200) and FITC or Texas Red conjugated anti-rabbit secondary anti-bodies (1:1000). The experiments demonstrated that all three subunits were present both in the cell bodies and in the processes of the pyramidal cells emphasising the significance of these channels in determining the electrophysiological features of these neurones.

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P23-07

KV4.2 K^+ CHANNEL SUBUNITS CONTRIBUTE TO THE TRANSIENT OUTWARD CURRENT OF THE RAT COCHLEAR BUSHY CELL

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Bushy cells have a distinguished role in the central processing of auditory information as they receive inputs from the cochlear nerve fibres and pass the incoming activity to the contralateral medial nucleus of the trapezoid body in an extremely faithful fashion. To understand the membrane characteristics of these cells, a depolarization-activated transient outward current has been investigated in a brain slice preparation taken from 6-14-day-old Wistar rats. The cell identity was confirmed by applying Lucifer yellow in the pipette solution.
Under whole-cell voltage-clamp the bushy cells produced a rapidly activating and inactivating outward current which was evident when the cells were depolarised to –50 mV from a holding potential of –100 mV. When the holding potential was changed to –60 mV the transient current almost completely disappeared. The half-inactivation voltage of this current was –77 ± 2 mV with a slope factor of -5.8 ± 0.3 mV (n = 7; T = 25 °C; Mean ± S.E.M.). The recovery from inactivation followed a single-exponential time-course (tau = 12 ± 1 ms at -100 mV; n = 3).
This current showed moderate TEA sensitivity as only 37 ± 20 % of the peak current was inhibited in the presence of 1 mM TEA (n = 9). In general, the channels responsible for the genesis of transient K^+ currents may be composed of Kv1.4, Kv3.4 or Kv4.2 subunits. In the present study specific blockers were applied to reveal whether Kv4.2 subunits are involved in the final assembly of these channels on bushy cells. Phrixotoxin-2, a specific inhibitor of the Kv4.2 containing K^+ channels inhibited 27 ± 5% of the transient current (n = 3), indicating the significance of Kv4.2 subunits in forming the functional channels responsible for the transient current of the bushy neurones of the ventral cochlear nucleus.

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P23-08

THE INVESTIGATION OF VASOMOTOR AND CARDIOPROTECTIVE EFFECTS OF NEW OPENERS OF KATP CHANNELS

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ATP-sensitive potassium (KATP) channels are known to play an important role in the control of the vascular tone and heart function under physiological and, especially, pathological conditions. As was shown by numerous investigation in whole animals, isolated hearts, and cardiac myocytes KATP channels are important downstream mediators of ischemic or pharmacological preconditioning. Pharmacological KATP openers protect the myocardium against ischemia-reperfusion damage.
We studied the effects of the new fluorine-containing openers of KATP channels - PF-5, PF-10, diazoxide-Fp and diazoxide-Fm - on isolated strips of the guinea pig aorta, aorta of rats, and isolated hearts perfused by Langendorff. The tested agents showed themselves powerful vasodilatory effects; these effects were dose-dependent and depended on the initial vascular tone. Norepinephrine and, especially, angiotensin II partially inhibited these vasodilator effects; i.e., it can be supposed that the above mentioned agents suppress the KATP channel activity. Angiotensin II-induced inhibition of these effects was stronger in preparations from spontaneously hypertensive rats. We should mention that PF-5 dilatory effects tested in normotensive and hypertensive preparations under conditions of K^+-depolarization were rather similar. Our data suggest that the PF-5 could be considered a prospective agent for hypotension therapy. PF-10 (1-10?M) enhance myocardial contractility and decrease resistance of the coronary vessels in a dose-dependent manner, and attenuate spontaneous alterations of the heart rate. Preliminary activation of KATP channels by PF-10 and PF-5 exert cardioprotective effects under conditions of ischemia-reperfusion (similarly to the well-known phenomenon of ischemic preconditioning). Our results allow us to believe that the tested substances can be considered as effective regulators of the vascular tone and heart function.

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P23-09

CHARACTERIZATION OF DIFFERENT A561 HERG MUTATIONS ASSOCIATED WITH VARIOUS FORMS OF LONG QT SYNDROME

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HERG channels underlie the rapidly activating delayed rectifier potassium current (IKr) in cardiac myocytes. HERG mutations have been linked to congenital long QT syndrome (LQT2) and to acquired long QT syndrome (ALQT).
We have recently identified in a French family a missense mutation (A561P) in the S5 region of HERG. This mutation was not associated with any phenotype until the carrier, a healthy young boy, had arrhythmia while being treated with clobutinol, an antitussive drug (ALQT mutation). Interestingly, two other mutations (A561V and A561T) at this position had already been described. Both were associated with LQT2. In order to address how mutations at the same site in HERG can be related with different phenotype severity, we examined the mechanisms for HERG channels dysfunction in the A561 mutations using electrophysiological methods and immunolocalization experiments in transfected COS-7 cells. We showed that: (i) clobutinol blocks WT HERG current in a dose-dependant manner with a half-maximum block concentration (IC50) of micromolar range. (ii) All mutations lead to channel loss-of-function due to defect in protein traffic towards plasma membrane. (iii) The mutated proteins have a dominant-negative effect on WT HERG, reducing the current by 70%. (iv) Co-expression of WT and A561P (ALQT) HERG induces a 10 mV shift, towards negative potentials, of the activation/V curve whereas co-expression of WT and A561T or A561V (LQT2) proteins does not modify the current voltage-sensitivity. This earlier activation is responsible for an earlier contribution of IKr current during cardiac action potential.
We conclude that the long QT syndrome is a disease of increasing complexity based on many mechanisms involving channel function alteration, abnormality in protein processing and trafficking, and channel pharmacological properties. Interactions between those mechanisms are likely to be determinant for understanding the pathophysiology of LQT2.

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P23-10

ELECTROPHYSIOLOGICAL EFFECT OF TERIKALANT IN DOG CARDIAC MUSCLE

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The class III antiarrhythmic agent RP 58866 and its active enantiomer, terikalant was generally considered as a selective blocker of the inward rectifier K^+current, Ik1. These drugs have demonstrated efficacy in experimental arrhythmias, suggesting that block of Ik1 may be a useful antiarrhythmic mechanism. However the selectivity of terikalant on Ik1 is uncertain, especially at high concentration.
Therefore the aim of the present study was to investigate the in vitro electrophysiological effects of terikalant in canine isolated ventricular muscle and Purkinje fibers by applying the standard microelectrode technique.
At a stimulation cycle length of 1000 ms, terikalant (1-5-10-20 μM) significantly prolonged the action potential duration (APD) in papillary muscle. In Purkinje fibers terikalant (2.5 μM) produced a marked APD prolongation. At concentration <5μM terikalant lengthened APD in a reverse frequency-dependent manner both in papillary muscle and Purkinje fibers. At concentrations higher than 5 μM the APD prolongation was not clearly rate dependent. In right ventricular papillary muscle terikalant concentrations equal or higher than 5 μM depressed the maximal upstroke velocity of the action potential (Vmax) in a frequency dependent manner (at CL=300-5000 ms) The onset kinetics of the terikalant induced Vmax block was rapid (2.5 beat-1) like Class I/B, and the offset (recovery) kinetics of terikalant induced Vmax block was intermediate (1377.4 ms) between Class I/A and Class I/B.
Terikalant, like other class III antiarrhythmic drugs, at concentration lower than 5 μM has reverse rate dependent effect on the repolarization but at higher concentration also blocks the inward sodium current (INa) in a use-dependent manner.

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P23-11

THE ANNEXIN II SUBUNIT P11 IS ASSOCIATED WITH THE K2P POTASSIUM CHANNEL TASK1

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Background potassium conductances control the resting potential and input resistance of cells, two key components of neuronal excitability. The channels responsible for these background currents form a new family of potassium channels. Open at rest, these channels (K2P channels) lack both voltage and time depedencies. K2P channels are insensitive to the classical K^+ blockers, and are regulated by physical and chemical stimuli including pressure, temperature, lipids, pH, and neurotransmitters. Among these channels, TASK1 possesses a C-terminus PDZ consensus binding site (SSV). In heterologous expression systems, wild type (wt) TASK1 is active whereas the deleted SSV mutant (TASK1*SSV) is inactive. In order to identify proteins associated with the TASK1 PDZ motif, we used the yeast two hybrid system to screen a heart cDNA library with the C-terminus of TASK1 as bait. We found that p11, the light chain of annexin II, interacts with TASK1. This interaction, fully reproduced in vitro using GST Pull-down as well as immunoprecipitation techniques, is specific and requires the integrity of TASK1 PDZ motif. By immunocytochemistry we showed that TASK1 wt is located at the plasma membrane in transiently transfected COS cells, whereas TASK1*SSV is not. Successive deletion of TASK1 C-terminus allowed us to identify a retention motif, immediately upstream the PDZ motif, corresponding to the KRR sequence. We propose that during synthesis, partially folded or misfolded TASK1 subunits are retained in the ER through the binding of ER resident proteins to their retention motif. Correctly folded TASK1 channels expose their PDZ motif which interacts with p11, masking the retention motif and allowing the forward transport of the channel to the plasma membrane. Since TASK1 contributes to the resting membrane potential of various neuronal cells, genetic defects disrupting the SSV motif of TASK1 could induce drastic changes in neuronal excitability, leading to pathological states.

