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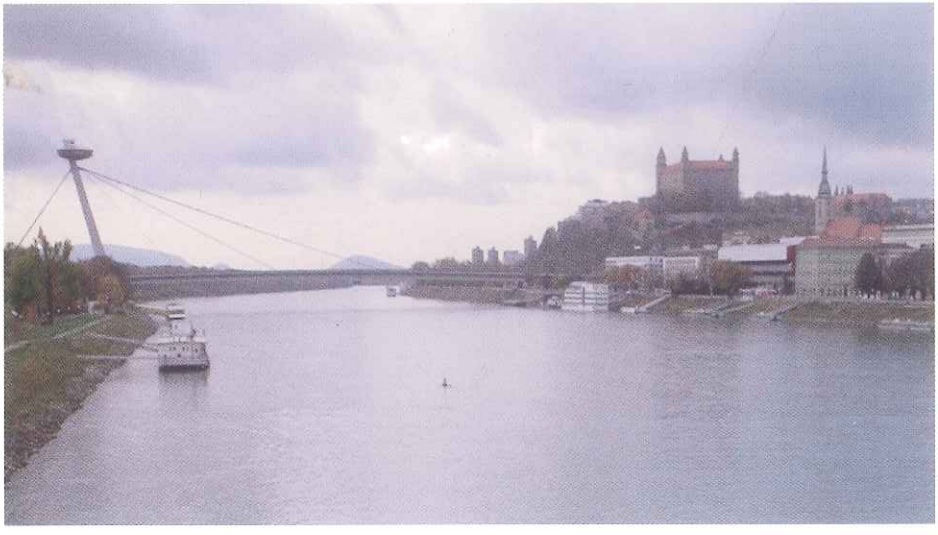

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Editorial Office
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Fax +46 8 304 273
Sue Chin Lwee, Editorial Secretary
(email aph@ki.se)

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Abstracts

Joint Meeting of The Slovak Physiological Society, The Physiological Society and The Federation of European Physiological Societies

September 11th – 14th, 2007, Bratislava, Slovakia

Edited by
V. Strbak, Bratislava
D. Jezova, Bratislava
T. Ravingerova, Bratislava

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SW01-1

Dynamic modulation of Ca^{2+} current by Ca^{2+} release in cardiac hypertrophy

Antoons G.[1,3], Volders P.G.A.[2], Stankovicova T.[1,4], Bito V.[1], Stengl M.[2,3,5], Vos M.A.[2,3], Sipido K.R.[1]
[1]Laboratory of Experimental Cardiology, University of Leuven, Belgium; [2]Department of Cardiology, Academic Hospital Maastricht, the Netherlands; [3]Department of Medical Physiology, University Medical Center, Utrecht, the Netherlands; [4]Department of Pharmacology, Comenius University, Bratislava, Slovakia; [5]Department of Physiology, Charles University, Medical School Plzen, Czech Republic; Karin.Sipido@med.kuleuven.be

In compensated cardiac hypertrophy the amplitude of Ca^{2+} transients is maintained or even larger than in control conditions. This is also observed in the dog with chronic atrioventricular (cAVB) block for 6 weeks. We studied the feedback of Ca^{2+}release from the sarcoplasmic reticulum on Ca^{2+} current inactivation and recovery, in particular during low plateau potentials in isolated LV midmyocardial myocytes from cAVB dogs, compared to weight-matched controls (CTRL). In the presence of beta-adrenergic stimulation with isoproterenol, we observed large window Ca^{2+} currents. These window currents were of equal magnitude in both groups, but the maximal current occurred at more positive potentials in cAVB. Window currents were accompanied by significant Ca^{2+} influx. Using a specific double clamp voltage protocol, we could quantify the availability of Ca^{2+} channels during and following Ca^{2+} release from the sarcoplasmic reticulum at steady membrane potential. Ca^{2+}release from the sarcoplasmic reticulum induced a pronounced degree of inactivation of the Ca^{2+} current followed by a significant recovery. This dynamic modulation was more pronounced in cAVB and could contribute to the higher incidence of early afterdepolarizations in cAVB. This is further discussed as part of the arrhythmic mechanisms in cardiac hypertrophy and contrasted to what is seen in heart failure.

SW01-2

The ryanodine receptor connection: from normal to cardiac arrhythmias

Fauconnier J., Lacampagne A., Bideaux P, Richard S.
Research unit for Cardiovascular Physiopathology, INSERM U637, University Montpellier 1, University Montpellier 2, France
srichard@montp.inserm.fr

Aims: Heart failure (HF) is associated with decreased contraction and propensity for arrhythmias, both of which are related to altered Ca^{2+} handling. The purpose of our study was to test the hypothesis of a link between the cardiac ryanodine receptor (RyR2) and Ca^{2+} -dependent ionic currents, such as I_{CaL} and I_{K1}, potentially involved in arrhythmias. **Methods:** Effects of HF (vs. Sham) were assessed 8 weeks after left coronary artery ligation (PMI) in 250-280 g Wistar rats. Echocardiography confirmed that PMI rats had impaired systolic function. Whole-cell patch-clamp experiments were performed (22-24°C). Abrupt changes in pacing rate (from 0.1 Hz to 2 Hz) were used to assess propensity of single ventricular myocytes to trigger arrhythmias. $[Ca^{2+}]_i$ transients and Ca^{2+} sparks were recorded using fluorescent Ca^{2+} dye Fluo-4 AM. Ca^{2+} load in the sarcoplasmic reticulum (SR) was estimated using caffeine. **Results:** In all HF cells, Ito was decreased, and the AP was prolonged. However, we identified two subsets of cells with distinct Ca^{2+} handling features. One set of cells exhibited increased Ca^{2+} sparks occurrence, decreased $[Ca^{2+}]_i$ transients and decreased SR Ca^{2+} load. We determined that leaky RyR2 were associated with inhibition of I_{K1}, slow inactivation of I_{CaL}, longer APs, loss of adaptation of AP duration to high pacing rate and triggering of delayed afterdepolarizations (DADs). The other type of cells had normal (vs. Sham) RyR2 activity, Ca^{2+} handling, I_{CaL} and I_{K1}, but high pacing rate promoted dramatic prolongation of APs (due to enhanced facilitation of I_{CaL}) with occurrence of EADs. **Conclusion:** The physiological status of RyR2 determines occurrence of either EADs or DADs with key roles of, respectively, I_{CaL} and I_{K1}.

SW01-3

Role of CaMKII for ECC coupling and hypertrophy/heart failure

Maier L.S.
Dept. of Cardiology and Pneumology/Heart Center, Georg-August-University Gottingen, Germany
lmaier@med.uni-goettingen.de

Calcium (Ca^{2+}) is the central second messenger in the translation of electrical signals into mechanical activity of the heart. This highly coordinated process, termed excitation-contraction coupling or ECC, is based on Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR). In recent years it has become increasingly clear that several Ca^{2+}-dependent proteins contribute to the fine tuning of ECC. One of these is the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) of which CaMKII is the predominant cardiac isoform. During ECC CaMKII phosphorylates several Ca^{2+} handling proteins including SR Ca^{2+} release channels or ryanodine receptors (RyR), phospholamban (PLB), and L-type Ca^{2+} channels with multiple functional consequences. CaMKII may also be co-localized to distinct target proteins. In addition, novel data suggest that non- Ca^{2+} transporters such as sarcolemmal Na+ and K+ channels may be regulated by CaMKII and thus be sensitive to Ca^{2+} handling properties and also influence them via electrophysiological effects. CaMKII expression as well as activity are reported to be increased in heart failure and CaMKII overexpression can exert distinct and novel effects on ECC in the heart. In animal models it was found that CaMKII overexpression can induce myocyte hypertrophy and heart failure with altered intracellular Ca^{2+} handling and protein expression leading to reduced SR Ca^{2+} content, typical for heart failure.

SW01-4

Mechanisms of dysfunction in mutant ryanodine receptors in cardiac pathology

Lai F.A.
Wales Heart Research Institute, School of Medicine, Cardiff University, United Kingdom; lait@Cardiff.ac.uk

Aims: The ryanodine receptor-calcium release channel (RyR) is an integral membrane protein responsible for the calcium efflux that triggers numerous calcium-activated physiological processes, including muscle contraction, neurotransmitter release and hormone secretion. Recently, approximately 70 single residue mutations in the cardiac RyR2 were identified in families that exhibit catecholaminergic polymorphic ventricular tachycardia (CPVT), a condition in which physical or emotional stress can trigger severe tachyarrhythmias that can lead to sudden cardiac death. Our laboratory aims to characterise the dysfunction underlying the cardiac pathology due to these mutant RyR2s. **Methods:** The RyR2 mutations in CPVT are clustered in the N- and C-terminal domains, as well as in a central domain. We have cloned the full-length cDNA encoding human RyR2 and generated expression plasmids with various CPVT mutations across the RyR2 sequence to enable the heterologous expression and analysis of calcium release mediated by the wild type and mutated RyR2. **Results:** Our studies employing confocal microscopy, calcium fluorometry, FRET and biochemical analysis suggest that the mutational locus may be a significant factor in the mechanism underlying RyR2-mediated calcium channel dysfunction. **Conclusion:** Further understanding the causes of aberrant calcium release via RyR2 should assist in the development of effective treatments for the ventricular arrhythmias that often leads to sudden death in both heart failure and in CPVT (Thomas et al. Biochem Soc Trans 34, 913-918, 2006; George et al. J Mol Cell Cardiol 42, 34-50, 2007).

SW01-5

Regulation and dysregulation of the ryanodine receptor activity: insights from mathematical modelling

Zahradnikova A., Valent I., Cocherova E., Zahradnik I.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia; alexandra.zahradnikova@savba.sk

Aim: Increased frequency of diastolic Ca sparks supposedly contributes to the increased propensity to arrhythmias in the failing myocardium. Another type of RyR malfunction that occurs in stress-induced arrhythmias is related to mutated RyRs. Both pathological conditions transpire at the cellular level as diastolic calcium waves. Several molecular mechanisms leading to calcium wave formation have been implicated in RyR dysregulation; these include changes in the interaction of RyR with FKBP12.6, in intramolecular interactions within RyRs, in the inhibition of the channel by Mg^{2+} ions, or in the RyR activation by luminal Ca^{2+}. **Methods:** We have tested the effects of these regulatory mechanisms, as well as the effect of RyR refractoriness in a mathematical model of calcium spark generation that incorporated various models of RyR gating. **Results:** The apparent sensitivity of RyRs to cytosolic Ca^{2+}, and thus also diastolic spark frequency, was changed by all regulatory mechanisms in a similar way but with different sensitivity. Increased spark frequency alone was not sufficient for initiation and propagation of arrhythmogenic calcium waves. Calcium wave formation was strongly dependent on the amplitude and duration of calcium sparks and on the rate of RyR recovery from refractoriness. **Conclusions:** All proposed molecular mechanisms may underlie the increase of diastolic calcium spark frequency. Importantly, however, wave generation required increased amount of released calcium during a spark and/or faster recovery of RyRs, which may be achieved only upon adrenergic stimulation, i.e., when this condition occurs *in vivo*.

SW02-6

Organization of intracellular energy metabolism in muscle cells: structure-function relationships

Seppet E.
Department of Pathophysiology, University of Tartu, Tartu, Estonia
enn.seppet@ut.ee

Aims: This study addresses the importance of strict structural organization of the slow-twitch muscle's cell in determining the type of control over mitochondrial oxidative phosphorylation and the nature of intracellular energy transfer between mitochondria and ATPases. **Methods:** The function of mitochondria and their interaction with ATPases was compared between the adult rat cardiomyocytes and m. soleus cells (RMC), both expressing regular and stable mitochondrial arrangement and cultured cardiac HL-1 cells with irregular and dynamic mitochondrial network, after permeabilisation of the cells with saponin. The mitochondrial function was assessed by respirometry and the expression of adenylate kinase (AK) and creatine kinase (CK) was assessed by RT-PCR, western blotting and native electrophoresis. **Results:** Respirometry revealed a 20-30 times lower apparent Km for exogenous ADP in stimulating respiration in the HL-1 cells than in RMC that suggests less restricted ADP diffusion in HL-1 cells. While in RMC the endogenous ADP flux from ATPases was reduced after activation of oxidative phosphorylation (OxPhos) due to direct ADP channelling (DAC) to mitochondria, the DAC was absent in HL-1 cells. The expression of mitochondrial (mit) and cytosolic (cyt) isoforms of CK and AK was revealed in both types of cells, but with much lower mit-CK protein and activity in HL-1 cells than in RMC. The mit-CK and mit-AK was found to be coupled to OxPhos in RMC, whereas in HL-1 cells mit-AK exclusively was coupled to OxPhos. **Conclusion:** High level of structural organization of RMC gives rise to formation of complexes of ATPases and mitochondria, with CK- and AK-networks and DAC ensuring energy transfer and metabolic feedback within the complexes. In contrast, the HL-1 cells having less organized structure rely upon a simple diffusion of adenine nucleotides facilitated by AK-network for energy transfer.

SW02-7

Mitochondrial potassium channels: pharmacology and properties

Szewczyk A.
Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Poland
adam@nencki.gov.pl

Aims: The ATP-regulated potassium channel (mitoK_{ATP} channel) is present in the inner membrane of heart mitochondria. The purpose of the study was to analyze regulation of mitochondrial potassium channels by ATP, pH and pharmacological modulators. **Methods:** In this study a single channel activity was measured after reconstitution of the inner mitochondrial membrane from bovine myocardium into planar lipid bilayer. **Results:** We provide direct evidence of vectorial pH regulation of mitoK_{ATP} channels. Alkalization (from pH 7.2 to 8.2) of the matrix side changed the channel conductance, open probability, mean open and closed dwell time distribution. The effect was reversed by changing pH back to the neutral value. The mitoK_{ATP} channel activity was not changed by alkalization of the cytosolic side of the planar lipid bilayer. Additionally, we observed that acidification from pH 7.2 to 6.2 in either the matrix or cytosolic compartments decreases the open probability of the channel. This effect is reversed by perfusion with pH 7.2 medium. Additionally, our results suggest that the mitoK_{ATP} channel is regulated by multiple phosphorylation events. The mitoK_{ATP} channel "run-down" was reversed by incubation with ATP/ Mg^{2+} complex on both sides of the planar lipid bilayer. **Conclusion:** We conclude that pH and ATP play an important regulatory role for the cardiac mitoK_{ATP} channel with respect to ischaemia-reperfusion phenomena.

Supported by Polish Mitochondrial Network and grant from Ministry of Research and Higher Education N301 05331/1676

SW02-8

Mitochondrial dysfunction and ROS formation. Crucial roles in functional and structural derangements of the ischaemic heart

Di Lisa F.
University of Padova, Padova, Italy
dilisa@civ.bio.unipd.it

Abstract not submitted.

SW02-9

Mitochondria and nitric oxide in cell death and cardioprotection

Borutaite V.
Institute for Biomedical Research, Kaunas University of Medicine, Kaunas, Lithuania
vilbor@vector.kmu.lt

Nitric oxide (NO) and related reactive nitrogen species have several effects on mitochondria that may impact cell death and survival of cells in cardiovascular system. NO can cause rapid but reversible inhibition of mitochondrial respiration which may synergize with hypoxia to induce cell death. NO and reactive nitrogen species may cause production of reactive oxygen species which may have signalling role in the cells or may induce cell death. Reactive nitrogen species also activate mitochondrial permeability transition pore leading to apoptotic or necrotic cell death. Recently we and others have found that low concentrations of NO may activate signalling pathways involving activation of protein kinase G and leading to increased resistance of mitochondria to opening of permeability transition pore. This may have important cardioprotective effect in heart ischaemia/reperfusion. The mechanism of such protective effect will be discussed.

SW02-10

The mitochondrial permeability transition pore – from molecular mechanism to cardioprotection

Halestrap A.P.
Department of Biochemistry and The Bristol Heart Institute, University of Bristol, Bristol, United Kingdom
a.halestrap@bristol.ac.uk

The molecular composition of the mitochondrial permeability transition pore (MPTP) will be considered briefly and evidence presented for a central role for the phosphate carrier in addition to a facilitating role for cyclophilin D (CyP-D) and a regulatory role for the adenine nucleotide translocase (ANT). The importance of both calcium and especially oxidative stress in opening the MPTP will be stressed. It will be shown how these factors cause MPTP opening during reperfusion following a prolonged period of ischaemia leading to the irreversible necrotic damage known as reperfusion injury. Preventing this MPTP opening is cardioprotective and the mechanism by which known cardioprotective strategies achieve this will be discussed with a particular emphasis on ischaemic preconditioning. This involves exposure of hearts to two or three brief cycles of ischaemia and reperfusion before the prolonged ischaemia. We will show how this reduces the oxidative stress experienced by mitochondria at the end of ischaemia and during early reperfusion. This, in turn, decreases the sensitivity of MPTP opening to calcium and so leads to less MPTP opening and necrotic tissue damage. We find no evidence that any protein kinases translocate to the mitochondria to induce these effects, nor for changes in the phosphorylation of mitochondrial proteins exerted by endogenous protein kinases. Rather decreased oxidation of critical thiol residues on the ANT can provide an adequate explanation for the protection. Other preconditioning protocols such as temperature preconditioning and urocortin also act in this way.

SW03-11

Pulsatility as a key aspect of HPA regulation

Lightman S.L., Atkinson H., Conway-Campbell B., Droste S., Harbuz M., de Kloet R., Linthorst A., McKenna M., Reul J., Seale J., Wiles C., Wood S.
Henry Wellcome Laboratories for Integrative Neuroscience & Endocrinology, University of Bristol, Bristol, United Kingdom
Stafford.Lightman@bristol.ac.uk

Aims: To define the ultradian pattern of corticosterone secretion in the rat, how this may be altered in different physiological states and the relevance of these patterns to nuclear signalling in the hippocampus. **Methods:** Rats were cannulated and attached to our automated blood sampling system. Some rats had simultaneous intracranial microdialysis for free corticosterone in the hippocampus and other rats were adrenalectomised and corticosterone replaced by automated intravenous infusion. Corticosterone was measured by radioimmunoassay and glucocorticoid receptor (GR) and mineralocorticoid (MR) translocation in the hippocampus assessed by subcellular fractionation of nuclear and cytoplasmic fractions and Western blotting. **Results:** Rats have an approximate hourly ultradian pattern of corticosterone release, amplification of which results in the classic circadian rhythm. This pattern shows sexual dimorphism, changes with age and is responsive to inflammation and lactation. Pulses of corticosterone in the blood are paralleled by tissue levels of free corticosterone in the brain. Each pulse results in a rapid translocation of both MR and GR to the nucleus. The rate of clearance of MR and GR from the nucleus are however very different, with MR remaining at high levels for over sixty minutes (the interpulse interval) and nuclear GR falling rapidly within thirty minutes. This rapid depletion of GR was blocked by the ICV infusion of MG-132, a specific irreversible inhibitor of 26S proteasome. **Conclusions:** Proteasome dependent rapid turnover of activated nuclear GR provides a mechanism for tissues to respond to the different patterns of corticosterone pulsatility seen in different physiological and pathological states.