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P23-12

SIGMA LIGANDS INHIBIT TUMOR CELL PROLIFERATION THROUGH K^+ CHANNEL MODULATION AND P27 ACCUMULATION

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Recent works have indicated that sigma-1 receptor modulates K^+ channels. In addition, recent observations suggest that sigma receptors modulate cell proliferation. However, the mechanism by which the sigma-1 receptor inhibit cell proliferation remains enigmatic. In the present study, patch-clamp and western blot assays were used in NCI-H209 (a Small Cell Lung Cancer line) and Jurkat cells (a Leucemic T cell line) to investigate the effects of sigma ligands on K^+ channels and cell proliferation. The sigma ligands (+)-pentazocine ((+)Ptz), igmesine (Ig) and ditolylguanidine (DTG) all reversibly inhibited voltage-activated K^+ current in both cell lines. The potency of sigma ligands induced-inhibition (10 μM) was Ig (61.7%) = (+)Ptz (57.8%) >> DTG (27.3%), which is in good agreement with the pharmalogical properties of the sigma-1 receptor. Addition of either TEA, a K^+ channels blocker, or one of those sigma ligands inhibited proliferative growth of Jurkat as determined by cell counts over 4 days. Furthermore, TEA and sigma ligands caused an accumulation of the cyclin dependent kinase inhibitor p27kip1 but not p21cip1 and a decrease in cyclin A expression in both cell types. This result is consistent with a cell cycle arrest in G1 phase. Several reports suggest that K^+ channels are involved in cell proliferation through volume regulation. Interestingly, we found that sigma ligands induced a strong delay in volume regulation when Jurkat cells were challenged with an hypotonic stress. Taken together, these results suggest that the sigma-1 receptor induced modulation of K^+ channels may play an important role in cell proliferation arrest in G1 phase through inhibition of cell volume regulation and accumulation of p27kip1.
This work was supported by the Association pour la Recherche sur le Cancer.

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P23-13

CHARACTERIZATION OF THE M-CURRENT IN RAT SENSORY NEURONS

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Neuronal hyperexcitability is a feature of epilepsy and both inflammatory and neuropathic pain. M-currents (IK(M)) play a key role in regulating neuronal excitability and mutations in neuronal KCNQ2/3 subunits, the molecular correlates of IK(M), have previously been linked to benign familial neonatal epilepsy. In this study, using whole-cell perforated-patch recording, we sought the presence of IK(M) in cultured dorsal root ganglion (DRG) neurons from 17 day old rats. In 30 small cells tested (capacitance: 20.4±1.1pF), of which 16/22 were sensitive to capsaicin, IK(M) was the dominant subthreshold sustained current. IK(M) was also present in the majority (9) of large cells (capacitance: >100pF, n=10) tested, but in contrast to small cells, was masked by large dendrotoxin-sensitive (100nM) and Cs^+-sensitive (1mM) currents. As in superior cervical ganglion (SCG) neurons, IK(M) in small DRG neurons activated at ~-60mV and deactivated slowly (tfast=76.4±9.9ms, tslow=583±134ms, n=9). IK(M) was inhibited by the M-channel blocker linopirdine (IC50: 2.1±0.2μM; n=8), its analogue XE991 (IC50: 0.26±0.01μM; n = 6) and Ba^{2+} (IC50: 0.3±0.04mM; n=4). Sensitivity to TEA ranged from low to intermediate (IC50: 0.2-4.7mM; n=7) indicating a variable expression of TEA-sensitive and -insensitive KCNQ subunits, which was confirmed by immunocytochemistry. As expected, retigabine (10μM) enhanced IK(M) in a voltage-dependent manner (EC50s: 0.18±0.02 μM and 1.19±0.07 μM at –20 and –50mV respectively, n=7). Furthermore, linopirdine (10μM) and retigabine (10μM) reduced and increased the threshold of firing, respectively. RT-PCR confirmed the presence of all four neuronal KCNQ subunits in whole DRG, though KCNQ4 was absent at the single cell level. Thus, in small DRG neurons, native M-channels are composed mostly of either homomeric KCNQ2 or heteromeric KCNQ2/3 subunits.

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P23-14

SUBCELLULAR LOCALIZATION OF THE DELAYED RECTIFIER POTASSIUM CHANNELS KCNQ1 AND ERG1 IN THE RAT HEART

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Several potassium channels are responsible for the repolarization of the cardiac action potential, these include transient outward and delayed rectifier potassium currents. In the present study the cellular and subcellular localization of the two delayed rectifier potassium channels KCNQ1 and ERG1 was investigated in the rat heart. Confocal immunofluorescence microscopy of atrial and ventricular cells revealed that both KCNQ1 and ERG1-immunoreactivity was confined to the peripheral sarcolemma and to a structure transversing the myocytes. Immunoelectron microscopy of ventricular myocytes showed that the ERG1 channel was selectively expressed in the transverse tubular system and its entrance while KCNQ1 was detected in both the periheral sarcolemma and in the T-tubules. Thus, while ERG1 displays a very restricted subcellular localization pattern, KCNQ1 is more widely distributed within the cardiac cells. The localization of these potassium channels to the transverse tubular system close to the Ca^{2+} channels renders them with maximal repolarizing effect.

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P23-15

COMPARISON OF AJMALINE- AND PROPAFENONE-INDUCED BLOCK OF ITO IN RAT VENTRICULAR MYOCYTES

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The effects of ajmaline on the transient outward current (Ito) was studied and compared with those of other class I antiarrhythmic drug propafenone in experiments on rat ventricular myocytes. In our previous work we have shown that the ajmaline-induced block of Ito is frequency-independent in the range of 0.33 - 3.3 Hz. In contrast, propafenone showed significant increase of Ito-block with increasing frequency in the same range. The present study was aimed to explain the observed differences. In experiments on isolated rat ventricular myocytes, Ito was recorded in response to imposed standard depolarizing pulses preceded by variable preconditioning using the whole-cell patch-clamp technique. The reconstructed variations of the degree of block induced by both drugs during a depolarizing pulse increased at first and then decreased in parallel with inactivation. The recovery from the rest of propafenone-induced block after repolarization proceeded slowly (half time around 40 ms). This slow process that is likely to account for the observed frequency dependence of a steady state block was not significant in the case of ajmaline. Consequently, the repeated depolarizations did not induce cumulative block within the explored frequency range. However, both drugs revealed a cumulative block in the range of 20 - 50 Hz if trains of short (10 ms) depolarizing pulses were applied after a 10 s period of rest. The present results show that in the presence of both drugs the inhibition of Ito is mediated mainly through interaction with open channel (Kd = 3.55 mM for propafenone and 3.7 mM for ajmaline). Furthermore, they are consistent with the hypothesis that the apparent affinity of the drug to its receptor is low in the resting and the inactivated state. The differences in the values of rate constants related to recovery from the block of inactivated channels may account for the observed differences in frequency dependence of Ito-block induced by ajmaline and propafenone.

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P23-16

CEREBROVASCULAR DYSFUNCTION IN INSULIN RESISTANT ZUCKER OBESE RATS

Busija D., Erdos B., Miller A.

Our previous in vitro studies indicated that cerebrovascular dilatory responses are reduced in fructose fed insulin resistant (IR) rats. In order to further our understanding of the effect of IR on the cerebral circulation, we examined the vascular function of the basilar artery (BA) using a cranial window preparation in anesthetized Zucker obese (ZO) and lean (ZL) rats. The resting diameters of the BAs were similar in the two experimental groups. Acetylcholine induced endothelium-dependent, NO-mediated dilations in the BAs, but the responses were significantly reduced in the ZO rats. Dilations to acetylcholine were 17±3% (10-6 M) and 26±4% (10-5 M) in ZL and 6±1% and 12±2% in ZO rats (n=6, $p<0.01$). In contrast, relaxations to sodium nitroprusside were similar in the two experimental groups. Iloprost, a prostacyclin analogue, induced BKCa-channel mediated relaxations in the BAs, however the responses in ZO rats [3±1% (10-7 M) and 10±1% (10-6 M)] were significantly lower compared to the ZL rats [6±1% and 17±1% (n=6, $p<0.05$)]. Similarly, relaxations to the KATP-channel opener cromakalim were impaired in ZO rats [5±1% (10-6 M) and 28±4% (10-5 M)] compared to ZL rats [17±3% and 43±3% (n=6, $p<0.05$)]. These findings demonstrate that both endothelium-dependent and smooth muscle K-channel-mediated cerebrovascular responses are severely impaired in IR. Supported by: NIH grants HL-30260, HL-46558, HL-50587 (DWB) and HL-66074, HL-65380 (AWM).

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P23-17

EFFECT OF THE ANTI-CUNVULSANT DRUG, RETIGABINE, ON SINGLE KCNQ2/3 CHANNEL ACTIVITY IN CHO CELLS.

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Retigabine is a novel anticonvulsant compound that is now in clinical phase II development. We have previously shown that it enhances whole cell currents generated by KCNQ2/3 K^+ channels when expressed in Chinese hamster ovary (CHO) cells, as well as currents through native M channels in rat sympathetic neurones [thought to be generated by KCNQ2/3 subunits]. In the present experiments, we have examined the effect of retigabine on the properties of single heteromeric KCNQ2/3 channels expressed in CHO cells, to see if the increase in macroscopic current amplitude caused by retigabine can be attributed to an increase in the number of functional channels, single channel conductance and/or open probability. KCNQ2/3 potassium channel subunits were co-expressed in CHO cells and currents through single channels recorded using cell-attached patches. Channels had a similar slope conductance in the presence (8.04 + 0.02 pS) and absence (7.6 + 0.01 pS) of 10 μM retigabine. The mean maximal open probability Po for single KCNQ2/3 channels was 0.13 + 0.02, with a half-maximal Po potential (Vo) of -28.7 + 1.4 mV for control recordings. Retigabine increased mean maximal Po to 0.38 + 0.04 and produced a hyperpolarising shift of (Vo) to -40.1 + 3.4 mV. Single KCNQ2/3 channels have multiple voltage-dependent kinetic components in their activity (CL-OS-CM-OL-CS), giving short,

medium and long closed times (tauCS, tauCM, tauCL) and short and long open times (tauOS and tauOL). In the presence of retigabine at 0 mV the combined duration and contributions of the longest closed time tauCL decreased ten-fold, while the short and long open times increased four-fold and two-fold respectively. Thus, the results suggest that retigabine enhances macroscopic KCNQ2/3 currents by increasing the channel open probability and modifying steady-state kinetics to favour the open channel configuration. Supported by the U.K.MRC, the Wellcome Trust and the EU FP5.

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P23-18

REGULATION OF CALCIUM-ACTIVATED POTASSIUM CHANNELS BY EXTRACELLULAR ATP
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Extracellular ATP is an important regulator of transepithelial transport in a number of tissues. In pancreatic ducts, results from our laboratory have shown that ATP modulates epithelial K channels via purinergic receptors, most likely of the P2Y2 and P2Y4 types, but so far the identity of the involved K channels is not clear. The aim of the present study was to find which K channels are expressed in pancreatic ducts, and to study regulation of individual K channel types via specific P2Y receptors in an expression system.
RT-PCR experiments showed that Ca-activated K channels of intermediate conductance (IK-channels) and big conductance (BK channels) are abundantly expressed in pancreatic ducts. Possible interactions between the purinergic receptors and the different type of K channels were examined in co-expression experiments in Xenopus laevis oocytes. cDNA for P2Y receptors and K channels were cloned into pXOOM vector. Channel activity was measured electrophysiologically in oocytes stimulated with ATP or UTP (0.1 – 1 mM, pH 7.4). BK channels seemed to be differentially regulated by P2Y2 and P2Y4 receptors. UTP or ATP stimulation of oocytes expressing BK channels and P2Y4 receptors resulted in a 30% increase in the current through the expressed channels (n=6), whereas the BK channels co-expressed with P2Y2 receptors were inhibited by 18-25% during exposure to nucleotides (n=10 for ATP, n=8 for UTP). In contrast, co-expression of IK channels with P2Y4 receptors resulted in a large activation of the current when stimulated by UTP (from 100 to 2000 nA, n=9). This study indicates that there is a differential interaction between the purinergic receptors and different types of Ca-activated K channels.

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P23-19

QT PROLONGING EFFECTS OF DOFETILIDE, A HERG POTASSIUM CHANNEL BLOCKER IN VARIOUS EXPERIMENTAL MODELS
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Investigation of the potential QT prolonging effects of new drug candidates is necessary in the early phases of drug research. For this purpose we applied isolated rabbit heart, anaesthetised rabbit and conscious dog models. In the isolated rabbit heart changes in left ventricular pressure (LVP), coronary flow (CF), heart rate (HR) and surface ECG were recorded, and corrected QT (QTc) values were determined. In the anaesthetised rabbit, mean arterial blood pressure (MABP), HR, and QTc derived from standard bipolar ECG were measured. In the conscious dog, HR and QTc were derived from the ECG. In order to validate these models, the effects of dofetilide, a class III antiarrhythmic drug, were investigated.
In the isolated heart LVP and CF were not affected by intracoronary perfusion with dofetilide (10-100 nmol/l), HR dose-dependently decreased from the basal HR (mean BHR: 104 bpm) (to 59 %) whereas QTc dose-dependently increased (to 162 % upon application of 100 nmol/l dofetilide). When the concentration was increased up to 1 µmol/l, the shape of the ECG curve was significantly distorted.
In the anaesthetised rabbit, MABP and HR (BHR: 326 bpm) slightly decreased upon i.v. infusion of increasing cumulative dosing of dofetilide. There was only a slight dose-dependent increase in QTc (to 115 % at 300 µg/kg, i.v.).
In the conscious dog, following oral administration of 0.2 mg/kg dofetilide the HR (BHR: 99 bpm) slightly and transiently decreased (to 90 %). The maximal QTc prolongation (to 116 % of control) was observed at 2 hours post-dose.
In summary, the applied methods are suitable to detect QT prolonging effects. However, in the in vivo models, the degree of QT prolongation that could be achieved with dofetilide was much less pronounced than in the isolated rabbit heart with relatively low heart rates.

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P23-20

KIR6.1 SUBUNIT OF K(ATP) CHANNEL IS PRESENT IN VENTRICULAR CARDIOMYOCYTES OF RABBIT BUT NOT OF RAT
Christe G., Garnier-Raveaud S., Cand F., Usson Y., Verdetti J.

It is widely accepted that the pore of ATP-sensitive K^+ (KATP) channels of cardiomyocytes is a tetramer of the Kir6.2 subunit. Here we report on the immunolocalization of Kir6.1 and Kir6.2 KATP channel subunits in ventricular cardiomyocytes. We used antibodies raised to short peptides with specific sequence to each of these subunits (Kir6.2, courtesy of Dr. Grigory Krapivinsky; Kir6.1, courtesy of Dr. Idelson, Alomone Labs, Israel). We verified the selectivity of these antibodies to subunits expressed in Xenopus Oocytes with SUR2A (Courtesy of Dr. Michel VIVAUDOU, CEA Grenoble, France). Ventricular cardiomyocytes were isolated from rabbit and rat hearts, fixed and permeabilized. Primary Abs were applied overnight at 4°C, Alexa 488 labelled secondary antibodies (Molecular Probes) were used to detect primary Abs under confocal microscopy. Kir6.2 was present in both species with a high level of fluorescence in T-tubules, where it colocalized with a-actinin. Kir6.1 labeling was detected in rabbit ventricular myocytes principally at the level of T-tubules, but with lower fluorescence intensity than Kir6.2 labeling. It was undetectable when anti-Kir6.1 antibody was saturated with its specific immunogenic peptide. In rat ventricular myocytes, no Kir6.1 labeling was found. These results point out to possible interspecific differences in Kir6.1 expression. It is still debated whether Kir6.1-Kir6.2 chimeric channels may form in cardiac cells (Seharaseyon et al. J. Biol. Chem. 2000, 275:17561-65; Babenko and Bryan J. Biol. Chem. 2001, 276:49083-92). This issue is important, as such chimeric channels might more readily respond to metabolic depression than canonical Kir6.2 tetrameric channels (Babenko and Bryan 2001). The present finding of both subunits in rabbit ventricular myocytes at the T-tubule membrane suggests more detailed examination of this possibility in native KATP as a possible regulatory mechanism of the metabolic sensing and protection in cardiomyocytes.

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P23-21

LARGE CONDUCTANCE K CURRENTS IN HUMAN CORNEAL EPITHELIAL CELLS.
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Purpose: The large conductance K channel in the corneal epithelium plays critical roles in regulating cell volume and the resting membrane potential. Recently we reported that this K current in the bovine corneal epithelium was also activated by some fatty acids including arachidonic acid. In this study on the human corneal epithelial cells, pharmacological properties of K currents were investigated to identify the channel type.
Methods: Human corneal epithelial cells (HCEC, Kurabo, Japan) were cultured and isolated at the end of passage 4 or 5. The cells were perfused by HEPES-buffered Ringer solutions. Whole-cell currents were recorded using the perforated patch configuration of the patch clamp technique.
Results: The zero potential of HCEC averaged -12 mV (n=78). HCEC expressed a noisy, non-inactivating outward current. No voltage-gated K current was detected. External application of flufenamic acid (0.1 mM, n=18) augmented an outwardly rectifying, noisy current with a reversal potential near the equilibrium potential of K (-80 mV), indicating high selectivity for potassium. Similar K current augmentation was induced by arachidonic acid (0.02 mM, n=10) and by palmitoleic acid (0.1 mM, n=8). The enhanced K current was blocked almost completely by subsequent application of 0.1 mM diltiazem. Gadolinium (0.5 mM) enhanced transiently (< 2 min) the fenamate-induced current augmentation.
Conclusions: The human corneal epithelium exhibited the large conductance K current. The transient current augmentation by gadolinium indicates its dual modulation of the K channel.