SW03-12

The role of Urocortin 2 in HPA axis activity and stress-related behaviours

Chen A.
Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel
alon.chen@weizmann.ac.il

Aims and Methods: Urocortin 2, a recently identified member of the corticotropin-releasing factor (CRF) family and a specific ligand for the type 2 CRF receptor, is expressed in discrete neuroendocrine and stress-related nuclei of the rodent CNS and by several peripheral tissues, with high expression levels in skeletal muscle. To determine the physiological role of Urocortin 2, mice null for Urocortin 2 were generated and HPA axis activity, stress-related behaviours and metabolic performances were examined. **Results and Conclusions:** Female, but not male, mice lacking Urocortin 2 exhibit a significant increase in the basal daily rhythms of ACTH and corticosterone and a significant decrease in fluid intake and depressive-like behaviour. The differential phenotype of Urocortin 2 deficiency in female and male mice may imply a role for Urocortin 2 in these gender differences.

SW03-13

HPA axis and gastroprotection

Filaretova L.
Laboratory of Experimental Endocrinology, Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia
filaretova@pavlov.infran.ru

Aims: For a long period it was generally accepted that glucocorticoids released during stress are ulcerogenic hormones. We designed some experimental studies to clarify the validity of the widely held view that the glucocorticoids released during activation of the HPA axis are harmful to the gastric mucosa. **Methods:** We examined the effect of glucocorticoid deficiency or the glucocorticoid receptor antagonist RU-38486 on stress-induced gastric erosion, parameters of general body homeostasis and local gastric function in rats. Glucocorticoid deficiency was created by different approaches and followed by corticosterone replacement. **Results:** The data obtained show that the reduction in the stress-induced corticosterone release, or its actions, aggravates stress-caused gastric erosion. It is suggested that an acute increase in corticosterone during stress protects the stomach against stress-induced injury. Accordingly our results various ulcerogenic stimuli (aspirin, indomethacin, ethanol, acetic acid), similar to stress, induce an increase in glucocorticoid production that helps the gastric mucosa to resist against a harmful action of the ulcerogenic stimuli. Glucocorticoids exhibit gastroprotective effect by both maintaining local defensive factors and inhibiting pathogenic elements. The contribution of glucocorticoids to gastroprotection is tightly related with their contribution to general body homeostasis. Glucocorticoids exert gastroprotective actions in co-operation with prostaglandins, nitric oxide and capsaicin-sensitive sensory neurons: their compensatory action is observed when the protective mechanism provided by either of these factors is impaired. **Conclusion:** The results suggest that glucocorticoids released during acute activation of the HPA axis are naturally occurring gastroprotective factors.

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SW03-14

HPA axis and mood disorders

Jezova D.
Institute of Experimental Endocrinology, Laboratory of Pharmacological Neuroendocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
daniela.jezova@savba.sk

Aims: A dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis has been reported in several psychiatric a neurological disorders. In depression, a dysbalance at all levels of the HPA axis has been reported. It is suggested that chronic exposure to stress situations and particularly the neurotoxic effects of glucocorticoids contribute to the development of mood disorders. This general belief neglects the already known variability and specificity of the stress response. **Methods:** We have studied several models of psychiatric disorders, such as chronic mild stress and spontaneous anhedonia as models of depression, and enriched environment (EE) as a model of increased neural plasticity. **Results:** The results show that a prolonged rise in plasma corticosterone may, but need not to be observed in the models of depression as well as in the EE inducing a positive physiological outcome. Main neurochemical changes observed in the brain were those in gene expression of CRH and ionotropic glutamate receptor subunits. Many intensive stress situations in humans, both under daily life conditions and in stress models, were not associated with increased cortisol release. Moreover, high trait anxiety in healthy subjects was accompanied by lower cortisol response during psychosocial stress. In anxious subjects, low cortisol responses during stress correlated with exaggerated perception of stress and worse mental performance. **Conclusions:** Our findings do not support the corticosteroid neurotoxicity hypothesis. More complex neuroendocrine changes have to be considered in evaluating the risk factors of the development of mood disorders.

The study was supported by grants of APVV LPP-0194-06 and VEGA 5064.

SW04-15

Uncoupling proteins – tissue specific functions?

Miroux B.
Faculte de Medecine Necker Enfants-Malades, Paris V Descartes, Paris, France
miroux@necker.fr

Abstract not submitted.

SW04-16

Differential roles for uncoupling proteins through the life cycle

Symonds M.E., Budge H., Gnanalingham M.G., Stephenson T.
Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham, NG7 2UH, United Kingdom
michael.symonds@nottingham.ac.uk

It is now established that the role and/or abundance of both UCP1 and 2 changes dramatically over the perinatal period and that the magnitude of this adaptation is dependent on maturity at birth. In sheep, for example, the brown adipose tissue specific UCP1 is lost soon after birth, whilst a similar transition is seen for UCP2 in the lung. In contrast, gene expression for UCP2 in adipose tissue peaks during the postnatal period coincident with the large increase in white adipose tissue deposition. The magnitude of these adaptations is substantially influenced by the prenatal environment and as such can be reprogrammed by changes in maternal dietary intake. Critically these responses persist into later life when they may not necessarily accompany increases in fat mass. At the same time UCP2 gene expression can be enhanced in a tissue specific manner that is dependent on the stage of embryonic or fetal development in which the mother is nutritionally manipulated. These adaptations are mediated in part by changes in tissue glucocorticoid action which similarly vary between tissues and stage of development. The talk will thus summarise our recent findings on the impact of prenatal maternal nutrient restriction and its long term influence on UCP abundance in individuals that are habituated to either a sedentary or active life style.

SW04-17

Dietary and developmental regulation of uncoupling proteins

Kopecky J.
Czech Academy of Sciences, Praha, Czech Republic
kopecky@biomed.cas.cz

Abstract not submitted.

SW04-18

Mitochondrial uncoupling proteins and 4-hydroxy-2-nonenal: a possible physiological role in protection against oxidative damage

Echtay K.S.
Department of Biomedical Sciences, Faculty of Medicine & Medical Sciences, University of Balamand, P.O Box 100 Tripoli, Lebanon
karim.echtay@balamand.edu.lb

Oxidative stress and mitochondrial oxidative damage have been implicated in the etiology of numerous common diseases such as degenerative diseases, diabetes, obesity and aging. Mitochondrial respiratory chain is a major source of superoxide and derived reactive oxygen species (ROS) in vivo. Oxidative stress results from overproduction of ROS, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes, particularly 4-hydroxy-2-nonenal (HNE). There is strong evidence that mild uncoupling of oxidative phosphorylation attenuates mitochondrial ROS production and protects against cellular damage. HNE induces mitochondrial uncoupling by specific and inhibitable interactions with the uncoupling proteins UCP1, UCP2 and UCP3, and with the adenine nucleotide translocase (ANT). These proteins are members of a large family of at least 35 anion carriers present in the mitochondrial inner membrane. This suggests a physiological role for HNE and/or other lipid peroxidation products in a negative feed-back loop mechanism which acts to prevent excessive mitochondrial ROS production and thus protects against the effects of oxidative stress.

SW04-19

Endocrine manipulation of uncoupling proteins

Mostyn A.[1], Clarke L.[2], Fowden A.L.[3], Symonds M.E.[1]
[1]Centre for Reproduction and Early Life, Institute of Clinical Research, University Hospital, Nottingham; [2]Faculty of Natural Sciences, Imperial College London, Wye, Kent; [3]Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom
alison.mostyn@nottingham.ac.uk

The endocrine system is a key regulator of uncoupling protein (UCP) abundance and function through the life cycle and is particularly important at birth. Cortisol, leptin and tri-iodothyronine (T_3), appear to differentially regulate UCPs in large and small mammals. The regulation of UCPs by these hormones during the transition from fetal to neonatal life is crucial for survival. Adrenalectomised fetal sheep have reduced UCP1 protein in brown adipose tissue (BAT); conversely cortisol infusion raises UCP1. The manipulation of fetal plasma cortisol also causes parallel changes in plasma T_3, both of which are positively related to UCP1 abundance. This suggests that an intact adrenal gland is essential for the up-regulation of UCP1 at birth, due to cortisol in combination with T_3. Postnatally, cortisol, leptin and T_3 decline rapidly, an adaptation followed by the loss of UCP1. Rodent studies demonstrate that leptin administration increases body temperature and UCP1 activity. Acute or chronic leptin administration to young sheep causes maintenance of body temperature and loss of UCP1 as well as tissue specific effects on UCP2 expression in the pancreas and lung. Acute leptin administration decreases UCP2, whereas chronic administration has the opposite effect in BAT. Chronic administration of T_3 or a β3 adrenoceptor (ZD) agonist to piglets demonstrates genotype specific responses in subcutaneous AT with T_3 reducing UCP3 and ZD increasing UCP2 with no effect on UCP3 thereby contrasting with rodent studies. The divergent responses of UCPs to endocrine mediators between species and ages suggest a varied role in promoting normal temperature regulation, metabolism and development.

SW05-20

The molecular bases of nicotine addiction

Maskos U.
Neurobiologie integrative des systemes cholinergiques, Institut Pasteur, Paris, France` umaskos@pasteur.fr

Understanding mechanisms underlying normal complex behaviours and the abnormalities that accompany most neuropathologies is a primary challenge in fundamental and biomedical research. Optimal use of the large body of genetic, molecular, electrophysiological, behavioural and imaging techniques that provide new insights into cellular organisation at the microscopic level, and functional circuits at the macroscopic level, is hampered by the usual dissociation of these techniques. Today, the crucial challenge lies in the integration of these approaches in order to target a unified scientific question at multiple levels. The subject of this presentation is the functional analysis of brain circuits with a multi-level approach, to understand how nicotine acts on the brain, affects cognition, and causes addiction. Our goal is, using a simple animal model, to explore the molecular mechanisms underlying addictive and cognitive behaviours in their relation to reward. Addiction to nicotine is typically an 'integrated' pathology including short-term receptor modification, and long term modification of circuit equilibrium and behaviours. Understanding such phenomena requires the development of new tools. Nicotine addiction presents a serious social and public health problem. Worldwide, 100 million people are expected to die this century from the consequences of nicotine addiction, yet nicotine is also known to enhance cognitive performance. Additionally, nicotinic acetylcholine receptors (nAChRs) are down-regulated in serious human psychiatric conditions like autism. Hence, the identification of the molecular mechanisms and circuits involved in nicotine reinforcement and cognition is urgent and requires the development of novel tools that allow genetic and molecular manipulation *in vivo*.

SW05-21

Acetylcholinesterase anchoring/genetics and cell biology of cholinergic targeting

Krejci E., Dobbertin A., Dembele K., Bernard V.
Biology of Neuromuscular junctions, Inserm U686, Universite Paris Descartes, Paris, France; eric.krejci@univ-paris5.fr

Aims: Acetylcholinesterase (AChE), the key the enzyme for the degradation of acetylcholine, is associated to the plasma membrane in the brain but also secreted in the extracellular space. The objective of this study was to evaluate the importance of the membrane anchor, PRiMA, in the localization of AChE. **Methods:** We generated a single PRiMA mutant by an insertion in the exon encoding the domain interaging with AChE. In addition double mutant was generated, in which, we used an allele of AChE in which the last exon encoding the domain interacting with PRiMA is deleted. In the striatum of simple or double mutants, we localized AChE with specific antibodies at light and electron microscopy. **Results:** In normal mouse, AChE is highly accumulated at the surface of the axon and of cell body and also in the secretory pathway. In PRiMA mutant, AChE is completely absent from the axon as expected. Surprisingly, AChE is only accumulated in the endoplasmic reticulum and completely absent in the Golgi apparatus and cell surface. In contrast, in the double mutant PRiMA/allele AChE, AChE is found both in the ER and the Golgi apparatus but absent from the cell surface. **Conclusion:** These results show that PRiMA is essential for AChE and has two functions: (1) targeting AChE to the axon and the cell surface and (2) maturation of AChE through the secretory pathway.

SW05-22

AChE knockout mice/receptor adaptation mechanisms in AChE-/- mice

Hrabovska A.[1], Farar V.[1], Brabec J.[2], Milotova M.[3], Duysen E.G.[4], Lockridge O.[4], Myslivecek J.[3]
[1]Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia; [2]Institute of Anatomy, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Institute of Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [4]Eppley Institute, University of Nebraska Medical Center, Omaha, NE, USA; anna.hrabovska@univ-paris5.fr

Aims: Viability of the mice with no acetylcholinesterase (AChE-/-) is achieved by many adaptation changes, among which down-regulation of muscarinic receptors (MR) in the brain and periphery was described. We hypothesize that in accordance to maintain neurotransmitter balance, the AChE-/- brain has decreased dopaminergic receptors (DR). **Methods:** (1) DR in the striatum of adult wild-type and AChE-/- mice were quantified by radioligand binding, using specific ligands for D1- and D2-like DR. (2) The results were confirmed by immunohistochemistry, using primary anti-D1A and anti-D2 antibodies. (3) Striatal sections were stained with DNA-specific dye. (4) cAMP contents were measured using an immunoassay kit. (5) PI-PLC activity was measured in striatal homogenates with radiolabeled PIP2 substrate. **Results:** KD and B_{max} values obtained in wild-type mouse for both D1-like and D2-like receptor ligands correlated with values published by other authors. The D1-like DR in AChE-/- striatum were reduced to 5% of normal. D2-like DR in the AChE-/- striatum were almost undetectable under our conditions. These results were confirmed by immunohistochemistry. DNA-specific staining in the striata did not differ in size, density and distribution between the two genotypes. PI-PLC activity did not differ in the mutant striata comparing to wt. **Conclusion:** Results suggest severe alteration of the dopamine pathway in AChE-/- striatum without changes in the neuronal density. This is accompanied by 40 to 64% reduction in MR. We conclude that the lower binding of the specific ligands in both systems must be caused by the decreased number of the receptors on the cell surface.

SW05-23

Conditional inactivation of the ChAT gene expression as a novel approach to investigate the physiology of cholinergic systems

Mallet J., Lecomte M-J., Santamaria J., Berrard S
LGN, CNRS, Batiment CERVI, Hopital de La Pitie-Salpetriere, Paris, France
mallet@chups.jussieu.fr

Aims: In the central nervous system, the cholinergic nuclei are localized mainly in the basal forebrain and brainstem, and in spinal cord motoneurons. Acetylcholine (ACh) is thus widely distributed throughout the brain and is implicated in a wide range of physiological functions such as learning, memory, attention, motor activity, sleep and wakefulness. Our aim is to analyze, by genetic means, the implication of ACh in the regulation of these integrated functions. **Methods:** The functional role of cholinergic neurons has been generally addressed by analyzing the effect of either pharmacological treatments or lesions of cholinergic structures. However, the principle classical techniques for lesioning are not cell specific and do not alter just cholinergic neurotransmission. In order to circumvent these limitations, our strategy relies on the selective inhibition of choline acetyltransferase (ChAT) expression in given cholinergic structures and at precise times once embryogenesis and synapse establishment have proceeded normally. **Results:** We have generated two major tools (i) a conditional knockout of the ChAT gene in mice, (ii) a lentiviral vector producing a sh-RNA which is able to efficiently trigger the inhibition of ChAT expression when delivered by stereotaxic injection. Both animal models are currently being used. They will be discussed together with our initial findings. **Conclusions:** Our strategy will be instrumental in deciphering the impact of neurotransmitter systems in higher brain functions and their involvement in neuropsychiatric affections.

STh06-24

Involvement of cardiac troponin I in endotoxaemia-induced contractile dysfunction

Cave A.C.
King's College London, London, United Kingdom
alison.cave@kcl.ac.uk

Systemic sepsis/endotoxaemia is associated with an intrinsic impairment of cardiomyocyte contraction, due in part to a decrease in myofilament calcium (Ca^{2+}) sensitivity associated with a sustained increase in cardiac troponin I (cTnI) phosphorylation at serines 23/24. These residues are required for the protein kinase A (PKA)-dependent reduction of myofilament Ca^{2+} sensitivity after β-adrenoceptor stimulation. Such sustained phosphorylation could arise from a sustained activation of the phosphorylating (kinase) pathways or an inhibition of the dephosphorylation pathways. We hypothesis therefore that (i) sustained cTnI phosphorylation is a major mechanism underlying the decreased contractility in septic cardiomyocytes and (ii) a dysregulation of protein phosphatase 2A (PP2A) underlies this increased phosphorylation. To investigate the functional significance of increased cTnI phosphorylation in endotoxaemia, we studied the contractile effects of systemic bacterial lipopolysaccharide (LPS) in transgenic mice with cardiac specific expression of slow skeletal TnI (ssTnI, which lacks the N-terminal protein extension containing PKA-sensitive phosphorylation sites in cTnI) and wild type (WT) littermate controls. Studies demonstrated that ssTnI mice showed significant protection against LPS-induced cardiac contractile dysfunction. PP2A is the main phosphatase which dephosphorylates cTnI. Studies revealed that increased cTnI phosphorylation in septic hearts was associated with dysregulation of PP2A in terms of decreased expression of the catalytic and regulatory subunits, an increased percentage of the inactive (demethylated) form of PP2A and a decrease in the specific methyltransferase (PPMT) that catalyses this reaction. Hence we conclude that cTnI phosphorylation is a major mechanism underlying the intrinsic depression of myocyte contractile function in endotoxaemia. Such sustained phosphorylation may arise from a dysregulation of dephosphorylating pathways mediated by PP2A.

STh06-25

Targeting essential myosin light chain/actin interaction in transgenic rats

Morano I.
Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany
imorano@mdc-berlin.de

Abstract not submitted.

STh06-26

Role of C-protein versus troponin I during PKA-dependent control of contractility

Cazorla O.[1], Szilagyi S.[1], Vignier N.[2], Vassort G.[1], Carrier L.[2], Lacampagne A.[1]
[1]INSERM U-582, Montpellier; [2]INSERM U-582, Paris, France
cazorla@montp.inserm.fr

β-adrenergic stimulation modulates cardiac contractility through protein kinase A (PKA), which phosphorylates cellular proteins, such as cardiac troponin I (cTnI) and cardiac C-protein (cMyBP-C). The relative contributions of cTnI and cMyBP-C to the regulation of myofilaments Ca^{2+} sensitivity are still controversial. In the present work we studied the PKA effect on myofilament Ca^{2+} sensitivity of left ventricular skinned myocytes isolated from young (5-weeks old) and old (55-weeks old) wild type mice (WT) and cMyBP-C deficient mice (KO) at two sarcomere lengths (SL: 1.9 and 2.3 μm). Without PKA stimulation and at the shorter SL, Ca^{2+} sensitivity was higher in KO than in WT. The difference disappeared at the longer SL. No difference in passive tension or maximal active tension was observed. PKA stimulation induced a desensitization of WT myofilaments at both SL but had almost no effect on young and old KO myofilaments. The results suggest that cMyBP-C contributes to the regulation of cardiac contraction and that TnI phosphorylation alone by PKA was not sufficient to induce myofilament desensitization.