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P23-22

ALLOSTERIC INTERACTION BETWEEN NICORANDIL AND NUCLEOTIDES ON SULFONYLUREA RECEPTORS 2A AND 2B
Yamada M., Kurachi Y.

To understand how nicorandil allosterically interacts with nucleotides on sulfonylurea receptors (SUR) 2A and 2B, we analyzed with the patch clamp method the effect of nicorandil on ATP-sensitive K^+ channels formed from Kir6.2 and either SUR2A and SUR2B. SUR2A and SUR2B are respectively cardiac and vascular SUR and differ only in the C-terminal 42 amino acids (C42). An invariant lysine in the Walker A motif in either or both of the nucleotide-binding domain (NBD) 1 and 2 was substituted with alanine (K708A and K1349A, respectively). In the presence of MgATP (1mM), nicorandil activated SUR2A/Kir6.2 and SUR2B/Kir6.2 channels with EC50 of 1.6 mM and 10 μM, respectively. In the presence of MgATP (1mM) and MgADP (100μM), nicorandil activated SUR2A/Kir6.2 channels with EC50 of 80 μM. All these responses were abolished by the K708A or K708A / K1349A mutation. The K1349A mutation did not change the EC50 of nicorandil for SUR2A/Kir6.2 channels in the presence of MgATP alone but increased the EC50 to 2.0 mM in the presence of MgATP and MgADP. This mutation increased the EC50 of nicorandil for SUR2B/Kir6.2 channels to 300μM in the presence of MgATP alone. Therefore, (A) The NBD1-ATP interaction is essential for the effect of nicorandil on both the channels. (B) The effect of nicorandil on SUR2A/Kir6.2 channels is mainly supported by NBD1-ATP interaction alone in the presence of MgATP but also by NBD2-ADP interaction in the presence of MgATP and MgADP, accounting for the more potent effect of nicorandil in the presence than absence of MgADP. (C) The effect of nicorandil on SUR2B/Kir6.2 channels is supported by both NBD1-ATP and NBD2-ATP interactions in the presence of MgATP. Furthermore, the NBD1-ATP interaction more strongly supports the effect of nicorandil on SUR2B than SUR2A. Thus, nicorandil much more potently activates SUR2B/Kir6.2 than SUR2A/Kir6.2 channels. (D) The C42 likely modulates allosteric interaction between nicorandil-binding sites and NBDs on SUR2A and SUR2B.

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P23-23

PHYSIOLOGICAL SIGNIFICANCE IN ALLOSTERIC MODULATION OF RGS PROTEINS BY PIP3 AND CALMODULIN
Ishii M., Kurachi Y.

Regulators of G-protein-signalling (RGS) proteins are a family of proteins, which accelerate intrinsic GTP-hydrolysis on a subunits of trimeric G-proteins and play crucial roles in the physiological regulation of G-protein mediated cell signaling. If RGS proteins were active unrestrictedly, it would completely suppress various G protein-mediated signallings as has been seen in the over-expression experiments of RGS proteins. Therefore, it is quite important to understand how the actions of RGS proteins are regulated in various physiological conditions. The modulatory mechanisms of RGS-action per se have, however, been poorly clarified. We recently showed in cardiac myocytes a physiological mode of action of a RGS protein (Circ Res 2001; PNAS 2002). The voltage-dependent formation of Ca^{2+}/calmodulin (CaM) facilitated the GTPase-activity of RGS protein via removing intrinsic inhibition mediated by a kind of phospholipid, phosphatidylinositol-3,4,5,-trisphosphate (PIP3). This modulation of RGS-action underlies the "relaxation" behavior of G-protein-gated K^+ (KG) channels in native cardiac myocytes. In order to elucidate their molecular mechanisms, we have performed further examination using co-sedimentation assay, which enables us to perform quantitative analyses on protein-lipid interaction. In results, we detected the specific interaction between RGS4 and PIP3 (but not other PIPs), which was abolished by Ca^{2+}/CaM. Interestingly, the allosteric modulation is exclusively performed within RGS domain, which is also responsible for GTPase-accelerating activity. We identified the clusters of positively charged residues (K99, K100) in helix 4 of RGS domain as a candidate of the switch of PIP3/CaM-modulation. Because the residues are conserved in almost all RGS protein subtypes, the allosteric modulation of RGS proteins should be important in the physiological regulation of G-protein signalling by various RGS proteins in different cell types.

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P23-24

BIOCHEMICAL EVIDENCE FOR THE INTERACTION OF THE HSK3 CHANNEL WITH A PROTEIN OF THE ENDOPHILIN FAMILY
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Small Ca^{2+} activated K^+ channels (SK) generate the slow afterhyperpolarisation (sAHP) following an action potential. The human SK3 channels (hSK3) are widely expressed, in liver, heart, skeletal and smooth muscle, and in the brain. For understanding the physiological role of hSK3 it is important to know modulating factors, protein targeting mechanisms, and localization. To find putative interaction partners we performed a screening for interacting proteins with a LexA-based yeast two-hybrid system. Recently we could show the interaction of the first 299 amino acids of hSK3 with SH3GL3, a member of the endophilin family, while other SH3 domain containing proteins like Abl, Lck, Fyn, Grb2, PI3K P85 did not interact.
To further confirm the interaction in vitro we performed a pulldown assay whereby the coding region of the N-tail of hSK3 was cloned into a prokaryotic expression vector fused to glutathione-S-transferase (GST) and the SH3GL3 gene into a prokaryotic expression vector fused to a His-tag. Both proteins were expressed in E. coli. Expression of both fusion proteins was confirmed by Western blotting. For the pulldown assay the ProFound Pull-down PolyHis Protein:Protein Interacion Kit was used following the manufacturers? instructions. Analysis of the experiment was done by Western blotting with antibodies against GST and SH3GL3. Our data indicate an interaction between hSK3 N-tail and SH3GL3 and hence confirm our previous results found with the yeast two-hybrid system. As SH3GL3 and hSK3 are both expressed in brain nerve terminals the interaction might be possible under physiological conditions and be relevant for channel function modulation or targeting of hSK3.
Supported by grants from the DFG (Gr848/8-2), the BMBF (iZKF Ulm, B7), and the Graduate College 460 "Diagnostic and Therapeutic Concepts in Molecular Medicin?.

Dept. of Applied Physiology, University Ulm, Germany

S24 PULMONARY CIRCULATION : FROM CELL TO INTEGRATIVE PHYSIOLOGY

ORAL SESSION

S24-1

CALCIUM OSCILLATIONS IN PULMONARY SMOOTH MUSCLE CELLS

Marthan R.

Using microspectrofluorimetry to measure the $[Ca^{2+}]i$ in single cells, studies from our laboratory and others have revealed that agonists controlling smooth muscle tone via activation of membrane receptors, such as angiotensin II, endothelin-1, noradrenaline, serotonin induce a complex temporal $[Ca^{2+}]i$ response in pulmonary artery smooth muscle cells (PASMCs). The agonist-induced $[Ca^{2+}]i$ response is composed of a series of cyclic increases in the $[Ca^{2+}]i$, so-called Ca^{2+} oscillations. The average percentage of oscillating cells is 50 to 80 % under identical experimental conditions. The concentration of agonist is the main factor that modulates the pattern of Ca^{2+} oscillations. In contrast to mediators acting at cell surface receptor, caffeine and ryanodine, known to act directly on the sarcoplamic reticulum (SR) always induce a transient or monotonic increase of $[Ca^{2+}]i$ that is never followed by oscillations. The amplitude of this transient $[Ca^{2+}]i$ -response is dependent on the concentration of caffeine. Therfore, agonist-induced Ca^{2+} oscillations appear to be underlain by a cytosolic Ca^{2+} oscillator. Both acute and chronic hypoxia (CH) alter resting $[Ca^{2+}]i$ value and Ca^{2+} transients in PASMCs. The molecular mechanisms of such alterations are not fully understood. We have investigated the effect of CH chronic on calcium signaling in PASMCs. In myocytes from CH rats, the $[Ca^{2+}]i$ response to agonists is altered with a disappearance of Ca oscillations and a decrease in the percentage of responding cells. However, both the amount of mobilized calcium and the sources of calcium implicated in the agonist-induced response are not changed. In conclusion, alterations in pulmonary vascular reactivity are related to hypoxia-mediated effects on various pathways implicated in PASMC calcium homeostasis. Further studies are required to identify the common mechanism(s) leading to these various effects in order to define new molecular therapeutic targets.

Inserm & Université Bordeaux 2, Bordeaux, France

S24-2

NON VOLTAGE-DEPENDENT CALCIUM INFLUX IN AN INTEGRATED MODEL OF PULMONARY MICROVESSELS

Guibert C., Marthan R., Savineau J.-P.

5-hydroxytryptamine (5-HT) is a potent pulmonary vasoconstrictor and contributes to hypoxic pulmonary vasoconstriction and pulmonary arterial hypertension. Small resistance pulmonary arteries are very important for blood flow regulation and very sensitive to hypoxia in the pulmonary vascular bed. Thus, we further investigated the mechanisms involved in the calcium signal to 5-HT in rat small intrapulmonary artery (IPA).
Effects of 5-HT were examined in isolated IPA (external diameter < 250 µm) from rat. Digital imaging with fura-PE3 was used to record intracellular calcium concentration and to follow external diameter of the vessels. Nerves and endothelium were detected by labeling the vessels with anti-neurofilament and anti-endothelial-nitric oxide synthase antibodies respectively.
Tetrodotoxin 0.5 µM and L-NAME 300 µM did not affect the concentration-dependent calcium increase induced by 5-HT. 5-HT 10 µM induced a sustained calcium variation which was sensitive to the inhibitor of the 5-HT2A receptors, ketanserin 0.1 µM, and insensitive to voltage-dependent L-type calcium channel blockers (nitrendipine and nicardipine 1 µM) or voltage-independent calcium channels antagonists (LOE 908, SKF 96365 and gadolinium 10 µM). The calcium response to 5-HT was also not modified by a sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor (cyclopiazonic acid, CPA, 10 µM) which depletes intracellular calcium store. CPA alone activated a capacitative calcium channel which was sensitive to LOE 908 and insensitive to SKF 96365 and gadolinium. The sustained calcium signal to 5-HT was partly blocked by inhibitors of arachidonic acid production (RHC 80267 50 µM and isotetrandrine 10 µM) and mimicked by application of exogenous arachidonic acid.
These results suggest that 5-HT-induced sustained calcium increase in small IPA is partly due to activation of a non-capacitative arachidonic acid sensitive receptor-operated calcium channel.

Laboratoire de Physiologie Cellulaire Respiratoire - INSERM E0356 - Université Bordeaux 2 - Bordeaux - France

OC24-1

SILDENAFIL PREVENTS CHANGE IN RHOA EXPRESSION INDUCED BY CHRONIC HYPOXIA IN RAT PULMONARY ARTERY

Sauzeau V., Rolli-Derkinderen M., Loirand G., Pacaud P.

Exposure to chronic hypoxia (CH) induces a sustained pulmonary hypertension associated with structural and functional changes in the pulmonary arterial bed, including alterations of contractile properties. The small G-protein RhoA and its effector Rho kinase constitute major components of the sustained rise in tension induced by vasoconstrictors. The aim of this study is to analyze the effect of CH on RhoA/Rho kinase signalling pathway in the rat pulmonary artery (PA).
CH (10% O_2, 2 weeks) induces a decrease in NO production/disponibility in rat PA, remodelling of PA and right ventricular hypertrophy. Maximal contraction of PA rings to endothelin-1, noradrenaline and the thromboxan A2 analogue U46619 is decreased by 27.7±2.8% (n=4), 45.2±7.0% (n=8) and 73.8±5.4% (n=4) in CH rats respectively. Contraction measurement in permeabilized strips of PA demonstrates that the CH-induced decrease in the response to agonists is due to the abolition of RhoA-mediated Ca^{2+} sensitization of the contraction. Real-time RT-PCR and western blot analysis reveal that CH induces decrease in RhoA mRNA (79.4±6.0%, n=4) and RhoA expression (81.1±8.0%, n=4) in the main pulmonary artery. Treatment of rats with the type 5 phosphodiesterase inhibitor, sildenafil (25 mg/kg/d) throughout 2 weeks of exposure to CH prevents CH-induced down-regulation of RhoA, reduction of contraction and PA remodelling.
These findings indicate that CH induced-down-regulation of RhoA expression, leading to the abolition of RhoA/Rho kinase-mediated Ca^{2+} sensitization of the contraction, is responsible for the decreased responses to contracting agonists in pulmonary artery of CH rats. These alterations are prevented by sildenafil, indicating a major role of NO/cGMP pathway in the CH-induced alteration of RhoA signalling in pulmonary artery.

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OC24-2

REGULATION OF T LYMPHOCYTE SURVIVAL BY RESIDENT CELLS IN ASTHMA

Darveau M.-E., Rouabhia M., Pagé N., Chakir J.

Background : Asthma is a chronic inflammatory disease where bronchial mucosa is infiltrated by activated T cell. Understanding the mechanisms that govern cell-apoptosis and factors involved may help to explain different aspects of this disease. Signals from tissue microenvironments and resident cells may be a potential mechanism involved in the unrelenting and survival of activated T cells. Objective: This study was designed to investigate the effect of resident cells on survival of T lymphocytes. Methods: We used a well designed engineered human bronchial mucosa (EHBM). Different tissues were produced : an EHBM containing both epithelial cells and fibroblasts, an EHBM with only fibroblasts included in the collagen gel, an EHBM with epithelial cells seeded onto the collagen gel without fibroblasts and T cells cultured in the gel. We performed immunofluorescence to determine the expression of CD45Ro, Bcl-2 and Bax. We also performed Tunel techniques to evaluate apoptosis of T cell cultured with EHBM. Results: We obtained a significant decrease of apoptotic T cells, when they were cocultured with fibroblasts alone (20% ± 6 compared to 32% ± 8), epithelial cells alone (15% ± 6 compared to 32% ± 8) or the two type of resident cells (20% ± 7 compared to 32% ± 8) obtained from asthmatics. Fibroblasts and epithelial cells from normal subjects showed no protective effect. The decrease of apoptosis in asthmatic cells was associated with a significant down-regulation (p =0.001) in Bax expression by T cells CD3+ when they were cultured with EHBM from asthmatics compared to T cells cultured in collagen gel. Conclusion: These results support the fact that bronchial resident cells can play a role in the amplification of inflammation in asthma by increasing the survival of T lymphocytes
(supported by CIHR and Canadian Asthma Society).

Centre de recherche de l'Hôpital Laval, Université Laval – Québec – Canada

OC24-3

MODULATION OF HUMAN PULMONARY VASCULAR TONE BY GUANOSINE 3'-5' CYCLIC MONOPHOSPHATE

Perrotin C., Pham-Minh H., Regnard J.F., Dall'Ava-Santucci J., Dinh-Xuan A.T.

Background. Guanosine 3'-5' cyclic monophosphate (cGMP) is a nucleotide second messenger that plays a key role in the mechanisms leading to vasodilatation in response to endothelium-derived nitric oxide (NO). It is possible to increase intracellular concentration of cGMP through stimulation of soluble guanylyl cyclase (sGC) by NO and its donors. Alternatively, it is also possible to keep a high level of intracellular cGMP through inhibition of type 5 isozyme of phosphodiesterase (PDE-5). The aim of our study was to assess the role of cGMP in the modulation of human pulmonary vascular tone and the effects of substances activating sGS and/or inhibiting PDE-5 in human pulmonary vascular smooth muscle.
Methods. Fourth generation pulmonary arteries (PA) were dissected from lungs obtained from 15 patients (3 women and 12 men, age range: 47-79 yrs) undergoing lobectomy for lung carcinoma. Pulmonary vascular rings were mounted in organ chambers and isometric tension was measured in responses to acetylcholine and various agonists stimulating sGC and antagonists inhibiting PDE-5.
Results. Sodium nitroprusside, a NO donor, induced a dose-dependent and maximally relaxed all PA vascular rings, an effect which was significantly greater than that observed with the endothelium-dependent vasodilator, acetylcholine. Relaxation of PA vascular rings was weaker in response to YC-1, a specific activator of sGS, as compared with that observed with zaprinast, a specific inhibitor of PDE-5.
Conclusion. Results from this study are consistent with a critical role of cGMP in the modulation of pulmonary vascular tone. Circumstantial evidence also suggests that sGS and PDE-5, two key enzymes that control cGMP intracellular concentration, are differentially regulated in human pulmonary vascular smooth muscle. Activation of the former and/or inhibition of the latter might be of therapeutical interest in human pulmonary vascular disease.

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OC24-4

REN-2 TRANSGENIC RATS HAVE REDUCED CHRONIC HYPOXIC PULMONARY HYPERTENSION
Hampl V., Bíbová J., Herget J., Charles X.