STh06-27

Regionalisation of MLC-2 phosphorylation and its dephosphorylation in diseases

Lacampagne A., Ait Mou Y., Szilagyi S., Vassort G., Cazorla O.
INSERM U637, CHU Arnaud de Villeneuve, F-34295 Montpellier, France
lacamp@montp.inserm.fr

Aim: The "stretch-sensitization" response is essential to the regulation of heart contractility. The cellular mechanisms of this modulation, the Frank-Starling law, are still uncertain. Moreover, their alterations in heart failure remain controversial. **Methods:** We combined in this study micromechanical experiments on skinned rat myocytes and biochemical analysis (Western blot) on skinned strips of myocardium both isolated from the epicardial and endocardial layer. **Results:** We show a non-uniform stretch-sensitisation of myofilament activation across the ventricular wall. Together with the length-dependent component seen in all cells attributable to interfilament lattice spacing, sub-endocardial cells that are stiffer than sub-epicardial cells also demonstrate a strain-dependent component. This later component of myofilament activation is not correlated with troponin I or titin. Instead, in sub-endocardial tissues specifically, we observe that strain activates phosphorylation of myosin light chain VLC2b. Strain-dependency of contractile properties and phosphorylation of VLC2b are lost in a rat model of post-myocardial infarction. **Conclusion:** VLC2b phosphorylation appears to be a strain-dependent modulator of activation tuned within normal heart and altered in pathology. A transmural gradient of strain-dependent component of myofilament activation predominant in endocardium allows this tissue to participate equally to epicardium in ventricular contraction despite less constraint. The lost of transmural adaptation after myocardial infarction contributes to the development of cardiac insufficiency.

STh06-28

Mechanisms of action of the Ca^{2+}-sensitizer levosimendan

Papp Z.[1], Hertelendi Z.[1], Szilagyi S.[1], Edes I.[1], Pollesello P.[2]
[1]Division of Clinical Physiology, Institute of Cardiology, University of Debrecen, Hungary; [2]Orion Pharma, Cardiology and Critical Care, Espoo, Finland
pappz@dote.hu

A number of maladaptive changes are amplified within the cardiovascular system during the progression of chronic heart failure that makes the decompensation phase difficult to handle. Levosimendan is a new Ca^{2+}-sensitizer for the treatment of acutely decompensated heart failure that has proved to be effective during the decompensation of chronic heart failure and acute myocardial infarction. Levosimendan differs from other cardiotonic agents that are used for acute heart failure in that it utilizes a unique dual mechanism of action: Ca^{2+}-sensitization through binding to troponin C in the myocardium, and the opening of ATP-sensitive K^+ channels in vascular smooth muscle. In general, these mechanisms evoke positive inotropy and vasodilation. Clinical studies suggested long-term benefits on mortality following short-term administration. It may therefore be inferred that levosimendan has additional effects on the cardiovascular system that are responsible for the prolongation of survival. Recently, levosimendan has also been shown to act on mitochondrial ATP-sensitive potassium ($mitoK_{ATP}$) channels, an action thought to protect the heart against ischaemia–reperfusion damage. This finding has suggested a possible application for levosimendan in clinical situations in which preconditioning would be beneficial (e.g. in pre- and perioperative settings in cardiac surgery). Collectively, these effects of levosimendan shift the disturbed cardiovascular parameters towards normalization, thereby halting the perpetuation of the vicious cycle of heart failure progression. This may contribute to stabilization of the circulation and improved life expectancy of patients with chronic heart failure.

STh07-29

$Ca_v1.4$ dysfunction in congenital stationary night blindness type 2 (CSNB2)

Striessnig J., Singh A., Gebhart M., Hamedinger D., Romanin C, Hoda J.C., Sinnegger-Brauns M., Koschak A.
Pharmacol. Toxicol., Inst. Pharmacy, Univ. Innsbruck; Inst. Biophys., Univ. Linz, Austria; joerg.striessnig@uibk.ac.at

Mutations in the genes of voltage-gated Ca^{2+} channel subunits (Ca^{2+} channelopathies) cause diseases in mice and humans. Although human mutations are rare, they provide a unique molecular approach to study the role of different channel isoforms for pathological processes underlying diseases such as migraine, epilepsy, ataxia, night blindness, cardiac arrhythmias and autism. CSNB2, an X-linked recessive congenital form of night blindness, is caused by mutations of retinal $Ca_v1.4$ α1 subunits. We investigated the functional consequences of CSNB2 mutations by electrophysiological analysis after heterologous expression in HEK-293T cells. We found that structural aberrations reduce channel function to various degrees and through different mechanisms. A C-terminal truncation mutant (K1591X) unmasked a novel regulatory mechanisms that controls the voltage- and Ca^{2+}-dependent gating of $Ca_v1.4$ as well as other L-type Ca^{2+}-channels. In $Ca_v1.4$ channels the last 55 amino acid residues participate in the formation of a C-terminal modulatory structure which completely prevents the Ca^{2+}-dependent inactivation of $Ca_v1.4$ and shifts channel activation to more positive voltages. FRET analysis revealed binding of this C-terminal peptide to proximal regions of the C-terminus known to serve as interaction sites for calmodulin. Our data represent an excellent example of how channelopathies can provide unique insight into $Ca_v1.4$ Ca^{2+} channel function. We will provide evidence that a similar C-terminal modulatory mechanism also controls the function of other L-type channels. A more detailed understanding of this intramolecular protein-protein interaction may lead to novel concepts for the selective modulation of Ca^{2+}-channel function by drugs.
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STh07-30

L-type Ca^{2+} channels in the cochlea

Knipper M., Engel J.
Univ. Tubingen, Inst. Physiol., Germany; Marlies.Knipper@uni-tuebingen.de

L-type Ca channels play a crucial role for hearing. Deletion of the $Ca_v1.3$ Ca^{2+} channel has been shown to lead to deafness due to loss of Ca^{2+}-dependent transmitter release of inner hair cells (IHC). Surprisingly deafness in mutant mice is correlated with loss of outer (OHC) but not inner hair cells. This was unexpected as OHCs are primarily specializing in electromotility for sound amplification, so the existence of Ca^{2+}-activated exocytosis in OHCs was doubted since decades. In $Ca_v1.3$(-/-) mutant mice specifically OHCs in the low frequency regions exhibit sensitivity for lack of the $Ca_v1.3$ Ca^{2+} channel that resulted in degeneration around the onset of hearing (P12). In contrast, a normal phenotype and detectable distortion-product otoacoustic emissions (DPOAEs) indicated functional OHCs in the higher frequency range of the cochlea. Ca^{2+} currents of ~170 pA could be measured in neonatal $Ca_v1.3$+/+ OHCs along the whole tonotopic axis. These currents declined in mature apical OHCs to ~50 pA at P12 and to 12 pA at P19. The more robust OHCs of the rat showed a similar developmental downregulation of the Ca^{2+} current amplitude that stabilized at ~60 pA. In particular in the low frequency regions of mature rat cochleae, $Ca_v1.3$ protein was visible in OHCs at places where afferent fibers make their contacts, in exact co-localization with the ribbon synapse protein CtBP2/RIBEYE. These findings strongly suggest a role of $Ca_v1.3$ channels for exocytosis in mature OHCs. Considering expected Ca^{2+} dependent processes in spiral ganglia neurons in addition to $Ca_v1.3$ other L-type Ca^{2+} channels may exist. As such $Ca_v1.2$ was detected in synaptic contacts of efferent fibres from the MOC (brainstem) as well as in spiral ganglia neurons.
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STh07-31

Voltage-dependent Ca^{2+} channels and cardiac automaticity: from ionic currents to genes

Mangoni M.E., Marger L., Aptel H., Striessnig J., Nargeot J.
Institut de Genomique Fonctionnelle (IGF), CNRS UMR 5203, Inserm U661, Universite de Montpellier I et II, France; matteo.mangoni@igf.cnrs.fr

Aim: The spontaneous activity of pacemaker cells in the sino-atrial node (SAN) controls the heart rhythm and rate (HR) under physiological conditions. SAN cells express a wide array of ionic channels, but we have limited knowledge about their functional role in the genesis and regulation of heart automaticity. **Methods:** We have studied pacemaker activity in mice lacking L-type $Ca_v1.3$ ($Ca_v1.3^{-/-}$) and T-type $Ca_v3.1$ ($Ca_v3.1^{-/-}$) Ca^{2+} channels. **Results:** We found severely slowed and erratic pacemaking in SAN and atrioventricular node (AVN) cells from $Ca_v1.3^{-/-}$ mice and attributed this phenomenon to abolition of $I_{Ca,L}$ in the diastolic depolarization range. In contrast, disruption of the gene coding for $Ca_v3.1$ channels abolished $I_{Ca,T}$ in SAN and AVN cells. $Ca_v3.1^{-/-}$ mice had moderately reduced HR and slowed AVN conduction. The differential role of $Ca_v1.3$ channels and "funny" f-channels (underlying the cardiac I_f current) in the genesis and regulation of cardiac automaticity was then studied. The f-channel blocker ivabradine was administrated intra-peritoneally to different mouse strains. In WT and $Ca_v1.3^{-/-}$ mice, ivabradine dose-dependently reduced the mean HR. At 3 mg/kg, mean HR reduction was ~23 and ~39% in WT and $Ca_v1.3^{-/-}$, respectively. **Conclusions:** Similarly to f-channels, $Ca_v1.3$ channels controls basal and maximal HR, and in addition, play a distinct role in stabilizing HR. Furthermore, both normal and intrinsic pacemaker activity can persist in the absence of $Ca_v1.3$ channels even after strong inhibition of f-channels. In conclusion, our work demonstrates that multiple voltage-gated Ca^{2+} channel mechanisms contribute to cardiac automaticity and deserve distinct roles with respect to "pacemaker" f-channels.

STh07-32

Role of $Ca_v1.2$ channels for hippocampal synaptic plasticity, excitability and spatial memory

Kleppisch T. [1], Lacinova L.[2], Moosmang S. [1], Haider N.[1], Langwieser N.[1], Klugbauer N.[3], Hofmann F.[1]
[1]Inst. Pharmakol. Toxikol., Techn. Univ. Munchen, Germany; [2]Inst. Mol. Physiol. Genetics, SAS, Bratislava, Slovakia; [3]Inst. Exp. Klin. Pharmakol. Toxikol., Albert-Ludwigs-Univ. Freiburg, Germany; kleppisch@ipt.med.tu-muenchen.de

Aims: Activity-dependent Ca^{2+} entry into postsynaptic neurons is important for learning, synaptic plasticity, modulation of cellular excitability and transcription. We sought to elucidate the functional contribution of Ca^{2+} influx via $Ca_v1.2$ channels to these processes in the hippocampus using mice with the $Ca_v1.2$ gene inactivated in principal hippocampal neurons ($Ca_v1.2^{HKO}$). **Methods:** Long-term potentiation (LTP) and cellular excitability were examined in the CA1 region of hippocampal slices from control and mutant mice using extracellular recording of excitatory postsynaptic potentials and current-clamp recording from individual pyramidal cells. Spatial learning was tested in a discriminatory water maze. Transcriptional activation through the extracellular signalling related kinase (ERK) – CREB – CRE pathway was assessed by immunoblotting and immunohistochemical analysis. **Results:** $Ca_v1.2^{HKO}$ mice displayed inferior performance in the discriminatory water maze. This deficit in spatial learning was paralleled by an impairment of protein synthesis-dependent NMDA receptor-independent LTP. CA1 pyramidal cells from mutant mice had normal resting membrane potential and input resistance, but showed attenuated excitability following somatic depolarisation (e.g. attenuation in spiking frequency). In addition, activation of the ERK pathway and CRE-dependent transcription by LTP-inducing stimuli was reduced in hippocampal neurons from $Ca_v1.2^{HKO}$ mice. **Conclusion:** These results suggest a critical function of the $Ca_v1.2$ channel for hippocampal memory formation likely due to modulating intrinsic excitability, facilitating CRE-dependent gene expression and maintenance of LTP.

STh07-33

The role of the $Ca_v1.2$ L-type calcium channel in smooth muscle tissues

Klugbauer N.[1], Moosmang S.[1], Hofmann F.[2], Lohmuller B.[2]
[1]Institut fur Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universitat Freiburg, Freiburg, Germany; [2]Institut fur Pharmakologie und Toxikologie, Technische Universitat Munchen, Germany
n.klugbauer@pharmakol.uni-freiburg.de

Aims: The $Ca_v1.2$ L-type calcium channel is involved in a large number of diverse functions such as the contraction of cardiac and smooth muscle, the release of hormones and the regulation of enzymatic activities. The purpose of our studies was to investigate the general role of the $Ca_v1.2$ L-type channel in vascular smooth muscle cells and to characterize the interaction between $Ca_v1.2$ and BK_{Ca}-channels. **Methods:** Since $Ca_v1.2$ L-type (-/-) mice die *in utero*, we used the tamoxifen-inducible Cre/loxP recombination system to inactivate the $Ca_v1.2$ gene specifically in smooth muscle cells. The interaction between $Ca_v1.2$ and BK_{Ca}-channel proteins was investigated using the yeast two hybrid split ubiquitin system. **Results:** The tissue specific deletion of $Ca_v1.2$ in the vascular smooth muscle revealed its dominant role for blood pressure regulation and its requirement for the autoregulation and maintenance of the vascular tone in response to depolarization and pressure. The unique large-conductance, voltage- and Ca^{2+}-activated K^+ (BK) channel limits the Ca^{2+} entry and thereby arterial contraction by repolarizing smooth muscle cells and closing of L-type channels. Several studies indicated that BK_{Ca} and L-type calcium channels are co-localized and functionally associated in the smooth muscle cell membrane. Using the yeast two hybrid split ubiquitin system we could show that both channels directly interact with each other without the need for scaffolding proteins. **Conclusion:** These results demonstrate that $Ca_v1.2$ channels are crucial for the regulation of blood pressure and the development of the myogenic tone and directly interact with BK_{Ca}-channels within macromolecular complexes.

STh08-34

Control of reproductive function by metabolic factors: the roles of leptin and insulin

Pralong F.P.
Service of Endocrinology, Diabetology and Metabolism, University Hospitals and Faculty of Medicine of Lausanne and Geneva, Switzerland
Francois.Pralong@chuv.ch

Variations in energy balance can have a profound impact upon reproductive activity, an effect mediated via peripheral metabolic signals such as leptin or insulin. The overall aim of the studies described below was to better understand the hypothalamic pathways and mechanisms of action implicated in the reproductive effects of these two satiety factors. The effects of leptin to modulate reproduction depend at least partially upon hypothalamic NPY neurons of the arcuate nucleus. Using mice knockout for the Y1 subtype of NPY receptors ($Y1^{-/-}$ mice), we identified a crucial role for Y1-dependent pathways downstream of NPY neurons to convey leptin signals upon GnRH neurons, the key activators of the reproductive neuroendocrine axis. A striking finding of these experiments was the observation that juvenile $Y1^{-/-}$ mice submitted to food restriction undergo normal sexual maturation, demonstrating the importance of Y1 for the sensing of decreasing leptin levels by these neurons. In contrast, GnRH-expressing neurons express functional insulin receptors, and insulin stimulation increases both the expression and the secretion of GnRH from cultured cells. The physiological relevance of these results is suggested by our finding that in adult male mice, circulating levels of luteinizing hormone are increased by peripheral insulin administration. Taken together, these data confirm that in addition to signalling satiety to the central nervous system, insulin stimulates the neuroendocrine reproductive axis, probably via a direct effect on hypothalamic GnRH neurons. Overall, our results provide new insights into the coordinated regulation of feeding and reproductive activity by the central nervous system.

STh08-35

Fetal nutrition and timing of puberty

Van Weissenbruch M.M.
Department of Pediatrics, VU University Medical Center, Amsterdam, The Netherlands
M.vanWeissenbruch@vumc.nl

Over the last decade growing evidence has been documented on the relationship between intrauterine growth retardation (IUGR) and pubertal development indicating changes in timing and progression of puberty. The influence of IUGR on the mechanisms behind the onset of puberty is still elusive. In a rat model of intrauterine growth retardation, based on ligation of the uterine arteries, the onset of puberty was delayed in female pups, with anovulation during the first cycle. The ovaries showed a lower number of follicles. The onset of puberty was also delayed in male pups. Testosterone production was lower in these growth-retarded rats compared with controls. The relationship between birth weight and the onset of puberty and pubertal progression in different cohorts of healthy children has been examined. In girls, no differences were observed in timing and progression of puberty, including age of menarche, between groups of different birth weights. In boys, a relatively delayed onset of puberty was observed in those with low birth weight, with a normally timed progression. In children with low birth weight, particularly boys, higher dehydroepiandrosterone levels were found compared with children with a normal birth weight, indicating an overactive adrenal gland in children with low birth weight. These data indicate that impaired fetal growth may have long-lasting effects on pubertal development. The fact that results of human studies on the relationship between fetal growth and the onset of puberty are often controversial may be explained by the heterogeneity of children born small for gestational age with respect to the intrauterine insult that they experience. From rat studies, it is clear that a serious intrauterine insult associated with growth failure can lead to dysregulation of puberty and gonadal function.

STh08-36

Leptin and other adipokine systems: gender dimorphism and role in reproduction and metabolism

Magni P.
Istituto di Endocrinologia, University of Milan, Milan, Italy
paolo.magni@unimi.it

The adipose tissue is regarded as an endocrine organ secreting a number of peptide hormones, collectively called adipokines and involved in the regulation of several physiological functions, including energy metabolism and reproduction. Leptin is the most studied adipokine. Its circulating levels mostly reflect the adipose mass and are modulated by male and female gonadal steroids in opposite ways. Leptin displays a relevant role in the control of food intake and energy expenditure, representing a signal of satiety to the hypothalamus and a stimulus to increase energy expenditure. Moreover, its rise at puberty is believed to contribute to the hypothalamic activation that leads to the development and maintenance of a mature reproductive function. Several effects of leptin on reproduction are mediated by direct and indirect effects on gonadotropin-releasing hormone (GnRH)-secreting neurons in the hypothalamus. Interestingly, cytokines structurally related to leptin and interleukin-6, like ciliary neurotrophic factor and leukaemia inhibitory factor (LIF) are also functionally correlated with leptin in these actions. The other adipose product adiponectin increases the sensitivity of peripheral tissues to insulin and induces fat oxidation. Conditions of insulin-resistance, like diabetes and obesity, are characterized by reduced adiponectin levels. Another adipokine, resistin, is associated with the induction of insulin resistance. It is also expressed in the rat testis and it appears to be a mediator of energy homeostatasis and reproduction. Thus, recent evidence suggests that the physiology of the adipose tissue appears to be an important component in the regulation of reproduction and metabolism, with potential roles in human disease.