Although there are indications that the rennin-angiotensin axis may participate in the regulation of the pulmonary circulation, its exact role is insufficiently characterized. We used transgenic rats harboring mouse Ren-2 renin gene to characterize its involvement in pulmonary vascular regulation under normal conditions and during chronic hypoxic pulmonary hypertension. Since homozygous rats rapidly develop heart failure, heterozygote (+/-) males were used in this study and compared to wild-type controls (-/-).
First, pulmonary artery pressure (PAP) was measured by catheterization in closed-chest, thiopental-anesthetized rats aged 50 and 80 days. Cardiac output (CO) was estimated as the ascending aorta blood flow by transonic flowmeter. While the systemic blood pressure (measured in anesthesia by carotid artery cannulation) was elevated in +/- rats at 50 days (139±4 mmHg) and 80 days (151±3 mmHg) compared to -/- rats (109±3 and 111±5 mmHg), PAP did not differ between the +/- and -/- rats at 50 days (17±1 vs. 16±1 mmHg) and at 80 days (19±2 vs. 18±1 mmHg). The groups also did not differ in CO at either age. In the next phase of the experiment, rats were exposed to hypoxia (10% O_2) for 2 weeks until they were 80 days old. As expected, chronic hypoxia elicited pulmonary hypertension in -/- rats: their PAP was 30±2 mmHg (compared to 18±1 mmHg in normoxic -/- controls; $P>0.0001$). In +/- rats, chronic hypoxia also elevated PAP (25±2 mmHg), but significantly less than in -/- rats ($P>0.05$). These differences were not due to differences in CO.
We conclude that chronic hypoxic pulmonary hypertension is milder in the Ren-2 transgenic heterozygotes, while their PAP in normoxia does not differ from -/- controls. Thus, the rennin gene is involved in the development of chronic hypoxic pulmonary hypertension. The Ren-2 transgenic rats are a useful model for further study of this phenomenon.

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S24-3

ROLE OF SEROTONIN AND ITS TRANSPORTER IN PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION
Adnot S., Eddahibi S.

Recent years have witnessed the identification of biological processes pivotal to the complex vascular changes associated with various forms of pulmonary hypertenion (PH). We recently reported that serotonin (5-hydroxytryptamine, 5-HT) and its transporter (5-HTT) play a critical role in the pulmonary arterial smooth muscle (PA-SMCs) hyperplasia and vascular remodeling associated with experimental hypoxic PH and human primary PH. Serotonin has a potent mitogenic effect on PA-SMCs and this effect is mediated by 5-HTT, not by serotonin receptors. In the experimental model of hypoxic pulmonary hypertension, 5-HTT expression is increased as a result of a direct effect of hypoxia on transcription of the gene. Mice that are deficient in 5-HTT are protected against the development of hypoxic pulmonary hypertension, whereas mice with 5-HTT overexpression develop more severe pulmonary hypertension that do wild-type mice. 5-HTT expression is increased in PA-SMCs and platelets from patients with PH as compared to controls. This 5-HTT overexpression in SMCs from patients persists when the cells are cultured. It is related in part to a functional polymorphism located on the 5-HTT gene promoter: thus, homozygosity for the (L) allele, the long gene promoter variant associated with a high level of gene transcription, is found in 65-75% of patients with primary PH as compared to only 25-30% of controls. It is likely that 5-HTT gene polymorphism, in combination with other factors, may play a crucial role in various forms of pulmonary hypertension. Recent data obtained in patients with advanced hypoxemic lung disease are consistent with this hypothesis.

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S24-4

COUPLING BETWEEN THE PULMONARY CIRCULATION AND THE RIGHT VENTRICLE
Naeije R.

Pulmonary hypertension is usually evaluated by mean pulmonary artery pressure, left atrial pressure, cardiac output measurements, and derived pulmonary vascular resistance calculations. While this approach has shown to be useful to define the resistive properties of the pulmonary vascular bed, it is insufficient for the quantification of right ventricular afterload. Ventricular function is optimally defined by a pressure-volume loop, but this approach has been limited until now by the technical difficulties of the continuous measurement of RV volume. We measured in intact anesthetized dogs instantaneous RV pressure and pulmonary arterial flow, and calculated an isovolumic beat maximal RV pressure (Pmax) from non-linear extrapolations of the isovolumic portions of the instantaneous RV pressure curve. Predicted Pmax was closely correlated to maximum RV pressure measurements obtained by direct pulmonary arterial clamping. End-systolic elastance (Ees) of the RV was calculated by drawing a straight line from the Pmax-end diastolic volume point tangential to the systolic portion of the RV pressure-volume curve. Effective pulmonary arterial elastance (Ea) was calculated by drawing a straight line from the Ees point to the end-diastolic volume point. Thus, we were able to obtain a load-independent measurement of RV contractility and an integrated estimate of the forces that oppose RV ejection, and most importantly, the coupling of both. Dobutamine increased and propranolol decreased Ees and the Ees/Ea ratio. We applied this approach to a model of congenital cardiac shunt-associated pulmonary arterial hypertension induced by three months systemic-to-pulmonary shunting in growing piglets. In this model, both RV Ees and pulmonary Ea were increased, with unaltered coupling. Chronic oral bosentan therapy, or acute iv prostacyclin, or inhaled NO had no intrinsic effect on RV contractility.

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S24-5

USING TRANSGENIC MICE TO UNDERSTAND MECHANISMS OF PULMONARY HYPERTENSION
Rodman D.M., Fagan K., West J.

Purpose: Utilize genetically engineered mice to understand mechanisms underlying pulmonary vascular disease.
Methods: Hemodynamic phenotyping of transgenic mice was performed using two techniques: 1) Measurement of PA systolic pressure via trans-thoracic right ventricular puncture and 2) isolated perfused mouse lung. Morphometrics were also performed on fixed tissue. The following transgenic mice constructed by others were used: nitric oxide synthase null mice, pre-pro-ET1 transgenic mice, prostacycline synthase transgenic mice. The following transgenic mice were constructed by us: dominant-negative

bone morphogenetic protein receptor-II (BMPRII) transgenics. The latter utilized a tissue-specific conditional approach using double transgenic mice.
Findings: 1) The role of endothelial vasodilator control of pulmonary vascular tone and structure was investigated in NOS null and PGIS transgenic mice. NOS3 was identified as the principle isoform modulating pulmonary vascular tone in the lung, although extrapulmonary NO production, likely from upper airway NOS2, contributed as well. Pulmonary specific overexpression of PGIS markedly reduced the development of hypoxic pulmonary hypertension in PGIS transgenic mice, suggesting that endogenous PGIS activity is rate limiting. 2) The role of ET-1 in the pathogenesis of pulmonary vascular disease was investigated in ET1 transgenic mice. A remarkable lymphocyte-predominant fibrotic vasculitis was seen. 3) dnBMPRII mice were constructed to determine the mechanisms through which loss of BMPRII results in familial pulmonary hypertension. Preliminary phenotyping of these mice will be presented.
Conclusions: Transgenic mice have proven to be a flexible and useful tool in dissecting he mechanisms underlying the pathogenesis of pulmonary vascular disease. Utilizing newer technology we have constructed conditional tissue-specific dnBMPRII transgenic mice. Phenotyping is in progress.

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POSTER SESSION

P24-01

HEART RATE AND MUSCLE PHOSPHATE METABOLISM PROVOKED BY BREATH-HOLDING AND SIMULATED DIVING IN MAN

Rasmussen T.C., Paulev P.-E., Quistorff B.

Face immersion in cold water is a strong stimulus for diving bradycardia and a widely used technique for simulated diving. The object of the present investigation was to measure the heart rate reduction and the phosphate metabolism in the antebrachial flexor muscles during breath-holding and during simulated diving in cold water, during a standardized handgrip manoeuvre.
The investigation was carried out on 7 healthy male volunteers including one of the authors. Handgrip manoeuvres were performed with the left arm in the nuclear magnetic resonance spectrometer and quantified using a plast tensiometer. The heart rate was counted using a plast stethoscope.
We found a relative bradycardia during breath holding at room air (up to 25%). During apnoea with the face immersed in cold water the heart rate was reduced up to 50%.
Evolutionary hypoxia adaptation implies that anaerobic pathways are favoured that maximize the generation of ATP per mol of oxygen. We found no sign of such an adaptation in the human forearm muscles neither during breath holding nor during face immersion in cold water. Oxygen sensitive tissues such as the brain and the myocardium may react differently.

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P24-02

MODULATION OF PULMONARY VASCULAR TONE BY THE SPECIFIC PHOSPHODIESTERASE TYPE 5 INHIBITOR SILDENAFIL.

Savineau J.P., Pauvert O., Lugnier C., Keravis Th., Marthan R., Rousseau E.