STh08-37

Novel peptide systems in the cross-talk of energy metabolism and reproduction: ghrelin and kisspeptins

Tena-Sempere M.
Department of Cell Biology, Physiology and Immunology, University of Cordoba, Spain; fi1tesem@uco.es

The close link between energy reserves, metabolic status and fertility is based on a complex network of regulatory signals, of peripheral and central origin, that participate in the joint control of energy homeostasis and reproduction, but whose nature remains to be fully elucidated. Recent experimental data suggest that the gut hormone ghrelin, regarded as signal of energy insufficiency, may operate also as pleiotropic modulator of the reproductive axis as (i) ghrelin is able to inhibit luteinizing hormone secretion in rodents and other mammals; (ii) ghrelin is a negative modifier of puberty onset in (male) rats; (iii) ghrelin and its canonical receptor are expressed in the gonads; and (iv) ghrelin conducts direct gonadal actions; testicular effects of ghrelin include inhibition of testosterone secretion, Leydig cell proliferation and expression of relevant Sertoli cell genes. In addition, evidence is also mounting that kisspeptins, encoded by the KiSS-1 gene and potent stimulators of the reproductive axis, play a crucial role for integrating energy status and fertility. Thus, expression of KiSS-1 gene at the hypothalamus is sensitive to nutritional status, and its diminished expression in situations of negative energy balance likely contributes to the suppression of reproductive function in such conditions. Moreover, leptin has been recently demonstrated to stimulate hypothalamic KiSS-1 gene expression, in different models of disturbed metabolism and hypoleptinaemia. These observations, together with the demonstration of leptin receptors in discernible KiSS-1 neurons at the hypothalamus, provide the basis for a leptin-kisspeptin pathway in the metabolic control of the reproductive axis. It is proposed that, in addition to 'classical' metabolic regulators (i.e. leptin and insulin), the gut hormone, ghrelin, and the hypothalamic neuropeptide, kisspeptin, are 'novel' players in the neuroendocrine networks that integrate energy balance and reproduction.

STh09-38

Role of glia in volume transmission in the CNS

Sykova E.
Department of Neuroscience, Institute of Experimental Medicine ASCR, Department of Neuroscience, Charles University, Second Medical Faculty, Prague, Czech Republic; sykova@biomed.cas.cz

Extrasynaptic volume transmission is mediated by the diffusion of neuroactive substances in the extracellular space of the nervous system (ECS) and plays an important role in short- and long-distance communication between nerve cells. The ability of a substance to reach extrasynaptic high-affinity receptors via diffusion depends on the ECS diffusion parameters, i.e. the size of the ECS volume and the presence of diffusion barriers represented by, for example, fine astrocytic and neuronal processes or the extracellular matrix (ECM). In many brain regions such as the corpus callosum, hippocampus, cerebellum and supraoptic nucleus, these barriers may facilitate the diffusion of substances in the ECS in one direction rather than in another, thus the diffusion is anisotropic. Ionic changes and amino acid release evoke cell swelling, which leads to a concomitant decrease in ECS volume and also in the apparent diffusion coefficients of ions, neuroactive substances and water. Using ion-selective microelectrodes or diffusion-weighted MRI, changes in the ECS diffusion parameters have been found in many physiological and pathological states in which glial remodeling and ECM changes are among the key factors influencing diffusion. Thus, the movement of substances in the ECS is affected not only by the size of the pores between cells, but also by glial structure and ECM composition changes, which may significantly influence the diffusion of neuroactive substances, extrasynaptic transmission, neuron-glia communication, the "spillover" of mediators, synaptic "cross-talk", hormonal release and cell migration.

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STh09-39

Gap junctions in glial physiology and pathophysiology

Giaume C., Koulakoff A., Houades V., Rouach N.
INSERM U840, College de France, Paris, France
christian.giaume@college-de-france.fr

Aims: Astrocytes constitute the brain cell population the most widely coupled by gap junctions that organize these cells as multicellular networks. Indeed, intercellular channels made by connexins provide direct cell-to-cell communication allowing astrocytes to work as groups of communicating cells rather than independent units. How are organized such astrocytic networks and what are the rules that govern their shape and function constitute critical questions to understand their interaction with neuronal circuitry. **Methods:** Gap junctional communication (GJC) was investigated by patch-clamp recording and dye-coupling in acute brain slices while the distribution of the two astrocytic connexins was assessed by immunohistochemistry and confocal microscopy. **Results:** In the somatosensory cortex, where layer IV neurons constitute anatomo-functional compartments, connexin expression is enriched in the barrels, while GJC is restricted in the barrel-to-barrel axis and favoured toward their center. In the CA1 region of the hippocampus, the extent of GJC is controlled by glutamate released from neurons in an activity-dependent manner and by bioactive peptides. The use of fluorescent glucose derivatives demonstrated that GJC allows intercellular trafficking of energetic compounds and rescues neuronal activity following glucose deprivation. **Conclusion:** Astrocytes are organized as communicating networks tightly regulated by neurons. The shaping of astrocytic networks depends on the local organization of neurons and on their activity indicating that they are dynamically controlled. Finally, the permeability properties of gap junction channels in astrocytes provide a basis for their contribution to the metabolic supply by glia to neuronal activity.

STh09-40

Vesicular release of "glio" transmitters and integration in neuronal-glial networks

Volterra A.
Universite de Lausanne, Lausanne, Switzerland
Andrea.Volterra@unil.ch

Abstract not submitted.

STh09-41

Physiology and pathophysiology of microglia

Kettenmann H.
Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany
kettenmann@mdc-berlin.de

Abstract not submitted.

STh09-42

Cell swelling-induced peptide hormone secretion, pathophysiological implications

Strbak V., Bacova Z., Orecna M., Hafko R.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
vladimir.strbak@savba.sk

Cell swelling (induced by hypotonicity or permeants) evokes exocytosis of material stored in secretory vesicles resulting in a secretory burst of peptide hormones or enzymes from various types of cells. Dynamics of this secretion is indistinguishable from that induced by specific secretagogue. Swelling-induced secretion possesses specific features indicating a unique signaling pathway. For instance glucose- and swelling induce insulin secretion through separate signal transduction pathways; hyposmotic stimulation is independent from the extra- and intracellular Ca^{2+}, does not involve other intracellular mediators of glucose stimulation, and could not be inhibited by noradrenaline. Swelling is a useful tool when natural or pharmacological secretagogue is unknown: Thyrotropin releasing hormone (TRH) release from the heart slices could be stimulated by hypotonic medium and stimulation overrides inhibitory effect of angiotensin II. Cell swelling-induced exocytosis possesses limited selectivity; cells specifically involved in water and salt regulation retain their specific response to osmotic stimuli: hypotonic medium evokes TRH but not oxytocin release from hypothalamic paraventricular nucleus (PVN). Specific response of intranuclear oxytocin secretion to osmotic stimulation in the PVN and the supraoptic nucleus could be obviated by $GdCl_3$ and at these conditions general response (hormone release) to swelling-inducing stimuli emerged. Swelling-induced hormone secretion could have pathophysiological implications. In ischaemia there is a shift to anaerobic glycolysis and production of metabolites which can increase the intracellular osmolarity by 130 $mOsmol.L^{-1}$ after 60 min, thus producing cell swelling. Peptides and proteins released after swelling could play an important role in the pathophysiology of ischaemia and be mediators of local or remote preconditioning when factors released at the place of ischaemia have protective effect.

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STh09-43

Calcium signalling and integration in neuronal-glial networks

Verkhratsky A.
Faculty of Life Sciences, The University of Manchester, Manchester M13 9PT, United Kingdom
alex.verkhratsky@manchester.ac.uk

Integration in the nervous system is achieved by signal processing within dynamic functional ensembles formed by highly complex neuronal-glial cellular circuits. The interactions between electrically excitable neuronal networks and electrically non-excitable glial syncytium occur through either chemical transmission, which involves the release of transmitters from presynaptic terminals of astroglial cells, or via direct intercellular contacts, gap junctions. Calcium ions act as a universal intracellular signalling system, which controls many aspects of neuronal-glial communications. In neurones, calcium signalling events regulate the exocytosis of neurotransmitters and establish the link between excitation of postsynaptic cells and integrative intracellular events, which control synaptic strength, expression of genes and memory function. In glial cells metabotropic receptor mediated release of calcium ions from the intracellular endoplasmic reticulum calcium store provide specific form of glial excitability. Glial calcium signals ultimately result in vesicular secretion of "glio"transmitters, which affect neuronal networks thus closing the glial-neuronal circuits. Cellular signalling through calcium ions therefore can be regarded as a molecular mechanism of integration in the nervous system.

STh10-44

Regulation of the dopamine degrading enzyme monoamine oxidase a by the circadian clock

Albrecht U.
Department of Medicine, Division of Biochemistry, University of Fribourg, Fribourg, Switzerland
urs.albrecht@unifr.ch

Reward-related behaviour exhibits a recurring pattern with a period of about 24 hours and hence is circadian. This indicates potential interactions between the circadian and the reward systems in the brain. First evidence for an involvement of circadian clock-associated genes in drug-related reward behaviour came from studies in *Drosophila*, which showed that cocaine sensitization is dependent on clock gene expression. Subsequently, we found that mice lacking the *Per1* or the *Per2* genes showed abnormal sensitization and conditioned preference to cocaine. To find a molecular link between the clock and the reward system, we studied genes coding for key enzymes in dopamine metabolism. We found that the gene of the dopamine degrading enzyme monoamine oxidase A (MaoA) is regulated by clock components. A mutation in the clock gene *Per2* leads to decreased expression and activity of MaoA in the mesolimbic dopaminergic system of the brain. As a consequence we find increased levels of dopamine leading to a depression-resistant like phenotype in *Per2* mutant mice.

STh10-45

The circadian rhythm of mice: in the lab and in nature

Daan S.
Zoological Laboratory, Centre for Behaviour and Neurosciences, University of Groningen, Haren, The Netherlands
S.Daan@biol.rug.nl

Abstract not submitted.

STh10-46

The *tau* mutation in mice and hamsters

Loudon A.S.I.[1], Meng Q-J.[1], Logunova L.[1], Lebiecki J.[1], Maywood E.S.[2], Brown T.[1], Glossop N.[1], Piggins H.[1], Chesham J.E.[2], Boot-Handford R.[1], Hastings M.H.[2]
[1]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; [2]MRC Laboratory of Molecular Biology, Division of Neurobiology, Cambridge, United Kingdom
andrew.loudon@manchester.ac.uk

Casein kinases (CK) are central regulators of the circadian clock. Mutations in CK1 and its target hPer2 cause sleep disorders in patients whilst the *tau* mutation of CK1ε in hamsters accelerates circadian rest/activity cycles and amplifies resetting responses to light. We re-created this mutation in mice and also generated whole-animal knock-outs by using the *Cre-loxP* system. *Tau* mutant mice express robust, short period circadian rest/activity rhythms comparable to mutant hamsters and patients, although re-setting responses to light are normal. In contrast, circadian period is lengthened in knock-out animals. Multi- and single-unit recording of SCN firing rates revealed periods comparable to genotype-specific behaviour. Clock gene mRNA and protein rhythms were also accelerated in both SCN and peripheral tissues, revealing for the first time the global impact of the mutation on the molecular pacemaker, and supporting a model of $CK1\varepsilon^{tau}$ as a gain of function accelerating clock protein turnover during circadian night. Studies of PER2 protein turnover using luciferase imaging of SCN slices and single cells reveal that circadian phase is accelerated in the early night, compressing this phase by 4 hours, but of normal phase and duration in the subjective day time. These real-time imaging data now reveal for the first time how a circadian mutation accelerates a target protein at a specific phase of the cycle, and support a model for accelerated PERIOD protein turnover in the early nocturnal phase. We extend these data to peripheral tissue in a predictive model which describes the impact of the *tau* mutation on re-setting responses to external stimuli.

STh10-47

Clock gene expression during development

Sumova A., Bendova Z., Sladek M., Kovacikova Z., El-Hennamy R., Laurinova K., Illnerova H.
Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; sumova@biomed.cas.cz

Aims: In mammals, the circadian oscillator within the suprachiasmatic nuclei (SCN) entrains circadian clocks in numerous peripheral tissues. Central and peripheral clocks share similar molecular core clock mechanism. The aim of the present work was to elucidate when during ontogenesis expression of clock gene in the rat SCN and liver starts to be rhythmic and entrained by photic and maternal cues. **Methods:** Daily profiles of clock gene mRNA levels were analyzed in the SCN and liver of fetuses at embryonic day 20, of pups at different postnatal ages from P1 till P30 and in adults by *in situ* hybridization and/or real-time RT-PCR. **Results:** The circadian rhythmicity in clock gene expression develops gradually from prenatal to postnatal period both in the SCN and in peripheral organs. The maternal SCN sets the phase of the developing rhythmicity in the fetal and early postnatal SCN clock. The external light-dark cycle starts to reset the rat SCN clock only after the first postnatal week. Full adjustment to the day-length, i.e., to the photoperiod, is accomplished only around the time of weaning. The rhythmicity of the newborn liver clock is entrained by maternal feeding regime and starts to be fully entrained by the SCN only around weaning. **Conclusion:** The results demonstrate gradual postnatal development of the molecular mechanism responsible for generation of rhythmicity within the central and peripheral circadian clocks. Maternal entrainment of the developing clocks prevails during the first week after birth and, thereafter, photic and photoperiodic entrainment starts to dominate.

STh10-48

Clock genes and rhythmic function of the cardiovascular system in rats

Zeman M.[1], Monosikova J.[1], Petrak, J.[2], Kovacikova Z.[1], Mravec B.[2], Krizanova O.[3], Kvetnansky R.[2], Herichova I.[1]
[1]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia; [2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [3]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia; mzeman@fns.uniba.sk

The cardiovascular system exhibits distinct circadian rhythms in its activity, mainly in blood pressure (BP), heart rate, peripheral resistance etc. Moreover, number of cardiovascular incidents follows a daily rhythm with maximum risk in the morning hours. It is expected that compromised cardiovascular anticipation observed during hypertension and diabetes can lead to higher risk of cardiovascular incidents. The circadian system plays a key role in anticipation of periodic loads from the environment. Circadian expression of clock gene in the master biological clocks localized in the suprachiasmatic nuclei (SCN) of the hypothalamus and in peripheral organs is important for coordinated function of different organs. In our study we determined daily rhythms in clock genes (*per2, Bmal1, clock*) expression in target organs (heart and kidney) and central brain structures involved in control of the cardiovascular system (SCN, paraventricular nucleus (n), dorsomedial n., n. ambigus, medulla caudalis ventrolateralis, n. tractus solitarii and circumventricular organs, the anteroventral third ventricle and area postrema). As animals models we used rats with streptozotocine induced diabetes and hypertensive rats TGR(mREN-27) with up regulated renin angiotensine system (RAS) exhibiting a "non dipping" BP profile. Our data suggest that up regulated RAS is important for "non dipping" profile of BP. Mechanisms down stream from the SCN are important for the BP rhythmicity but existence of distinct population of angiotensine II sensitive neurons in the SCN that control BP is still not excluded.

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SF11-49

Structure, function and pharmacology of gap junctions

Dhein S.
Clinic for Cardiac Surgery, Heart Centre Leipzig, University of Leipzig, Germany
dhes@medizin.uni-leipzig.de

Abstract not submitted.

SF11-50

Basic electrical and diffusional properties of gap junctions

Weingart R.
Institute of Physiology, University of Bern, Bern, Switzerland
weingart@pyl.unibe.ch

Aims: In the tissues of the cardiovascular system, adjacent cells are functionally coupled by means of gap junction channels (GJC), predominantly made of the connexins Cx37, Cx40, Cx43 and Cx45. Diffusional coupling (signalling molecules, metabolites) and electrical coupling (ions) via GJCs are essential for the tissue homeostasis and the impulse propagation. The purpose of this presentation is to elaborate on the biophysical properties of these channels. **Methods:** Gap junctions (GJ) can be studied in terms of electrical (conductance) or diffusional properties (permeability). Hence, a voltage-clamp technique or a fluorescence detection method, respectively, has been used to perform experiments on cell-pair preparations. **Results:** Co-expression of more than one type of connexin renders it possible to form different classes of channels (homomeric-homotypic, homomeric-heterotypic, heteromeric-homotypic, heteromeric-heterotypic). The electrical properties of different types of channels will be described at the multichannel and single-channel level. The data gathered led to a generalized electrical model for GJs and GJCs. The diffusional properties of GJCs are less well studied. While earlier investigations focused on all-or-nothing responses, more recent studies have been aimed at quantitative properties. Currently, data are available for homotypic channels only. **Conclusion:** Up-to-date methods enable accurate determinations of the biophysical properties of GJs and GJCs. It turns out that structurally different GJCs exhibit functionally different properties. Conceivably, GJCs possess diverse molecular mechanisms designed to adjust the cell-to-cell transfer properties to the actual biological requirements.

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SF11-51

Gap junctions in diseased human heart

Kostin S.
Max-Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
sawa.kostin@mpi-bn.mpg.de

Abstract not submitted.

SF11-52

Gap junctions and atherosclerotic plaque development

Oviedo-Orta E.
Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom
E.Oviedo-Orta@surrey.ac.uk

Atherosclerosis is the main underlying cause of cardiovascular disease and it is characterised by a chronic inflammatory process of the arterial wall. Monocytes, macrophages, lymphocytes and dendritic cells have been shown to play a key role in atherosclerotic plaque development. It is known that these cells establish direct intercellular communication through gap junction channels among themselves and with other cellular components of the arterial wall. These hexameric channels are constructed of connexins (Cx), their basic protein subunits. Differential expression of connexins within the arterial wall has been shown to be associated with specific stages of the development of atherosclerotic plaques both in mouse and human lesions. Reports derived from studies using animal models have recently revealed opposite roles in inflammation leading to atherosclerosis for Cx37 and Cx43. We have also shown that different subsets of CD4+ T lymphocytes establish gap junctional communication with macrophages in vitro and that this interaction can be regulated by pro-atherogenic molecules such as oxidised-low density lipoproteins (oxLDL). Altogether, the evidence available demonstrates the importance of this type of intercellular cross talk in the pathophysiology of cardiovascular disease and provides new insights for understanding the mechanisms of its development.

SF11-53

Gap junctional communication between myocardial and stem cell-derived cardiomyocytes

Van Veen T.
Department of Medical Physiology, University Medical Center Utrecht, Utrecht, The Netherlands
A.A.B.vanVeen@med.uu.nl

Abstract not submitted.

SF12-54

Oxygen sensing in fetoplacental vessels

Hampl V., Jakoubek V., Klescova K.
Charles University, Second Medical School, Prague, Czech Republic
vaclav.hampl@lf2.cuni.cz

Aims: Despite its crucial importance in fetal physiology and pathology, the regulation of fetoplacental vasculature is poorly understood. Based on similarity in function with the adult pulmonary circulation we hypothesized that acute hypoxia elicits fetoplacental vasoconstriction by mechanisms similar to those known in the pulmonary vessels. We also proposed that chronic hypoxia produces morphological and functional changes in the fetoplacental vasculature analogous to chronic hypoxic pulmonary hypertension. **Methods:** We used isolated perfused cotyledon of human placenta, isolated perfused rat placenta, isolated fetoplacental vessels and their smooth muscle cells, and morphological methods. **Results:** Acute hypoxia causes marked fetoplacental vasoconstriction by inhibiting voltage-gated K^+ channels and subsequent Ca^{2+} influx completely carried by L-type Ca^{2+} channels. Chronic hypoxia in the rat (10%O_2 during the last week of the 3-week pregnancy) elevated fetoplacental vascular resistance and increased reactivity to vasoconstrictor stimuli (acute hypoxic challenge and angiotensin II). Morphologically, we found a random mixture of increased and reduced wall thickness of peripheral fetoplacental vessels in chronic hypoxia. The average lumen diameter, however, was consistently reduced. **Conclusions:** We conclude that fetoplacental vessels, particularly their smooth muscle cells, are exquisitely responsive to hypoxia. Their response to acute hypoxia is rather similar to that in the pulmonary circulation. The response to chronic hypoxia does not include the marked thickening of the vascular wall typical for hypoxic pulmonary hypertension; nevertheless, it does result in reduced lumen area and increased vascular resistance. It is likely that this mechanism significantly contributes to serious neonatal disorders such as intrauterine growth restriction.

SF12-55

Hypoxia and cardiorespiratory control

Pequignot J.M., El-Hajjaji F-Z., Nguyen C., Denavit-Saubie M.
Integrative, cellular and molecular Physiology UMR CNRS 5123, Universite Claude Bernard Lyon I, UPR 2216: Neurobiologie genetique et integrative CNRS, Gif sur Yvette, France; Jean-Marc.Pequignot@univ-lyon1.fr

Aims: Long-term exposure to hypoxia elicits a gradual increase in ventilation termed "*ventilatory acclimatization to hypoxia*" (VAH). The purpose of this study was to define the neurochemical remodelling induced by sustained hypoxia in the brainstem areas involved in regulation of VAH and its role in modulating VAH. **Methods:** We assessed (1) the VAH in intact or chemodenervated rats subjected to 10% O_2 in nitrogen for 2 weeks, (2) the expression of tyrosine hydroxylase and HIF-1α in brainstem and (3) the hypoxic ventilatory response after pharmacological induction of HIF-1. **Results:** In chemodenervated rats, the hypoxic ventilatory response was strongly blunted. After few weeks, the chemodenervated rats recovered their initial hypoxic ventilatory response but the pattern of response differed strikingly from that observed in intact rats. The HIF-1α expression was restricted to catecholaminergic (tyrosine hydroxylase-immunoreactive) neurones located in brainstem cardiorespiratory areas of hypoxic rats. In normoxic rats the overexpression of HIF-1α protein induced by desferrioxamine, an iron chelator, elicited an overexpression of tyrosine hydroxylase in the dorsomedian brainstem. The overexpression of tyrosine hydroxylase did not modify the basal ventilation but in contrast was associated with a moderate increase in hypoxic ventilatory response. **Conclusion:** The functional remodelling after chemodenervation revealed a central effect of hypoxia on the activity of cardiorespiratory brainstem regions. The plasticity of brainstem catecholaminergic cells plays a pivotal role in acclimatization to hypoxia. HIF-1 may participate in the control of brainstem catecholaminergic neurones within cardiorespiratory areas involved in integration of chemo-afferent inputs or central O_2-sensing.

SF12-56

Vascular remodelling

Gonzalez C., Rocher A., Agapito M.A., Obeso A.
Departamento de Bioquimica y Biologia Molecular y Fisiologia, CIBER de Enfermedades Respiratorias, Facultad de Medicina, Universidad de Valladolid, Valladolid, Spain; constanc@ibgm.uva.es

Vascular remodelling is a wide term which includes any change in the molecular and morphological structure of blood vessels whether physiological or pathological. In a wider sense it will include the development of blood vessels from precursor cells or vasculogenesis, the sprouting and non-sprouting (intussusception) of new blood vessels from the pre-existing ones (angiogenesis or neovascularisation) and the change in the proportions of the different cell stirps composing the vessel wall leading to either vessel enlargement (positive remodelling) or reduction (negative remodelling). From this wide perspective vascular remodelling occurs physiologically, during embryonic and fetal life, in the normal process of wound healing and in female reproductive cycle (uterus and ovary); and pathologically, in retinopathy of prematurity and diabetic retinopathies, in tumour growth and pulmonary and systemic arterial hypertension. Combinations of remodelling mechanisms are common in many pathological processes: thickening of the arterial walls is accompanied by formation of new blood vessels aimed to nourish the grown arterial layers. Among the many factors triggering and controlling vascular remodelling, hypoxia and the transcriptional regulator of hypoxia-responsive genes, hypoxia inducible factor, are of prime importance in processes such as tumour vascularisation and pulmonary circulation remodelling. In my presentation I shall review some of the general factors involved in vascular remodelling in pulmonary circulation. Recent findings in models of sustained hypoxia, animal models of chronic obstructive pulmonary disease and perinatal hyperoxia shall be presented.

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SF12-57

Hypoxic regulation of gene expression

Fandrey J.
Institut fuer Physiologie, Universitaetsklinikum Essen, and Zentrum fur Medizinische Biotechnologie (ZMB), Universitaet Duisburg-Essen, Germany
joachim.fandrey@uni-due.de

The activation of hypoxia-inducible factor-1 (HIF-1) has been recognized as the key event in adaptation to hypoxia. HIF-1 is a hetero-dimeric transcription factor complex composed of the O_2-labile α- and the constitutive α-subunit. Under hypoxic conditions HIF-α accumulates due to the lack of degradation, is translocated into the nucleus and after hetero-dimerization with the α-subunit binds to respective hypoxia response element in oxygen-regulated genes. Normoxic degradation of HIF-α depends on the activity of prolyl hydroxylases (PHDs). PHDs hydroxylate HIF-α depending on the PO_2 and mark HIF-α for proteasomal degradation. Under hypoxia HIF-α evades hydroxylation and accumulates. Recent advances in imaging subcellular three-dimensional distribution of HIF-α and interacting proteins by 2-photon-microscopy have provided first insights into localisation of the O_2 sensing process. Direct observation of the assembly the HIF-1 complex in living cells will further improve our understanding of cellular adaptation to hypoxia.

SF12-58

Hypoxia and Alzheimer's disease

Peers C.
Faculty of Medicine and Health, Univ. of Leeds, Leeds, United Kingdom
C.S.Peers@leeds.ac.uk

Various respiratory and cardiovascular diseases leave individuals hypoxic for prolonged periods. For the central nervous system, the most extreme O_2 deprivation follows a stroke, but other diseases also compromise its O_2 supply. Prolonged hypoxia predisposes individuals to developing dementias, primarily Alzheimer's disease (AD). We have found that hypoxia (2.5 – 1% O_2, 6-48h) alters aspects of neuronal and astrocytic function, and these are associated with production of amyloid β peptide (Aβ), the main pathogenic factor in AD. In neurones, hypoxia selectively up-regulated L-type Ca^{2+} channels; this was prevented by inhibition of secretases required for Aβ formation *(Webster et al. 2006)*. Parallel studies employing recombinant channels indicated that Aβ acted post-transcriptionally to alter Ca^{2+} channel trafficking such that more channels were present in the plasma membrane *(Scragg et al. 2005)*. In astrocytes, hypoxia altered Ca^{2+} signalling via disruption of mitochondrial Ca^{2+} buffering and inhibition of Na^+/Ca^{2+} exchange *(Smith et al. 2003; Atkinson et al. 2006)*, effects which were also associated with Aβ formation *(Smith et al. 2004)*. Thus, Aβ formation is linked with hypoxic remodelling of cell functions, any of which can contribute to disruption of Ca^{2+} homeostasis and so to neurodegeneration of AD. We are currently investigating the mechanisms by which production and degradation of Aβ is modified by hypoxia.

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Atkinson L., et al (2006) *Neuroreport* 17, 649-652; Scragg J. L., et al (2005) *FASEB J.* 19, 150-152; Smith I. F., et al (2004) *J. Neurochem.* 88, 869-877; Smith I. F., et al (2003) *J. Biol. Chem.* 278, 4875-4881; Webster N. J. et al (2006) *Neurobiol. Aging* 27, 439-445.

SF12-59

Is AMP-activated protein kinase the primary metabolic sensor and effector in O_2-sensing cells?

Evans A.M.
Department of Biomedical Sciences, School of Biology, Bute Building, University of St.Andrews, St. Andrews, Fife, KY16 9TS, United Kingdom
jevans1@email.uky.edu

Early detection of an O_2 deficit in the bloodstream of mammals is essential. Thus, highly specialised cells have evolved to monitor O_2 supply and regulate, for example, ventilation-perfusion matching in the lung and breathing patterns. Thereby arterial pO_2 is maintained within physiological limits. Two O_2-sensing systems incorporating such cells are: (A) the pulmonary arteries, in which detection of a fall in airway pO_2 by smooth muscle and endothelial cells leads to constriction in order to divert blood flow to oxygen rich areas of the lung; (B) the carotid bodies, in which detection of a fall in arterial pO_2 by type I cells precipitates excitation-secretion coupling and increased sensory afferent discharge along the carotid sinus nerve to elicit corrective changes in breathing patterns via the brain stem. One finding common to each cell type is that mitochondrial oxidative phosphorylation is inhibited over a range of pO_2 that is without effect on cell types that do not serve to monitor O_2 supply. That apart, the precise nature of the coupling mechanism(s) remains controversial. However, one consequence of a reduction in mitochondrial oxidative phosphorylation would be a rise in the cellular AMP/ATP ratio, which would in turn augment the AMP-activated protein kinase (AMPK) signalling cascade. Thus, we have proposed that AMPK may underpin hypoxia-response coupling in O_2-sensing cells. Our experimental findings provide strong support for this proposal *(for review see Evans, 2006)*.

Evans, A.M. (2006). *J. Physiol.*, 574, 113-123.

SF13-60

Suppression of neuroimmune response at term in the rat

Pittman Q.J., Harre E.M., Mouihate A.
Hotchkiss Brain Institute and Institute of Infection Inflammation and Immunity, University of Calgary, Calgary, Alberta, Canada
pittman@ucalgary.ca

Aims: In several mammals, including rats, the febrile response to the neuroimmune stressor, lipopolysaccharide (LPS) is attenuated at near term and recovers in the post partum period. **Methods:** We have carried out a series of physiological and neurochemical investigations to attempt to identify the mechanisms underlying the reduced neuroimmune response. **Results:** Levels of pro-inflammatory cytokines (IL-1β, IL-6, IFNγ, and TNFα) in the plasma taken 2h after LPS were similar at gestational day (G) 15, G22 or lactation day (L) 5 suggesting identical responses to TLR4 activation. In addition the anti-inflammatory cytokines, IL-1ra and IL-10, and the immunosuppressive hormone, corticosterone, were similar after LPS at the 3 stages of reproduction. Cytokines are known to cause fever by initiating the synthesis of prostaglandin E (PGE). To test the hypothesis that PGE synthesis or action was altered at term, we used semi-quantitative Western blots to measure levels of inducible COX-2, the rate limiting enzyme for the synthesis of PGE that is found in endothelial cells of the brain vasculature. We found that COX-2 levels in the hypothalamus are reduced at G22 compared to G15 or at L5. Pro-inflammatory cytokines activate COX-2 through a number of signaling pathways, so we explored if these were similarly altered. However, the reduced COX-2 expression was not associated with alterations in activation of transcriptional factors NFκB, STAT 3 and STAT5 or of ERK1/2. **Conclusions:** The reduced fevers at term are associated with a reduction in PGE synthesis in the brain, but the mechanisms responsible remain elusive.

SF13-61

Birth and perinatal behaviour: activation of the central oxytocin system

Meddle S.L.
Centre for Integrative Physiology, College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, United Kingdom
s.l.meddle@ed.ac.uk

Aims: Oxytocin is known to play a pivotal role in rat parturition as it acts within the brain to facilitate its own release. In rodents oxytocin has profound influences on social behaviour including the onset of maternal behaviour in the peripartum period. Here we examined the oxytocin system in the brain during pregnancy, parturition and the post partum period. **Methods:** We quantified in the rat brain (1) oxytocin receptor (OTR) mRNA (using a ^{35}S-riboprobe) throughout the peripartum period (2) OTR activation at parturition using double immunocytochemical labeling for the expression of the immediate early gene Fos and OTR and (3) oxytocin and OTR cell activation (Oxytocin or OTR and Fos double immunocytochemistry) during exhibition of one component of maternal behaviour, aggressive protection of the offspring from an intruder. **Results:** Our data show that not only does OTR mRNA increase dynamically in brain regions such as the supraoptic nucleus, olfactory bulbs, preoptic area, brain stem and limbic regions perinatally, but that OTR positive-cells in specific brain regions express Fos at birth. Moreover our results demonstrate Fos activation in the oxytocin system during maternal aggression. **Conclusion:** These findings provide evidence for dynamic changes in oxytocin sensitivity within oxytocin-producing and brainstem regions that mediate feedback from the uterus at parturition. The alteration in oxytocin sensitivity and the activation of oxytocin neurones in specific brain regions during maternal aggression presents further evidence for the crucial role this hormone plays in orchestrating maternal behaviour.
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SF13-62

Placental hormones and the onset of labour: the paradigm of corticotrophin-releasing hormone

Grammatopoulos D.K.
Warwick Medical School, University of Warwick, United Kingdom
d.grammatopoulos@warwick.ac.uk

The onset of labour is characterised by dramatic changes in uterine tissue responsiveness to external hormonal stimuli. These signals, many arising from the placenta, target the myometrial smooth muscle to facilitate inhibition of quiescent pathways and activation of pro-contractile mechanisms. An important signal appears to be corticotropin-releasing hormone (CRH), the hypothalamic peptide that controls mammalian survival and response to stressful stimuli. CRH is secreted by human placenta during pregnancy; however, its role during pregnancy and labour is still an enigma. CRH plasma levels of rise exponentially as pregnancy progresses towards term and might be an important predictor of the duration of human gestation. Furthermore, there are substantially increased concentrations of maternal circulating CRH in abnormal pregnancy states; in some women with idiopathic preterm labour, concentrations of CRH increase up to 10 weeks before the development of any symptoms. Placental CRH targets multiple feto-maternal tissues, including the myometrium, placenta and fetal adrenals. These tissues express a wide network of specific G-protein coupled receptors. Current evidence suggests that activation of CRH-Rs is involved in the mechanisms regulating uterine transition from relaxation to active contractions, in placental vascular tone, and modulation of fetal adrenal function. These receptors have various functional properties, depending on the receptor subtype, the ability of agonists to activate specific signalling cascades and the stage of pregnancy. In addition, their function is dependant upon other intracellular signals via communication between signalling cascades, suggesting potential multiple roles of CRH and other CRH-like peptides during pregnancy and labour.

SF13-63

Peripheral cytokines and labour mechanisms

Szekeres-Bartho J.
Department of Medical Microbiology and Immunology, Medical School Pecs University, Pecs, Hungary; szjuli@main.pote.hu

Pregnancy is a natural model of an optimal immune regulation in a graft-host relation. In order to satisfy contradictory interests of mother and foetus, a balance is established to protect the mother from infections and tumours and at the same time to prevent an immunological attack towards the foetus. In humans there is a well-established relationship between peripheral cytokine pattern and the outcome of pregnancy. It has been suggested that a Th2 dominant cytokine production favours the maintenance of normal pregnancy, whereas significantly increased Thl cytokine expression might represent the underlying phenomenon leading to pregnancy termination. The effect of progesterone on the immune system of pregnant women is at least in part receptor-mediated. Following recognition of fetal antigens, activated maternal g/d T cells express progesterone receptors, and upon progesterone binding produce a mediator; named PIBF. By signalling via a novel form of IL-4 receptor PIBF induces a TH2 dominant cytokine response, facilitating the production of IL-3, IL-4 and IL-10 by activated peripheral lymphocytes, and suppressing that of TH1 cytokines, such as IL-12 and IFN-γ. Through altered cytokine production PIBF inhibits NK mediated killing in an indirect way. Neutralization of endogenous PIBF activity in pregnant mice by specific anti-PIBF antibody causes a significant reduction in the number of viable foetuses, and this is associated with an increased splenic NK activity, together with reduced IL-10 and increased IFNγ production of the spleen cells. Ninety per cent of pregnancy loss is corrected by treatment of the pregnant animals with anti-NK antibodies. These data suggest that in mice PIBF contributes to the success of pregnancy and that the major part of its pregnancy-protective effect lies in keeping NK activity under restraint.

SF13-64

Co-ordinated uterine and brain mechanisms switching on oxytocin neurons perinatally

Douglas A.J.
Centre for Integrative Physiology, University of Edinburgh, Edinburgh, United Kingdom; adouglas@staffmail.ed.ac.uk

Pulsatile oxytocin secretion into the blood is important for inducing uterine contractions during birth. Magnocellular oxytocin neurones become activated during parturition, exhibiting increased firing rate with superimposed synchronised burst firing that generates the pulses of oxytocin secretion *(Summerlee 1989)*. However, in parturition the underlying mechanisms of activation of oxytocin neurones are not well understood. We have used an anaesthetised rat model to investigate oxytocin neurone activity during birth. Administration of oxytocin pulses i.v. at term pregnancy enhances strong uterine contractions and, as in conscious rats, induces the birth of pups. This triggers enhanced magnocellular oxytocin neurone activation, including increased firing rate and Fos expression. Possible mechanisms that underlie this activation include activation of the nucleus of the tractus solitarius (NTS) noradrenergic pathway, which relays uterine signals to the hypothalamus. NTS neurones that project to the supraoptic nucleus (SON) and that express tyrosine hydroxylase (rate limiting enzyme in noradrenaline synthesis pathway) become activated during parturition. Uterine contraction causes intermittent noradrenaline release within the SON *(Douglas et al 2001)*, and infusion of alpha1-adrenergic antagonist into the SON prevents the uterine-contraction induced Fos expression. Also, alpha1-adrenergic agonist, phenylephrine, given centrally greatly accelerates the speed of birth of pups during spontaneous parturition. Since phenylephrine facilitates burst firing of oxytocin neurones in vitro *(Wang and Hatton 2004)*, we hypothesise a role for brainstem noradrenergic neurones in the generation of oxytocin neurone bursting activity. Therefore, co-ordinated uterine and brain mechanisms at term pregnancy generate a positive feedback loop which precipitates birth. *Supported by The Wellcome Trust*

SF14-65

Differential mechanisms of synaptic plasticity control hippocampal interneuron networks

Pelkey K.[1], Topolnik L.[2], Lacaille J-C.[2], McBain J.C.[1]
[1]NICHD/LCSN, NIH, Bethesda, MD, USA, [2]Physiol, U. Montreal, Montreal, PQ, Canada; mcbainc@mail.nih.gov

mGluR7 activation and surface expression regulates bidirectional plasticity of feedforward inhibition in the hippocampal mossy fiber (MF) pathway. At naïve MF inputs to stratum lucidum interneurons (SLINs) surface mGluR7 activation during high-frequency stimulation (HFS) yields presynaptic long-term depression (LTD) through a PKC-dependent mechanism. This activity-induced LTD is mimicked by pairing basal synaptic stimulation with mGluR7 activation by exogenously applied agonist (L-AP4, 400 μM for 5 minutes). Surprisingly, L-AP4-induced LTD does not simply occlude HFS-induced LTD but rather unmasks the ability of HFS to induce presynaptic MF-SLIN long-term potentiation (LTP). We now demonstrate that L-AP4-induced LTD unlocks the ability of MF-SLIN inputs to enhance release in response to increased cAMP levels. As previously reported transmission at naïve MF-SLIN synapses was not significantly affected by forskolin treatment (50-100 μM for 5 minutes) remaining at 87% of control transmission assayed prior to forskolin application (n=4). In contrast, following L-AP4-induced MF-SLIN LTD to 63% of control responses, forskolin returned (de-depressed) MF-SLIN EPSCs to 142% of control responses (n=7). Finally, we found that conversion of depressing MF-SLIN inputs to potentiating ones is also revealed with multiple rounds of HFS. After two rounds of HFS MF-SLIN EPSCs were reduced to 55% of control responses (obtained prior to any HFS), but following subsequent HFS EPSCs de-depressed to 99% of initial control values measured prior to any HFS conditioning stimulation. We propose that MF-SLIN LTP proceeds by a cAMP-dependent cascade that can only be engaged following agonist-induced mGluR7 sequestration from MF-SLIN terminals, yielding state-dependent cAMP sensitivity of MF-SLIN transmission.