Cyclic nucleotides (cAMP and cGMP) are involved in the control of pulmonary vascular tone. Cyclic nucleotide level is controlled by specific phosphodiesterases (PDE) which are the only enzymes hydrolysing cyclic nucleotides. Recently, drugs acting on specific cGMP PDE (type 5) have been shown to have a potent pulmonary relaxant effect. In the present work we have investigated the presence of PDE5 isozyme and the effect of sildenafil (viagra), a potent PDE5 inhibitor, on the specific cyclic nucleotide phosphodiesterase (PDE) activity, smooth muscle tone and calcium signaling in the rat main pulmonary artery (MPA).The PDE activity was measured in cytosolic and microsomal fractions. Total cAMP and cGMP-PDE activities were mainly present in the cytosolic fraction. Sildenafil (0.1 μM) reduced by 72% cGMP-PDE activity whereas zaprinast (10 μM), a relatively selective PDE5 inhibitor, reduced this activity by 63%. Western blot analysis revealed the expression of PDE5 mainly in the cytosolic fraction of MPA. Sildenafil concentration-dependently inhibited (IC50 = 3.4 nM) the activity of MPA PDE5 partially purified by HPLC. Sildenafil (0.1 nM-50 μM) concentration-dependently relaxed MPA rings pre-contracted with phenylephrine (0.5 μM). The potency of sildenafil (IC50 = 11 nM) was similar to that of a nitric oxide donor, sodium nitroprusside, but higher than that of zaprinast (IC50 = 600 nM). In isolated MPA myocytes, which had been loaded with the calcium fluorophore indo-1, sildenafil (10-100 nM) antagonized ATP- and endothelin-1- induced calcium oscillations but had no effect on the transient caffeine-induced $[Ca^{2+}]$i-response. This study demonstrates the presence of a functional and highly sildenafil-sensitive PDE5 isozyme in rat MPA. Inhibition of this isozyme mainly accounts for the potent pulmonary vasodilator action of sildenafil which involves alteration in the inositol triphosphate-mediated calcium signaling pathway.

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P24-03

PULMONARY HEMODYNAMICS DURING AN INTERMITTENT EXERCISE TEST IN COPD PATIENTS

Lonsodrfer-Wolf E., Bougault V., Richard R., Oswald-Mammosser M.

The purpose of our study was to evaluate the behavior of the pulmonary arterial pressure (PAP) during a 30 minute intermittent exercise test (IET) in patients with chronic pulmonary obstructive disease (COPD).
Methods: Seven COPD patients, (six males, one women, 58 ± 5 years), with a FEV1 of 47 ± 11% of predicted, and seven healthy volunteers (38 ± 5 years) underwent a maximal exercise test (MET) in the morning and an IET in the afternoon of a same day, with a catheter inserted in the pulmonary artery. The 30 minute IET consisted of six stages alternating four minutes of work set on the first ventilatory threshold determined during the MET, with one minute work set on 90% of the maximal tolerated power of the MET, which corresponded in our series, to the second ventilatory threshold for the healthy subjects. Ventilatory parameters, mean pulmonary artery pressure (PAP), cardiac output (CO) using the Fick's method, heart rate (HR) were measured, and stroke volume (SV) was calculated. Results: PAP increased during the first minutes of the IET (without reaching the maximal pressure measured at the end of the IET), with a further subsequent significant decrease until the end of the test. CO increased and then remained stable, HR increased regularly reaching the maximal value of the MET at the end of the 30 minute IET. After an initial increase, SV decreased significantly throughout the IET. Total pulmonary vascular resistance (TPVR) decreased in COPD patients, but less than in healthy subjects. Conclusion: i) Despite bouts of hard work, there is no dramatic increase in PAP during the IET in our patients. ii) When comparing the behavior of PAP during the IET in COPD to that observed in younger normal subjects, there was no difference in the slope of decrease of PAP, although PAP was consistently higher in COPD than in normal subjects. iii) TPVR decreased in COPD patients but to a lesser extent than in healthy but also younger subjects.

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P24-04

SURFACTANT LUNG LAVAGE AND HIGH-FREQUENCY JET VENTILATION (HFJV) IN EXPERIMENTAL MECONIUM ASPIRATION

Sevecova D., Calkovska A., Drgova A., Javorka M., Javorka K.

Background: Combined therapeutic approach is needed to overcome respiratory failure due to meconium aspiration syndrome (MAS) in newborns. Effects of surfactant lung lavage and HFJV on gas exchange, dynamic lung-thorax compliance (Cdyn), right-to-left pulmonary shunts (RLS) and removal of meconium were evaluated in a rabbit model of MAS. Methods: Suspension of human meconium (25 mg/ml, 4 ml/kg) was instilled into the tracheal cannula of conventionally ventilated (CV, f. 30/min) adult rabbits. When respiratory failure developed, lung lavage (10 ml/kg in 3 portions) was performed either with diluted surfactant (Curosurf, 100 mg phospholipids/kg) or saline during CV or asymmetric HFJV (f.300/min, inspiration time Ti 70%). After the lavage, animals were further ventilated for 1 hour either with CV or HFJV (Ti 50%). Blood gases and lung function parameters were evaluated before and after meconium administration and 10, 30 and 60 min after the lavage. Removal of meconium was estimated spectrophotometrically and by mecocrit method. One-way ANOVA with LSD post-hoc test was used for between-group comparisons, considered $p<0.05$ for statistically significant. Results: Improvement in oxygenation index after the lavage was observed in both surfactant groups ($p<0.05$), although was slightly pronounced in conventionally ventilated animals of Surf-CV group. Higher elimination of CO_2 after the lavage was found in Surf-HFJV group vs. all remaining groups ($p<0.05$). Cdyn increased and RLS decreased after the surfactant lavage in both surfactant groups compared to saline controls ($p<0.05$). Removal of meconium was higher in surfactant groups than in controls ($p<0.05$) and also higher in Surf-HFJV vs. Surf-CV ($p<0.05$). Conclusion: Surfactant lung lavage with HFJV improved gas exchange, increased lung compliance and reduced pulmonary shunting by the similar manner as surfactant lavage with CV, and in addition, increased the removal of meconium.

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P24-05

ACCURACY OF 2-KM WALK TEST PREDICTED VO_2MAX IN ACTIVE SENIORS

Rance M., Lazaar N., Boussuge P-Y., Bedu M., Van Praagh E., Duché P.

Direct measurement of maximal oxygen consumption (VO_2max) requires maximal effort of the subject and substantial laboratory readiness. In large, middle-aged and elderly populations, this direct time consuming method is inappropriate. The 2-km Walk Test is a simple test, which offers VO2max prediction equations for working age population. Purpose: To explore the accuracy of VO_2max prediction equations of the 2-km Walk Test in active seniors aged 60-76. Methods: Twenty-seven active women and men (67±4 yr) participated in the study. VO_2peak (l/min) was measured during cycle ergometry by direct gas analysis from a maximal test (step: 30 Watts, time: 2min30). VO_2peak related to body mass (BM) was then calculated (ml/min/kgBM). Subjects completed also the 2-km Walk Test (UKK INSTITUTE). VO_2max (l/min; ml/min/kgBM) was then predicted from age, sex, Body Mass Index, heart rate and walking time measured during the 2-km Walk Test. Relationships between predicted VO_2max and measured VO_2peak values were observed calculating correlation coefficients. Measured values were compared with 2-km Walk Test prediction equations via mean difference analyses, and bias was explored using Bland-Altman analyses. Results: Predicted VO_2max and measured VO_2peak were high correlated ($r>0.72$, $p<0.001$). Predicted VO_2max (1.33±0.5 l/min; 22±6.8 ml/min/kgBM) was not significantly different from measured VO_2peak (1.37±0.49 l/min; 20.19±4.8 ml/min/kgBM). By using Bland-Altman plots, predicted VO_2max showed no significant bias. Conclusion: The 2-km Walk Test offers an accurate VO_2max prediction in an active senior population though a pretty important bias could be observed in few subjects. This test can be used to evaluate VO_2max in a population presented these characteristics.

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P24-06

INHALED NITRIC OXIDE IMPROVES HEMODYNAMIC CHANGES FOLLOWING ACUTE MASSIVE CO_2 PLUS 10% N_2O PULMONARY

Mobacher K., Diemunsch P., Doutreleau S., Lupescu R., Schaefer A., Plobner P., Mettauer B., Piquard F., Geny B.

Background : Nitrous oxide (N_2O), used during laparoscopic anesthesia, accumulates in CO_2 pneumoperitoneum (10% of N_2O is often observed) and gazeous embolism is a rare but life-threatening complication.
Objectives : To determine the pulmonary deleterious effects of an acute CO_2 + 10% N_2O embolism in experimental pigs and to investigate the potential beneficial effect on inhaled NO in such setting.
Methods : Seven anesthetized, ventilated and instrumented pigs underwent three randomly designed gazeous pulmonary embolisms (2cc/Kg during 1 minute) through the jugular vein without or with either inhaled NO at 7 and 22 ppm.
Results : CO_2 + 10% N_2O increased heart rate (from 82.4±8.4 to 92.3±9.4 bpm, P = 0.02), mean pulmonary artery pressure (from 16.9±09 to 29.9±2.3 mm Hg, P<0.002), systemic vascular resistances (from 1.25± 0.12 to 1.60±0.12 dynes.cm-5.s, P=0.04), pulmonary vascular resistances (from 0.36± 0.03 to 0.78±0.15 dynes.cm-5.s, P=0.02) and central venous pressure (from 2.6±0.6 to 3.0±0.7 mm Hg, P<0.005). It decreased mean arterial pressure (from 57.5±3.4 to 50.8±2.3 mm Hg, P=0.02), end CO_2 expiratory pressure (from 36.0±1.8 to 23.7±2.0 mm Hg, P<0.01) and cardiac output (from 3.5±0.5 to 3.2±0.4 L.min-1, P=0.02). NO inhalation attenuated pulmonary hypertension by 34±8 %, P<0.05, and $PetCO_2$ decrease was reduced by 31± 7%, P<0.05.
Conclusion : Thus, inhaled NO, a selective pulmonary vasodilatator, protects against CO_2 + 10% N_2O gazeous-induced pulmonary hypertension. The dose of 7 ppm might therefore be of therapeutic value during surgical laparoscopy. There is no need to increase the dose of inhaled NO to 22 ppm.

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P24-07

EFFECT OF PHOSPHODIESTERASE INHIBITION ON PULMONARY HYPERTENSION IN CHRONIC HYPOXIC RATS

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Pulmonary hypertension (PH) is characterized by vasoconstriction and vascular remodelling. In smooth muscle cells, cGMP is a vasodilating messenger. Nitric oxide (NO) stimulates the guanylyl cyclase (GC) to produce cGMP, which is hydrolysed by the phosphodiesterase 5 (PDE5). This study was designed to investigate the effect of the NO-donor, molsidomine, and the PDE5 inhibitor, sildenafil, in pulmonary arteries and as treatments for PH in chronic hypoxic rats. Rat pulmonary arteries were

dissected and mounted in wire myographs. Concentration-response curves (CRCs) for sildenafil and the active metabolite of molsidomine, SIN-1, were performed in endothelium-intact arteries in the absence and presence of an inhibitor of soluble GC, ODQ (3 uM). The rats were divided in 5 groups: A normoxic and a hypoxic control group, and three hypoxic groups receiving either sildenafil, molsidomine, or the combination. After two weeks, systemic blood pressure, right ventricular pressure (RVP), and right ventricle to left ventricle plus septum ratio (RV/LV+S) were measured. Sildenafil evoked maximal relaxations of 40±7% (n=10) in isolated endothelium-intact arteries. Sildenafil relaxation was inhibited by endothelial cell removal, and abolished in the presence of ODQ. SIN-1 caused relaxations of 81±4% (n=6), and ODQ shifted the curve to the right. In vivo, RVP and (RV/LV+S) were increased in hypoxic rats. In all three treated hypoxic groups, pulmonary pressure was reduced, and molsidomine and the combination treatment attenuated (RV/LV+S). Systemic blood pressure was not affected by any treatment or hypoxia. In conclusion, sildenafil and molsidomine relax pulmonary arteries by GC-dependent mechanisms and attenuate the development of PH in chronic hypoxic rats, but there is no synergistic effect of the two drugs.

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P24-08

COMPARATIVE ASPECTS OF IN VITRO REACTIVITY OF RAT AND HUMAN RESPIRATORY SMOOTH MUSCLE.
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Human bronchial fragments were removed from 6 patients undergoing resection for pulmonary carcinoma. We compared the reactivity of preparations, with and without epithelium (denudation was performed with CHAPS, 10mg/ml for 10 minutes). Tracheal preparations were obtained from 12 Sprague Dowley rats, 6 controls and 6 ovalbumin-sensitized animals in order to induce asthmatic-like hypereactivity. Preparations were introduced in isolated organ bath filled Krebs-Henseleit solution, kept at 37°C, bubbled with a 95%O_2 and 5%CO_2. Contractions and relaxations were recorded usinf a force transducer (FORT 10, WPI Inc.), connected to a computerized data aquisition unit (BIOPAC, AQKNOWLEDGE software).
For human preparations, acetylcholine-induced contractions ranged between 0.185±0.04g at 10-5M, and 0.75±0.03g at 10-3M. Similar effects were registered for metacholine, but carbachol produced a more pronounced effect ($p<0.01$). In denuded preparations, maximal contraction to acetylcholine was significantly increased ($p<0.05$).
In healthy rats, the amplitude of acetylcholine-induced contractions was 0.22±0.05g for a 10-7M concentration, and 1.1±0.02g for 10-3M. Metacholine induced similar effects, while carbachol presented a marked contractile ($p<0.05$). In ovalbumin-sensitized animals, statistically significant differences were noticed between acetylcholine-, metacholine, and carbachol-induced responses.
These results suggest that cholinergic response is similar in the studied species, with differences regarding the amplitude of the contractile response, probably species-related. Epithelium removal could be considered as a model of bronchial hyperreactivity, comparable with sensitization performed in animal models.

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P24-09

AIRWAY RESPONSIVITY IN OVALBUMIN SENSITIZED RATS
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The objectives of the our study were:
1) to standardize a sensitization method to ovalbumin
2) to study the contractile response of normal and sensitized rat tracheal smooth muscle, before and after administration of glucocorticoids or synthetic antileukotrienes.

The experiments were performed on 4 groups of Sprague-Dowley adult rats: group I: control; group II: ovalbumin-sensitized rats; group III: ovalbumin-sensitized rats treated with Montelukast, a synthetic leukotriene receptor antagonist; group IV: ovalbumin-sensitized rats treated with glucocorticoids. Tracheal spirals were introduced in organ chambers filled with Krebs-Henseleit solution, bubbled with 95% O_2 and 5% CO_2, at 37°C. Isometric force was measured using a force transducer and the results were recorded using a computerized data acquisition unit.
In both controls and sensitized rats, muscarinic agonists (10-7 - 10-4M) induced contractions, the relative magnitude of response being: carbachol>metacholine>acetylcholine. In OVA-sensitized rats, reduced contractile reactions were noticed, but the basal tone was significantly higher. In all animals, Pirenzepin administration induced a prominent relaxation of rat precontracted TSM, but its action was more pronounced in sensitized rats (100%). In both controls and sensitized animals, ipratropium bromide induced an augmented relaxation compared to the one induced by Pirenzepin, and shifted the dose-response curve to acetylcholine to the right. In sensitized animals treated with either Montelukast or glucocorticoids, the relaxing response to pirenzepin was significantly higher.
Our research revealed the beneficial effect of synthetic antileukotrienes and glucocorticoids, which restored the normal basal tone and the contractile reserve of TSM, improving the effects of associated bronchodilator agents.

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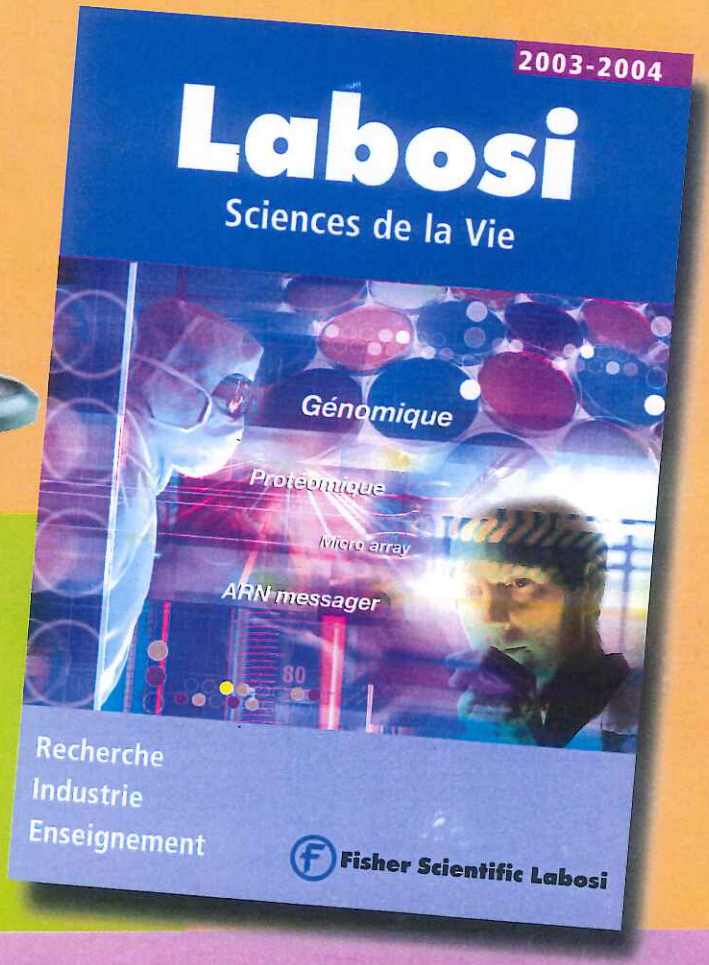

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