SF14-66

Disinhibition induced synchrony in the hippocampus: emergence, threshold and pacemaker phenomena

Wittner L.[1,2], de la Prida L.M.[1,3], Cohen I.[1], Huberfeld G. [1], Miles R.[1]
[1]INSERM U739, Paris, France; [2]Inst for Psychology, Budapest, Hungary; [3]Institute Cajal, Madrid, Spain; rmiles@chups.jussieu.fr

Suppression of fast GABAergic signalling induces periodic population bursts initiated in the hippocampal CA3 region. We examined the emergence of this activity, the existence of a threshold firing frequency for initiation of bursts and factors underlying initiation at specific sites in the CA3 region. Multi-unit records show that synchronisation of CA3 population discharges induced by bicuculline (10μM) or picrotoxin (50μM) does not emerge suddenly but evolves through partially synchronous activities. They consist of recurring bursts of firing associated with a field potential which increases in amplitude until fully synchronous firing is established. Partially synchronous bursts are terminated by a silent period corresponding to an intracellular hyperpolarisation mediated by $GABA_B$ receptors. During full synchrony, all pyramidal cells discharge during synchronous bursts of duration 50-100 ms. Each burst is preceded by an acceleration of multi-unit firing to a frequency higher than at any other time between bursts. Threshold is modulated by changing cellular excitability or altering excitatory synaptic efficacy and exceeded by induced firing in single pyramidal cells. Extracellular records show that population bursts are always initiated in the CA3a region. Our data suggests that the pacemaker region is defined by both an enhanced recurrent synaptic connectivity and a higher cellular excitability. CA3a cells are more likely to fire discharge in bursts than CA3b cells. Estimates from axonal and dendritic distributions suggest that they receive ~20% more recurrent synapses. CA3a pyramidal cells tend to discharge before population bursts and trigger firing in multi-unit records with a higher probability than CA3b cells so fulfilling two requirements for a pacemaker role.

SF14-67

Maturation of population coherence in the hippocampus

Crepel V.[1], Aronov D.[2], Jorquera I.[1], Represa A.[1], Ben-Ari Y.[1], Cossart R.[1]
[1]INMED, INSERM U29, Parc Scientifique de Luminy, BP. 13, Marseille, France; [2]M.I.T, Cambridge, MA, USA
cossart@inmed.univ-mrs.fr

Correlated neuronal activity is instrumental in the formation of networks, but its emergence during maturation is poorly understood. In my talk I will present how the development of population coherence from embryonic to postnatal stages in the hippocampus can be followed by multibeam two-photon calcium microscopy combined with targeted electrophysiological recordings. In particular I will describe a new form of immature correlated activity (SPAs : Synchronous Plateau Assemblies) that synchronizes at birth small cell assemblies coupled by gap junctions. These neuronal assemblies generate synchronous nonsynaptic calcium plateaus associated to recurrent burst discharges. I will present the mechanisms responsible for the generation of SPAs. I will demonstrate how, in the developing hippocampus, delivery is an important signal that triggers the first coherent activity pattern, which is silenced by the emergence of synaptic transmission.

SF14-68

Interneurons and development of spinal cord network activity; substrates for locomotion

Furlan F.[1], Sibilla S.[1], Guasti L.[2], Grandolfo M.[3], Ballerini L.[1]
[1]Physiol, Univ. Trieste, Italy [2]Pathol, Univ. Firenze, Italy; [3]Neurobiology, SISSA-ISAS, Trieste, Italy; ballerin@psico.units.it

GABAergic interneurons in the spinal cord display late embryonic and early postnatal expression and repression in accordance with network operation. We investigated the dynamic distribution of GABAergic neurons together with their excitability at different embryonic stages by using mouse organotypic spinal cultures at 8 and 14 days in vitro (DIV). By immunocytochemistry for the GABA-synthesizing enzyme (GAD67), we showed that at 8 DIV, GAD67-labeled neurons were numerous and evenly distributed along the ventral/dorsal axis of the spinal slice. At 14 DIV, GAD67-positive neurons decreased of more than four-fold and displayed a clear dorsal distribution. Next we addressed the temporal expression of ERG, a potassium conductance expressed only transiently by developing spinal interneurons. At 8 DIV ERG-positive cells were numerous, and scattered throughout the slice. As observed for GAD67-positive cells, at 14 DIV a five-fold decrease in ERG positive neurons was accompanied by a dorsal to ventral gradient. Confocal microscopy analysis of simultaneous immunostaining with anti-GABA and anti-ERG antibodies revealed that ERG-positive cells represented mainly GABAergic interneurons. Patch clamp recordings confirmed these data, showing that ventral interneurons expressed functional ERG currents [$I_{K(ERG)}$] only transiently. We next examined the role of the $I_{K(ERG)}$. In organotypic cultures, only ventral interneurons with fast adaptation and GABA-immunoreactivity, and only after one week in culture, were transformed into high frequency bursters by E4031, a selective inhibitor of $I_{K(ERG)}$. These data suggest that, during an early stage of spinal cord development, the excitability of GABAergic ventral interneurons depended, at least in part, on the function of $I_{K(ERG)}$.

SF15-69

Molecular mechanisms underlying kcnq1 voltage-dependency: the channel gate is locked closed by the S4-S5 linkers

Choveau F., Rodriguez N., Dahimene S., Boudin H., Rose T., Le Henaff C., Merot J., Escande D., Baro I., Loussouarn G.
L'institut du thorax, INSERM U533, Nantes, France
gildas.loussouarn@nantes.inserm.fr

Aims: Voltage-dependent potassium channels are tetramers of six transmembrane domain (S1-S6) proteins. Recent crystallographic data demonstrate that the tetrameric pore (S5-S6) is surrounded by four voltage sensor domains (S1-S4). One key question remains: how do the voltage sensors (S4) regulate the pore gating? We tested the hypothesis that the S4-S5 linker is a voltage-dependent ligand binding to the S6 C-terminus and locking the channel in a closed state. **Methods:** We designed plasmid-encoded peptides corresponding to portions of the S4-S5 linker and the S6 C-terminus of the voltage-gated potassium channel KCNQ1 and evaluated their effects on the activity of the KCNE1-KCNQ1 fusion protein. **Results:** Two different S4-S5 peptides inhibited the KCNQ1 function, consistent with the hypothesis. For example, L251-L266 decreased the current density from 22.6 ± 2.9 pA/pF (n = 57) to 10.78 ± 2.6 pA/pF (n = 25) and slowed down the activation kinetics. Two S6 C-ter peptides upregulated KCNQ1, consistent with the S6 C-terminus being the receptor for the endogenous S4-S5. Absence of effect of these peptides on Herg channel suggests the specificity of the interaction. Both kinetic and structural models of the channel were challenged by addition of peptides bound states. The computerized data illustrate well the experimental effects of S4-S5 and S6-C-ter peptides on the channel activity. **Conclusion:** Our results suggest a mechanistic model in which the channel gate is locked closed by the four S4-S5 linkers in a voltage-dependent manner. We anticipate these results to reflect a general mechanism for voltage-gated channels.

SF15-70

Nervous system K_v7 channelopathies

Lerche H.
Department of Neurology, University of Ulm, Ulm, Germany
holger.lerche@uni-ulm.de

Abstract not submitted.

SF15-71

Gating and assembly of $K_v7.1$ channels: multi-modular structures

Attali B.
Department of Physiology & Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel; battali@post.tau.ac.il

Aim: K_v1 (KCNQ1) potassium channels are members of the superfamily of voltage-gated K^+ channels. The $K_v7.1$ pore-forming subunit interacts with the KCNE1 auxiliary subunits to form the slow I_{KS} K^+ current which plays a major role in repolarizing the cardiac action potential. Mutations in either $K_v7.1$ or KCNE1 genes produce the long QT (LQT) syndrome, a life-threatening ventricular arrhythmia. **Methods:** Using patch-clamp, crystallographic and biochemical techniques, we examined the biophysical and structural properties of two important gating modules of $K_v7.1$, the pore and the C-terminus. **Results and Conclusions:** Here we show that long QT mutations located in $K_v7.1$ C-terminus impair calmodulin (CaM) binding which affects both channel gating and assembly. The mutations produce macroscopic inactivation and dramatically hinder channel assembly. KCNE1 forms a ternary complex with $K_v7.1$ and Ca^{2+}-CaM which prevents inactivation, facilitates channel assembly and mediates Ca^{2+}-sensitive stimulation of I_{KS}-current. The C-terminus proximal half associates with one CaM constitutively bound to each subunit where CaM is critical for proper folding of the whole intracellular domain. The distal half directs tetramerization, employing tandem coiled-coils. The first coiled-coil complex is dimeric that undergoes concentration-dependent self-association to form a dimer of dimers. The outer coiled-coil is parallel tetrameric, whose details have been elucidated based on 2.0 Å crystallographic data. Both coiled-coils act in a coordinate fashion to mediate the formation and stabilization of the tetrameric distal half. Functional studies including characterization of structure-based and LQT mutants prove the requirement for both modules and point to complex roles for these modules including folding, assembly, trafficking and regulation.

SF15-72

Regulation of neural K_v7 channels

Hernandez C.C., Zaika O., Shapiro M.S.
Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; shapirom@uthscsa.edu

Neural M-type (KCNQ/K_v7) K^+ channels control somatic excitability, bursting and neurotransmitter release throughout the nervous system. Their activity is regulated by multiple signaling pathways triggered via stimulation of numerous receptors. One pathway involves depletion of phosphatidylinositol 4,5-bisphosphate (PIP_2) in the membrane, whose interaction with the channels is thought necessary for their function. Another pathway requires IP_3-mediated intracellular Ca^{2+} (Ca^{2+}_i) signals and calmodulin action. We seek to localize the sub-domains on the channels critical to their differential affinity for PIP_2, and the mechanisms of receptor-specific modulation of M-type channels via these two intracellular pathways. cDNA clones for wild-type and mutant K_v7 channels were expressed in mammalian CHO cells and studied via single-channel patch or perforated-patch voltage clamp. Recordings from chimeras between high-PIP_2 affinity $K_v7.3$ and low-PIP_2 affinity $K_v7.4$ channels indicate the linker between the 1st and 2nd homologous domains in the carboxy-terminus determines PIP_2 affinity, and is a possible binding site. Point mutants within this linker in $K_v7.2$ and $K_v7.3$ further localize the critical region to a cluster of basic residues. In superior cervical ganglion sympathetic neurons, muscarinic M_1, angiotensin II AT_1, bradykinin B_2 and purinergic P2Y agonists suppress M current (I_M). In these neurons, stimulation of B_2 and P2Y, but not M_1 nor AT_1, receptors induce Ca^{2+}_i signals. Expression of IP_3 scavengers or IP_3 phosphatases, and wild-type or dominant-negative calmodulin, shows bradykinin and purinergic, but not muscarinic nor angiotensin, suppression of I_M to require IP_3 accumulation and Ca^{2+}_i signals in concert with calmodulin. We group these receptors into two fundamental modes of action.

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SF15-73

Functions of K_v7 channels in principal cortical neurons

Yaari Y.
Department of Physiology, Hebrew University–Hadassah School of Medicine, Jerusalem, Israel
yaari@md.huji.ac.il

K_v7 (KCNQ) channel subunits are widely expressed in peripheral and central neurons, where they give rise to a muscarinic-sensitive, subthreshold and noninactivating K^+ current (M-current). Until recently it was thought that M-current contributes to spike frequency adaptation during sustained depolarisations, but is too slow to influence the repolarisation of solitary spikes. This concept, however, was based mainly on experiments with muscarinic agonists, whose multiple effects on membrane conductances may overshadow the distinctive effects of M-current block. Using more selective blockers of K_v7 channels (linopirdine and XE991) we have recently shown that M-current plays a crucial role in spike termination in principal cortical neurons, such as CA1 and CA3 pyramidal cells. A solitary spike in these neurons is followed by a distinct, albeit subthreshold, spike afterdepolarisation (ADP). Blocking M-current generated by perisomatic K_v7 channels greatly facilitates the spike ADP beyond spike threshold, thereby converting solitary ('simple') spikes to high-frequency bursts of 3-7 spikes ('complex' spikes). This dramatic effect is not seen when other K^+ currents are selectively blocked. Thus, perisomatic K_v7 channels normally down-regulate the spike ADP and limit its duration, thereby precluding its escalation to a spike burst. Intriguingly this action is strongly modulated by intracellular Ca^{2+}, which can facilitate the spike ADP and induce bursting by inhibiting K_v7 channel activity.

SF15-74

K_v7 channel modifying drugs

Hansen H.
NeuroSearch A/S, Ballerup, Denmark
heh@neurosearch.dk

Abstract not submitted.

ST16-75

The role of the Educational Task Force in the FEPS organization

Snoeckx L.H.E.H.
Dept. Physiology, Maastricht University, The Netherlands
l.snoeckx@fys.unimaas.nl

Physiology teaching in Europe is one of the important issues to be addressed by FEPS. Because of such important new developments as change from classical curricula to curricula integrated with other fields and to curricula based on Problem Based Learning (PBL), disappearance of departments of Physiology resulting in loss of visibility of Physiology as a discipline on its own right, it was felt that a Task Force was needed. The first main goal of this Task Force should be to identify the common grounds of an academic European Physiology program, including the description of end-terms and competencies. This information could be very useful for the various national societies since they could help in establishing the generally accepted disciplinary end-terms. Aside from that, a survey is needed of the various approaches of educational methodologies, as well as of the Physiology-related educational research which is performed in each country. Furthermore, experts in various domains of educational methodologies have to be identified. Results of these inquiries will be published on the FEPS website, as well as published in international journals. Specific topics will be selected by the Task Force group to be presented on the annual workshop on Teaching in Physiology, which will be organized in concert with the FEPS meeting.

ST16-76

Collaborative learning as the base for developing innovative learning environments in higher education

Valcke M.
Department of Education, Professor Instructional Sciences, Ghent University, Belgium
martin.valcke@ugent.be

Based on a five-year research programme, this keynote will focus on key design guidelines to develop effective and efficient innovative learning environments in higher education. A variety of models will be discussed to develop collaborative learning in the domain of medicine, pharmacy, business sciences, education, teacher training, etc. Evidence-based approaches will centre on scripting, role assignment, task design, group structure, course development, tutoring, coaching and evaluation. The research models will centre on the necessity to adopt complex evaluation approaches to take into account the full complexity of the teaching and learning context.

ST16-77

A student-driven wiki-library for educative materials: concept, implementation, and evaluation for the Medical Curriculum Munich

Berger M., Stormann S., Fischer M.R.
"Schwerpunkt Medizindidaktik", University of Munich Hospital, Ludwig-Maximilians-University (LMU), Munich, Germany
michael.berger@med.uni-muenchen.de

Problem: The new study regulation for medical licensing in Germany has caused significant reform of the medical curricula in Germany. At Munich University (LMU), the medical curriculum Munich (MeCuM) was introduced in 2004. More individual responsibility of the students regarding their academic goals as well as a problem-oriented thought process are essential features of the new curriculum. Through our project, an open web based platform for the design of curriculum-specific teaching and study material has become accessible to students and faculty of the MeCuM. **Methods:** Students created an online library in harmony with the curricular structure of the MeCuM. The technical implementation was carried out with the MediaWiki-Open-Source software. Contents can be edited from all students or faculty of the MeCuM in an anonymous or personalized manner. An online questionnaire was carried out to evaluate the project. **Results:** Our online library as a wiki is the first solution of this kind in medical education in Germany. The concept was implemented without technical difficulties and is linked to the official websites of LMU Munich. The website's contents were visited 417,808 times from December 2005 through June 2007. Online-based evaluation showed that the project is positively recognized, and students are motivated to actively contribute. **Discussion:** The project closely reflects the academic curriculum of the MeCuM and creates additional opportunities for interaction between students and teachers. The design as an open source project allows easy handling as well as high control and ability to update the contents, but integrity and accuracy of the data content require further effort. Further evaluation will be essential for improvements. The integration of faculty as reviewers and tutors is planned. Sustainability of the project is dependent upon continued student leadership. Other medical faculties are interested in this concept.

ST16-78

Integrating e-learning in physiology – the Kiel experience

Karsten G.
Dept. of Physiology Christian-Albrechts-University of Kiel, Germany
g.karsten@physiologie.uni-kiel.de

At the medical faculty of the University of Kiel, e-learning is integrated into the physiology curriculum covering lectures, lab courses, seminars, propaedeutics and a physiology forum. In "blended learning" scenarios this method supplements more traditional methods of teaching and learning with the objective to support students' learning. For example, face-to-face teaching in lectures focuses on the presentation of concepts and interrelations. Students use the faculty's e-learning platform NICKELS (http://www.e-learning-service.de/) in order to repeat the content of the lectures, to enhance their knowledge by working on special e-learning modules for self determined learning, to get feedback about their learning progress and to pose questions to the lecturer. In physiology lab courses, e-learning is utilized to supplement the traditional manuals, e.g., with videos demonstrating the performance of the experiments. A skills lab ("introduction to clinical medicine") in the 4th term aims at integrating clinical and preclinical knowledge. The learning of medical examination techniques is coupled with the repetition of the anatomical and physiological basics as well as with typical patient cases in which these techniques are applied. This novel approach was developed in the innovative joint project "CliSO – Clinical Skills Online" with the medical faculty of the LMU Munich. Two platforms that complement each other were connected with regard to technique and content: NICKELS which is specially suited for systematic learning and communication and CASUS which focuses on case based learning. Before attending a face to face lab course where the skills are learned and practiced in small groups, students work through a patient case, repeat basic knowledge and get first standardized demonstrations of the skills in videos via the e-learning platforms. Evaluations reveal that this approach is very much appreciated by the students.

Oral Presentations

OW01-1

Arrhythmogenic Ca^{2+} waves in ventricular myocytes initiated by local activation of Ca^{2+} release channels

Hagen B.M., Ni J., Kao J.P.Y., Lederer W. J.
Molecular Cardiology Research Unit, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland, USA
hagen@umbi.umd.edu

Aims: Propagating waves of elevated intracellular Ca^{2+} ($[Ca^{2+}]_i$) are abnormal events that occur at the subcellular level in diverse models of heart dysfunction. Such Ca^{2+} waves activate the Ca^{2+}-dependent arrhythmogenic inward current that contributes to early and delayed afterdepolarizations. Although abnormally high levels of Ca^{2+} within the sarcoplasmic reticulum (SR) are suspected to underlie such Ca^{2+} waves, in some animal models of heart disease Ca^{2+} waves can develop when SR Ca^{2+} levels are relatively low. In this study we used sensitizers of RyR2 (the cardiac SR Ca^{2+} release channel) to activate Ca^{2+} waves in the presence and absence of SR Ca^{2+} overload. **Methods:** Ca^{2+} waves initiated by sub-millisecond local UV photolysis of a caged caffeine analog or caged Ca^{2+} were viewed by confocal microscopy in cells loaded the Ca^{2+} indicator Fluo-4. Whole-cell patch-clamp technique was used to measure membrane current and control voltage. **Results:** In isolated wild-type murine ventricular myocytes, localized UV photolysis initiated one of two responses: (1) a transitory but confined increase in $[Ca^{2+}]_i$, or (2) a Ca^{2+} wave. Analysis suggests that, unlike Ca^{2+} waves seen in Ca^{2+} overload that can be initiated by a single Ca^{2+} spark, Ca^{2+} waves under normal SR Ca^{2+} load required the activation of a larger volume ($40.2 \pm 7.5\ \mu m^3$) of Ca^{2+} release units. **Conclusions:** Arrhythmogenic Ca^{2+} waves can be initiated without overloading the SR with Ca^{2+}, but do require the activation of ~17 adjacent RYR2 clusters. How this may occur and the modeling implications of these findings will be discussed.

OW01-2

The effects of luminal calcium on the stability of coupled-gating between ryanodine receptors

Gaburjakova J., Gaburjakova M.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia
jana.gaburjakova@savba.sk

Aims: In cardiac muscle, the intracellular trigger for contraction is a transient rise in intracellular free Ca^{2+} released from the sarcoplasmic reticulum (SR) through a major intracellular Ca^{2+} channel - ryanodine receptor (RYR). Two or more RYR channels reconstituted into bilayer lipid membrane (BLM) can open and close either independently (single gating) or simultaneously (coupled gating). The coupled gating phenomenon has been suggested as an attractive candidate for a termination mechanism of Ca^{2+} release from the SR required for periodic contraction and relaxation of cardiac muscle. **Methods:** Using the method of reconstitution of a channel into BLM we investigated the potential effect of luminal Ca^{2+} on the stability of coupling between RYR channels isolated from the rat heart. We introduced a parameter of coupling stability for each detected simultaneous opening and closing and further averaged for experiments performed under identical conditions. **Results:** We found that the stability of coupling during simultaneous opening of RYR channels was significantly lower in comparison to the simultaneous closing under same experimental conditions. Furthermore, high concentration of luminal Ca^{2+} (53mM) as well as the absence of luminal Ca^{2+} noticeable destabilized functional coupling between RYR channels during opening, in contrast to lower tested concentrations (8mM-20mM). **Conclusion:** We provided experimental evidence that the stability of coupling between RYR channels depends on the functional state of channels. Furthermore, we pointed for the first time to the regulation role of luminal Ca^{2+} in the strength of interaction between coupled RYR channels in the heart.
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OW01-3

Novel stochastic model for calcium release unit in cardiomyocyte

Moskvin A.S., Ryvkin A.M., Solovyova O.E., Kohl P, Markhasin V.S.
Ural State Univ., Russia; Inst. Immunol. Physiol., Ekaterinburg, Russia; Lab. Physiol., Univ. Oxford, United Kingdom; alexandr.moskvin@usu.ru

Aims: The main goal of the study was to propose a simple, biophysically reasonable electron-conformational theory for the ryanodine receptor channel (RyR) and, on that basis, present a stochastic model to describe RyR cluster and Ca^{2+} release unit (RU) in cardiomyocytes. **Methods:** In addition to a fast electronic degree of freedom, the RyR channel is characterized by a slow classical conformational coordinate, Q, obeying Langevin dynamics, which specifies the RyR channel calcium conductance. The RyR gating is specified by conformational dynamics, Ca^{2+} induced direct electronic transitions, quantum tunnelling and thermal transitions. To account for the RyR cooperativity effects we have introduced the RyR-RyR conformational coupling. **Results:** We have reproduced all the features of single RyR gating both under stationary conditions and Ca^{2+} stimulus, including the activation-inactivation phenomenon. Calcium RU modelled by the 11×11 RyR cluster revealed different regimes depending on the sarcoplasmic reticulum (SR) Ca^{2+} loads and lumenal Ca^{2+} refilling rate. The optimal mode of RU functioning during Ca^{2+}-induced calcium release implies a fractional release with a robust termination due to a decrease in SR Ca^{2+} load. SR overload leads to instabilities with strong spontaneous low-frequency modulations of Ca^{2+} release and clear tendency to auto-oscillation regime with spontaneous RyR channel opening and closure. **Conclusion:** Electron-conformational model can successfully describe all the features of RyR gating and RU functioning. The model may be used to construct the appropriate discrete-state Markov scheme with physically clear picture of the underlying mechanisms of transitions and algorithms to estimate the appropriate probabilities.
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OW01-4

The role of triadin 95 on calcium homeostasis of skeletal muscle cells

Gonczi M.[1], Fodor J.[1], Sztretye M.[1], Dienes B.[1], Szabo L.[2], Szentesi P.[1], Csernoch L.[1]
[1]Department of Physiology, University of Debrecen, Debrecen, Hungary; [2]Department of Electrical Engineering, Sapientia Hungarian University of Transilvania, Targu Mures, Romania; gonczi_monika@hotmail.com

The 95kDa triadin (Trisk 95), the main isoform in skeletal muscle sarcoplasmic reticulum, interacts with both the ryanodin receptor and a calsequestrin. Earlier reports revealed that Trisk 95 overexpression suppresses depolarization-induced calcium transients on primary cultures of rat skeletal muscle while the caffeine-induced calcium release remains unaffected. To date there are no data on how Trisk 95 overexpression or downregulation would affect the elementary events of calcium release (ECRE). In the present study spontaneous ECRE and calcium transients were studied on C2C12 cells and on primary cultures of skeletal muscle. Liposome- or adenovirus-mediated Trisk 95 overexpression and RNA interference with triadin translation were used to change the level of the protein in these cells. Stable overexpression of Trisk 95 in C2C12 cells significantly decreased the amplitude and frequency of calcium sparks, and the frequency of embers. Similarly, adenoviral transfection of Trisk 95 in primary cultures of mouse skeletal muscle cells significantly decreased both the frequency and the amplitude (0.26±0.01 Hz and 0.56± 0.03 ΔF/F0, n=71) of spontaneous calcium transients as compared to control myotubes (0.75±0.04 Hz and 0.68±0.02 ΔF/F0, n=169). Primary cultures of rat skeletal muscle cells expressing endogenous triadin 95 readily generated spontaneous calcium transients (1.01±0.22 Hz and 0.62±0.05 ΔF/F0, n=553), but rarely produced calcium sparks. Transfection of these myotubes with specific shRNA sequence significantly reduced the triadin-specific immunopositivity. Functional experiments on these cells revealed that ECRE appeared with higher frequency. The results suggest that Trisk 95 negatively regulates excitation-contraction coupling by suppressing the elementary calcium release events.

OW02-5

The effect of aging on mitochondrial enzymes and protein oxidation in rat myocardium. The role of hydroxynonenal

Tatarkova Z., Racay P., Sivonova M., Pavlikova M., Dobrota D., Kaplan P.
Jessenius Faculty of Medicine, Comenius University; Department of Medical Biochemistry, Martin, Slovakia; tatarkova@jfmed.uniba.sk

Aims: The exact cellular and molecular mechanisms of the aging process are unclear, but there is growing evidence, that age-related changes are consequences of oxidative stress. It has been suggested that mitochondria are one of major sources of free radicals and also an important target for their damaging effects. Recent studies have suggested that hydroxynonenal (HNE), is an important mediator of cellular damage associated with oxidative stress. Therefore, in the present study we examined the effect of HNE on cytochrome c oxidase (COX) activity, oxidative modifications of mitochondrial lipids and proteins in rat heart. **Methods:** For experiments were used hearts from rats of three age categories 6, 15 and 26-month old. Fluorescence studies were performed as reported Kaplan et al. (2000). HNE and malondialdehyde were measured according to standard kits. COX activity was measured spectrophotometrically. **Results:** The activity of COX was 44.6 ± 1.6% ($p<0.001$) loss in senescent hearts, whereas the COX I protein level was unchanged. Lipid peroxidation in mitochondrial membranes was increased progressively with age. Activity of COX was inhibited, with concomitant increase in endogenous HNE level. Compared to adult rats, there was significant increase in protein dityrosines, lysine conjugates and decrease in sulfhydryl groups content. **Conclusion:** Our results suggest that loss of COX activity during aging may be due in part to oxidative modifications of mitochondrial proteins and/or lipids. We propose that mitochondrial dysfunction increases in age-dependent manner and is mediated, in part, by modification of specific mitochondrial proteins by the lipid peroxidation product HNE.

OW02-6

Dynamics of cerebellar granule neurones' response to oxygen and/or glucose deprivation in primary cultures

Zagrean A.M.[1], Ceanga M.[1], Mihai G.[1], Le Duc D.[1], Spataru A.[1], Toescu E.C.[2]
[1]Center for Excellence in Neuroscience, Department of Physiology, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; [2]Department of Physiology, Medical School, University of Birmingham, Birmingham, United Kingdom ; azagrean@gmail.com

Aim: Understanding neuronal response to conditions of impaired energy metabolism (hypoxia/ischaemia) is crucial in developing new neuroprotection models. We have used primary cultures of cerebellar granule neurones to assess the neuronal damage resulting from 3-hr exposure to oxygen-deprivation (OD–5% dissolved oxygen), glucose-deprivation (GD), or both in combination (oxygen-glucose deprivation; OGD). **Methods:** Neuronal viability and survival was assessed by use of a combination of fluorescent dyes (cytosolic calcein-AM and nuclear dyes Hoechst33342 and propidium iodide (PI)) and ATP measurements (luminescent assay). **Results:** Significant differences were evident when analysing in detail the dynamic response of the neuronal cultures during this period of metabolic stress. Exposure to OGD induced a degree of cellular death (%PI-positive neurones), larger than that evoked by either OD and GD combined (20.6 ± 0.1% in OGD vs. 9.9 ± 0.1% in GD and 2.9 ± 0.1% in OD). However, morphological inspection of the 3 culture conditions showed that while in OD and GD there were less than 10% and 40% morphologically compromised cells, in OGD these accounted for more than 80%. To investigate this process, we defined, using the cytosolic and nuclear fluorescent dyes (calcein and Hoechst, respectively) a nuclear/cytosolic ratio (NCR) parameter allowing us to identify and separate a population of neurones metabolically impaired (i.e., swollen, with decreased NCR). We also show that the temporal dynamics of this population in OGD cultures reflected the differential decrease in ATP during this period. **Conclusion:** Understanding if and how these metabolically impaired cells can be rescued could open new therapeutic avenues.

OW02-7

Neuronal activation of hypothalamic nuclei involved in food intake and energy homeostasis after prolonged high fat intake in mice

Pirnik Z.[1], Zelezna B.[2], Maletinska L.[2], Kiss A.[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic
ueenpirn@savba.sk

Aims and methods: The main regions of hypothalamus involved in food intake and energy homeostasis are the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMH) and lateral hypothalamic area (LHA). The PVN and DMH are involved in initiating and maintaining of food intake, VMH is considered as satiety and LHA as feeding centre. ARC is accessible to blood circulating signals of energy balance. In our study we used single Fos and double Fos-hypocretin (Fos-Hcrt) and Fos-oxytocin (Fos-OXY) immunostainings to identify neuronal activity in the above mentioned hypothalamic nuclei after 16 weeks of high fat diet (HF) consumption in C57Bl/6 male and female mice. HF diet contained 13, 60, and 27% calories as protein, fat, and carbohydrate, respectively. **Results:** We observed noticable Fos activation of neurons in ARC, VMH and DMH in male vs. female mice independently to consumption of applied diet. On the other hand, prolonged HF diet lead to a significant rise of Fos in orexinergic Hcrt LHA neurons in female mice. The activity of anorexinergic OXY neurons in PVN was not altered in any genders. **Conclusion:** These results may speak out for gender differences in the activity of hypothalamic nuclei regulated food intake and energy balance, which can be in female ones further altered by prolonged HF intake.

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OW02-8

The influence of UCP blockade on the endothelial dysfunction with 1 type experimental diabetes mellitus

Prysyazhna O.D., Sagach V.F.
O.O. Bogomoletz Institute of Physiology NAS of United kingdomraine, Kiev, United kingdomraine
prys@biph.kiev.ua

Aim: Contemporary studies indicate the significant role of uncoupling proteins (UCP) increased expression in the development of complications of diabetes mellitus. An increase of UCP expression leads to the proton leak growth and the reduction of the effectiveness of the mitochondrial respiratory chain work. These changes, in turn, lead to reduction of ATP synthesis and increase of the oxidative stress. The purpose of this work is to investigate the influence of inhibitor UCP genipin introduction on the indices of endothelial dysfunction in the rats with stz-induced diabetes mellitus. **Methods:** Streptozotocin 50 mg/kg was intraperitoneally injected to male rats of the weight 180-220g to reproduce experimental model of diabetes mellitus I type. Endothelium-dependent reactions of aorta and coronary vessels of rats with experimental diabetes mellitus after the single intraperitoneal introduction 10 mg/kg of UCP blocker genipin were studied. **Results:** Experimental diabetes mellitus leads to the appearance of endothelial dysfunction that is revealed by the essential disturbance of endothelium-dependent reactions of the aorta and the coronary vessels both. The genipin injection brings to the partial restoration of disrupted reactions. It points to the role of growth of UCP expression in the appearance of the endothelial dysfunction with I type diabetes mellitus. **Conclusion:** The application of UCP blockers is the forward-looking method of angioprotection and curing the vascular complications of diabetes mellitus.

OW03-9

EEnhanced activity preconditioning via NMDA but not AMPA receptors induced c-Fos and delayed kainate-induced degeneration of hippocampal CA3a neurons

Pavlik A., Mohammadi S., Krajci D., Al-Sarraf H., Alexander G.
Department of Physiology and Anatomy, Faculty of Medicine, Kuwait University; Alfred@hsc.edu.kw

Aims: Intraventricular (icv) injection of kainate (KA) causes an acute excitotoxic lesion to hippocampal CA3 region. NMDA was shown to protect neurons against *in vitro/in vivo* induced cell death. We used *in vivo* approach to study NMDA or AMPA preconditioning of KA–induced neurodegeneration. **Methods:** Preconditioning injections (icv) of NMDA or AMPA (with cyclothiazide) to anesthetized rats (urethane, 1.5 g/kg) were followed by KA (0.15 μg, icv). Light and electron microscopy enabled to evaluate neurodegeneration. c-Fos or Hsp70 immunohistochemistry (IHC) was used to estimate neuronal activation/severe cellular stress, respectively. Quantitative evaluation of morphology and IHC data were complemented by ANOVA with post-hoc analysis of significant differences. **Results:** KA alone depressed c-Fos induction in CA3a already by one hr post injection (-69%, CA3a vs. CA3b, $p \leq 0.01$), causing neurodegeneration with necrotic and apoptotic signs. On the contrary, c-Fos induced by NMDA injection, which preceded KA by one hour, was still elevated in CA3a at 1 hr (+169%) and 2 hrs after KA (+114%, both $p \leq 0.01$). The onset of neuronal degeneration was delayed in similar way indicating transient neuroprotection. However, c-Fos and neurons of CA3a deteriorated by 4 hrs to the same level as those seen after KA alone. NMDA-induced c-Fos expression was completely blocked by MK-801. However, preconditioning with AMPA failed to show any preventive effect. In addition, none of the above treatments induced Hsp70. **Conclusion:** Enhancing synaptic activity of neurons through NMDA specific signalling might be the way to rescue neurons suffering from excitotoxicity in the ischemic or injured brain.
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OW03-10

Electrophysiological correlates of brain stem dysfunction in panic disorder

Jagla F., Jergelova M.
Department of Brain Physiology, Institute of Normal and Pathological Physiology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia;
fedor.jagla@savba.sk

Aim: The lower brain stem neurotransmitter dysfunction is suggested as possible neurobiological basis of panic disorder (PD). We supposed the changes in visual-oculomotor integration which can support the above suggestion. **Methods:** The electrooculographic recordings of gaze refixation accuracy, the gain of the optokinetic nystagmus (OKN), the EEG potentials related to the gaze refixations and the P300 wave triggered by voluntary blocking the reflexive gaze refixations were analysed in PD outpatients at least 1 year after the last panic attack. The patients were working regularly without taking a specific medication. **Results:** The greater interindividual variability of the OKN gain as well as its decrease in relation to increased velocity of moving visual stimuli was found in PD patients. Moreover, their gaze refixations were significantly more inaccurate as compared to 5% inaccuracy in healthy controls. In PD patients the gaze refixation related potentials over the frontal eye fields were of longer duration and when recorded over the parietal eye fields the longer preparation for gaze refixation and attenuated correlates of the oculomotor muscle units at the onset of the gaze refixation were found. **Conclusions:** The results confirm suggested disordered early processing of sensory stimuli in PD. Since the changes persist after successive treatment they point to the considerable fluctuation of the overall activation level in neuronal loops participating in oculomotor circuits. The OKN and gaze refixations with their central correlates are proposed as a part of the battery of electrophysiological diagnostic tests in PD.

OW03-11

Impact of anxiety on the treatment of emotions in schizophrenia: an event-related potentials study

Montedoro C., Rosier V.
Cognitive Neuroscience Unit, University of Louvain and Clinique Sans Souci, Brussels, Belgium
calogero.montedoro@psp.ucl.ac.be

Aims: Observe the influence of anxiety, evaluated with the Spielberger anxiety-feature scale (1993), on the emotional recognition within a population of 14 schizophrenic patients and 7 control subjects. **Methods:** We subdivided the population of subjects in three groups according to the anxiety score obtained (1: control, 2: moderate anxiety, 3: strong anxiety). Participants were confronted with a visual face-detection task, in which they had to detect deviant faces amongst a train of standard stimuli (neutral faces). Deviant faces changed either on identity (different identity, neutral expression), or on emotion (same identity, happy, fearful or sad expression). **Results:** Anxiety influences, for all deviant stimuli, the amplitude of the P100 (F: 8.95; sig: 0.002), N170 (F: 7.26; sig: 0.005), P300 (F: 6.95; sig: 0.006) and the N400 (F: 5.519; sig: 0.014). With regard to latency, anxiety slows down data processing for all deviant stimuli on the P100 (F: 5.38; sig: 0.015) and P300 (F: 6.151; sig: 0.009). On the N170 component, we observed an increased latency only for fear (F: 7.691; sig: 0.004). Finally, we observe a laterality effect and an anxiety-deviant stimuli interaction. **Conclusion:** ERP results suggest that anxiety has an influence on each stage of the emotional treatment.

OW03-12

Prenatal testosterone influence on reelin expression associated with the pathogenesis of autism

Kelemenova S., Celec P., Hodosy J., Palffy R., Behuliak M., Ostatnikova D.
Comenius University, Faculty of Medicine, Department of Physiology, Bratislava, Slovakia
skelemenova@gmail.com

Testosterone as a sex steroid hormone plays a crucial role in neurodevelopment. Autism is the most genetically based neurodevelopmental disorder, although the genetic basis of its manifestation is not understood. Different gene defects have been observed in different autistic cases, including the gene encoding reelin protein. It has a major role in neuronal migration and during prenatal development of neuronal connections. Autistic brain has been described as a hyper-masculine, because of high systemization and spatial cognitive abilities. High prenatal testosterone levels seem to result in masculinised brain anatomy and behavioural patterns. It was found that testosterone influences the expression of reelin in brains of male starlings. Thus it is possible that the linkage between testosterone levels and reelin mediated neuronal development exists also in mammals, including humans. **Aims:** Our purpose was to reveal the possible relationship between testosterone levels and reelin expression and to explain one of the possible mechanisms of Autism pathogenesis. **Methods:** Pregnant female rats were exposed to high testosterone doses during 2 weeks of pregnancy. In newborns, reelin expression was measured in blood and specific brain regions using Real Time PCR and Western Blot analysis. Outgrowth rats were tested for cognitive spatial visualization using Morris water maze tasks. Reelin expression was also evaluated. **Results and Conclusions:** Reelin expression was measured in the brain and blood of newborn rats, where mother rats were treated with testosterone in comparison with control group treated with oil. Male and female rats were assessed separately. Results were statistically evaluated.

OW04-13

Heart rate variability is altered in non-complicated diabetic patients

Viggiano A.[1], Vicidomini C.[1], Monda M.[1], Carleo R.[2], Carleo D.[1], Messina G.[1], Viggiano A.[1], Viggiano E.[1], De Luca B.[1]
[1]Dept. Experimental Medicine, Second University of Naples, Naples, Italy; [2]Complex Operative Unit in Diabetology, S. Gennaro Hospital, Naples, Italy
andrea.viggiano@unina2.it

Aims: The aim of the present preliminary study was to describe a simple protocol for the analysis of the heart rate variability that can reveal the different autonomic control between non-complicated diabetic patients and normal subjects within 15 min. **Methods:** The power spectrum of the HRV was evaluated on 5-min long ECG recordings in both the supine and the seated positions in 30 non-complicated non-insulin-dependent diabetic (NIIDM) patients and in 30 healthy volunteers. **Results:** In healthy subjects the LF value was higher in seated position than in supine position, while in diabetic patients the LF value in supine position did not differ from that in seated position and did not differ from that in healthy subjects in supine position. **Conclusion:** The present work demonstrates that the protocol described reveals a different autonomic regulation of the heart rate in healthy subjects and in NIIDM patients even if there is not a clinically evident autonomic neuropathy.

OW04-14

The heart rate and blood pressure variabilities in children and adolescents with obesity

Tonhajzerova I., Javorka M., Trunkvalterova Z., Chroma O., Javorkova J., Javorka K., Lazarova Z.
Department of Physiology, Clinic of Children and Adolescents, Jessenius Faculty of Medicine, Comenius University; University Hospital, Martin, Slovakia
tonhajzerova@jfmed.uniba.sk

Aim: Obesity is a very serious problem even in children and adolescents. The purpose was to study cardiovascular autonomic regulation using heart rate and blood pressure variabilities analyses in obese children and adolescents. **Methods:** Twenty obese and 20 nonobese (control) subjects (12-18 years) were examined at rest in lying position. Evaluated parameters: Heart rate variability (HRV): the mean R-R interval, rMSSD, pNN50, spectral powers in high (HF), low frequency (LF) bands and total power (TP); blood pressure variability (BPV): the mean systolic and diastolic blood pressure, spectral powers in low and high frequency bands. The spectral power in high frequency band of HRV (HF-HRV) was considered as an index of parasympathetic activity, the spectral power in low frequency band of BPV (LF-BPV) was taken as an index of sympathetic control. **Results:** Obese subjects have significantly shortened mean R-R interval and higher mean systolic and diastolic blood pressure compared to controls. HRV parameters: The pNN50, rMSSD, logHF, logTP were significantly lower in obese compared to nonobese group. BPV parameters: No significant differences were found in these parameters. **Conclusion:** Higher mean values of heart rate and blood pressure, decreased parasympathetic activity (↓ rMSSD, pNN50, logHF, logTP) without alteration in sympathetic vasomotor control - was found in obese children and adolescents. We suppose that impaired parasympathetic regulation is present before sympathetic dysfunction.

OW04-15

Effect of proadrenomedullin N- terminal 20 peptide and calcitonin on contractile force and rate in isolated rat hearts

Kaygisiz Z.; Department of Physiology, Medical Faculty, Eskisehir Osmangazi University, Eskisehir, Turkey; ziyak@ogu.edu.tr

Aims: There are conflicting results dealing with effect of proadrenomedullin N-terminal 20 peptide (PAMP) on coronary perfusion pressure (PP), contractile force (CF) and heart rate (HR). Little is known about effect of calcitonin on PP, CF and HR. Furthermore, action of human and rat PAMP as well as salmon and rat calcitonin has not been compared. Therefore, we have studied possible effect of these peptides on PP, left ventricular developed pressure (LVP) as an index of CF and HR in isolated rat hearts. **Methods:** The hearts were perfused with modified Krebs-Henseleit solution under constant flow (12 ml/min) condition. Rat PAMP, human PAMP, rat calcitonin and salmon calcitonin were infused to separate groups of hearts. **Results:** Rat PAMP (n=7) did not affect PP. However, it increased HR (at 10^{-9} M from 257.83±9.55 to 282±9.98 beats/min, mean±SE, $p<0.05$ at 10^{-8} M from 259.83±10.02 to 289.83±7.8 beats/min, $p<0.001$ and at 10^{-7} M from 249.66±7.67 to 280.5±10.1 beats/min, $p<0.001$). Rat PAMP at a dose of 10^{-9}, 10^{-8} and 10^{-7} M decreased LVP from 90.5±7.39 to 78.66±5.08 mmHg, ($p<0.01$), from 88±4.04 to 73±4.95 mmHg ($p<0.01$) and from 79.83±3.59 to 64.83±4.05 ($p<0.001$), respectively. Rat calcitonin (n=7) did not alter PP and slightly decreased LVP. The peptide at a dose of 10^{-8} M also slightly decreased HR. Rat calcitonin at 10^{-7} and 10^{-6} M concentrations significantly decreased HR from 263.83±10.93 to 247±14.57 beats/min ($p<0.01$) and from 285±12.96 to 264±15.93 beats/min ($p<0.001$), respectively. Human PAMP and salmon calcitonin did not change significantly PP, LVP and HR when all the respective concentrations applied. **Conclusion:** Our results suggest that rat PAMP may produce positive chronotropic and negative inotropic effects. In contrast, rat calcitonin may induce a negative chronotropic effect.

OW04-16

Dose dependent effects of monoxidine on the parasympathetic activity

Turcani M; Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait; mturcani@gmx.net

Aims: Moxonidine is successfully used to reduce blood pressure (BP) in essential hypertension. However, its administration to heart failure patients was associated with increased mortality. Hypothesis was tested that a parasympatholytic effect could be responsible for this unexpected outcome of the MOXCON trial. **Methods:** Wistar male rats (8) were implanted with telemetric transmitters to monitor BP, ECG, temperature and animal activity. Moxonidine (40, 120, 360, 1080 μg/kg) was applied s.c. in four successive days. Cardiovascular and respiratory oscillations were analysed. **Results:** Low dose moxonidine (120 μg/kg) reduces overall intensity of the autonomic modulation of the cardiovascular system (-59% total variance (TV) of heart rate (HR), $p<0.05$; -66% TV of mean BP, $p<0.05$). Sympathetic system was affected more (-62% low frequency variance (FV) of diastolic BP, $p<0.05$) than the parasympathetic system (-15% cardiac vagal index, $p<0.05$). High dose (1080 μg/kg) attenuated the total BP variance (-56%, $p<0.05$) but augmented total HR variance (+41%, $p<0.05$). Sympathetic activity was diminished stronger than with the low dose (-85% low FV of diastolic BP, $p<0.05$), however the parasympathetic modulation was intensified (+170% RMSSD of HR, $p<0.05$). Baroreflex sensitivity was increased as well (1.12ms/mmHg control vs. 2.37ms/mmHg after high dose moxonidine, $p<0.05$). A dose dependent reduction in the respiratory frequency was also registered (-17% after high dose, $p<0.05$). **Conclusion:** Lower doses of moxonidine seem to have parasympatholytic effects while higher doses are parasympathomimetic. Depression of respiration might be related to the negative impact of moxonidine in the heart failure patients. Activation of different receptors, i.e. imidazoline and alpha2-adrenergic receptors could be responsible for these dose dependent differences in moxonidine effects.
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OW05-17

Vasopressin increases expression of preproendothelin-1 in rat aortic smooth muscle A7R5 cells. Role of intracellular calcium

Krenek P.[1], Hamaide M.C., Morel N., Wibo M.
Lab. Pharmacol., Univ. cathol. de Louvain, Brussels, Belgium, [1]Faculty of Pharmacy, Comenius Univ., Bratislava, Slovakia; krenek@fpharm.uniba.sk

Aim: Endothelin-1 is a strong vasoconstrictor and growth factor expressed mainly in the endothelium. Under pathological conditions, the peptide can be produced in vascular smooth muscle cells (VSMC). Regulation of preproendothelin-1 (pET-1) expression in VSMC has not been extensively studied. Our aim was to study vasopressin-induced expression of pET-1 in a rat aortic smooth muscle cell line, A7r5. **Methods:** Subconfluent serum-deprived cells were used. Gene expression of pET-1 was quantitated using radioactive RT-PCR followed by phosphorimager analysis. Ribosomal Protein Large-32 (RPL) was used as a housekeeping gene. **Results:** A7r5 cells expressed pET-1 mRNA under basal conditions. Vasopressin concentration-dependently increased the expression of pET-1 with a maximum about 3-fold of the basal value and EC50 of 5.5nM. Transcription blockade with actinomycin D suppressed induction of pET-1 by vasopressin. However, actinomycin D chase experiments showed that vasopressin also stabilized pET-1 mRNA, increasing its half-life from approx. 20-50min. Cycloheximide, an inhibitor of protein synthesis, caused an induction of pET-1 and a superinduction of pET-1 by vasopressin. Removal of extracellular calcium had no effect on pET-1 induction, while chelation of intracellular calcium with BAPTA decreased basal and vasopressin-stimulated pET-1 expression. Thapsigargin stimulated pET-1 expression, which was blocked by BAPTA. **Conclusions:** A7r5 cells express pET-1 mRNA and this expression is increased by vasopressin. Transcriptional and posttranscriptional mechanisms are involved in the regulation of pET-1 biosynthesis in these cells. Release of calcium from intracellular stores, rather than calcium influx from the extracellular space, is important for pET-1 expression in these cells.

OW05-18

S256 and S261 phosphorylation dynamics in AQP2 trafficking

Tamma G.[1,2], Hoffert J.[3], Konings I.B.M.[1], Knepper M.[3], Deen P.M.T.[1]
[1]Dept. Physiol., Radboud Univ. Nijmegen Medical Center, Netherlands; [2]Dept. Gen. Environm. Physiol., Univ. of Bari, Italy; [3]National Heart, Lung, and Blood Inst., Bethesda, USA; g.tamma@biologia.uniba.it

Aim: To increase renal collecting duct water reabsorption, vasopressin binds its V2-receptor and activates a cAMP-dependent pathway, resulting in phosphorylation of aquaporin-2 (AQP2) water channels at Ser256 (p256) and its translocation to the apical membrane. With quantitative phosphoproteomics on rat renal medulla, we found that, besides S256, AQP2 can also be phosphorylated at S261, S264 and S269 and that AVP reduced pS261. We investigate the reciprocal change in S256 and S261 phosphorylation in AQP2 trafficking. **Methods:** MDCK-AQP2 cells were unstimulated (O/N pre-incubated with $5x10^{-5}$M O/N indomethacin), stimulated with 10^{-5}M forskolin for 45min, or incubated with 10^{-7}M phorbol esters (TPA) for 30min following forskolin stimulation. Subsequently, cells were analysed by immunofluorescence or immunoblot. **Results:** In MDCK-AQP2 cells, forskolin induced the translocation of AQP2 from vesicles to the apical membrane, which was reversed with subsequent forskolin/TPA treatment. Immunoblots revealed that forskolin increased pS256 and decreased pS261 AQP2, which were reversed with forskolin/TPA. Similar changes in phosphorylation were found for AQP2- S256A, which mimicks constitutively non-phosphorylated and always localizes to vesicles, and AQP2-S256D, which mimicks constitutively phosphorylated AQP2 and locates in the apical membrane with/without forskolin, but is internalized with forskolin/TPA. Moreover, similar changes in pS256 were found in MDCK cells expressing AQP2-S261A or AQP2-S261D, while both constitutively localize to vesicles. **Conclusion:** Our data indicate that vasopressin induces a reciprocal change in S256 and S261 phosphorylation, which is reversed with forskolin/TPA. These changes occur independent of the localization of the AQP2 protein.

OW05-19

Mineralocorticoid receptor activation is implicated in the adaptation of cardiac potassium currents to pregnancy in rats

Bassien-Capsa V.[1], Chorvatova A.[1,2]
[1]Research Centre of CHU Sainte-Justine; [2]Department of Pediatrics, University of Montreal, Canada; valerie.bassien-capsa@umontreal.ca

Aim: Potassium currents are known to be implicated in cardiac remodelling during pregnancy, yet little is known about their hormonal regulation in this condition. Aldosterone, the hormone acting through its mineralocorticoid receptors (MRs), has been shown to decrease potassium currents in cultured cardiomyocytes. Moreover, aldosterone levels are increased in human and rat pregnancy. Our aim is therefore to examine the possible role of MRs in potassium current adaptations, in left ventricular cardiomyocytes, during rat pregnancy. **Methods:** Pregnant rats, one day before parturition, were compared to non-pregnant ones (NP). One group of pregnant rats was treated with potassium canrenoate (20 mg/kg/day), a MRs antagonist, in drinking water for the last seven days of pregnancy. Patch clamp technique was applied to study potassium currents. **Results:** Pregnancy induced a decrease in the density of the transient outward potassium currents, I_{to}: 5.2±0.4 pA/pF (n=11) vs. 8.8±0.8 pA/pF in NP (n=20), at +50mV; $p<0.05$. MR blockade prevented the lowering of I_{to}: 7.5±1.3 pA/pF (n=10), $p<0.05$. Our results also uncovered a decreased density of the inwardly rectifying potassium current, I_{K1}, in pregnancy: -7.5±1.3 pA/pF (n=14) vs.-13.8±1.8 pA/pF in NP (n=14), at -120 mV; $p<0.05$. MR blockade had a tendency to further decrease I_{K1} density: -4.1±0.6 pA/pF (n=10). **Conclusions:** Our data indicate that MRs are implicated in the adaptation of potassium currents during pregnancy. Understanding the regulation of ionic currents during pregnancy is crucial to determine their possible variations in pathological conditions, such as gestational hypertension, where aldosterone blood levels are decreased.

OW05-20

Phosphatidylinositol-bisphosphate regulates intercellular coupling in cardiac myocytes

Hofgaard J.P.[1], Banach K.[2], Mollerup S.[1], Jorgensen H.K.[1], Olesen S.P.[1], Holstein-Rathlou N.H.[1], Nielsen M.S.[1]
[1]Danish National Research Foundation Centre for Cardiac Arrhythmias and Dept. Biomed. Sci., Panum Inst., Univ. Copenhagen, Denmark; [2]Dept. Cell. Mol. Physiol., Stricht School of Med., Loyola Univ. Chicago, USA;

Aims: Phosphatidylinositol-(4,5)bisphosphate (PIP_2) levels in the plasma membrane regulates many channels and transporters relevant to cardiac tissue. Whether PIP_2 regulates gap junctional intercellular coupling (GJIC) in cardiomyocytes is unknown, but could add to the risk of arrhythmia during stimulation of Gαq-coupled receptors Hypothesis: PIP2 regulates GJIC and stimulation with Gαq-coupled agonists reduces GJIC partly due to a reduction of PIP2. **Methods:** GJIC was measured by dye transfer after localized electroporation of Lucifer Yellow in cultured cardiomyocytes from neonatal rats. Conduction velocity (CV) was measured in cardiomyocytes grown on electrode arrays. **Results:** One hour wortmannin exposure reduced GJIC indicating that PIP2-depletion inhibits GJIC. In contrast, hypertonic shock, which increases PIP2, increased GJIC. GJIC was inhibited by AngII and noradrenaline stimulation. To test if the reduction in GJIC after agonist stimulation was caused by PIP2-depletion; myocytes were stimulated by AngII and then allowed to recover in control medium with or without wortmannin. GJIC fully recovered in control medium whereas no recovery occurred in the presence of wortmannin. Inhibition of PKC did not affect the response to either AngII or noradrenaline. Also inhibition of arachidonic acid production did not affect the response to agonist stimulation. In beating myocytes CV was reduced by AngII stimulation. After wash-out, CV recovered and this recovery was prevented by inhibition of PIP2 production. **Conclusion:** Reductions in PIP2 inhibits GJIC in cardiomyocytes, and stimulation by physiologically relevant agonists can reduce PIP2 and thereby GJIC by this mechanism. This reduction lowered CV, which could lead to increased susceptibility to arrhythmias.

OW06-21

Chronic low-dose administration of L-NAME increases nitric oxide synthase activity and vasorelaxation in Wistar rats

Bernatova I.[1], Kopincova J.[1], Puzserova A.[1], Janega P.[1,2], Babal P.[2]
[1]Inst. Normal Pathol. Physiol., Centre of Excellence for Cardiovascular Research, SAS; [2]Dept. Pathol. Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovakia; Iveta.Bernatova@savba.sk

Aims: N^G-Nitro-L-arginine methyl ester (L-NAME) is a non-specific nitric oxide (NO) synthase inhibitor, commonly used for the development of NO-deficient hypertension. The aim of this study was to investigate the effect of chronic low-dose administration of L-NAME on NO production, vascular function and structure of the heart and selected arteries of rats. **Methods:** Adult male Wistar rats were treated with L-NAME in the dose of 1.5 mg/kg/day in drinking water for 8 weeks. **Results:** Basal blood pressure (BP) of rats (determined by tail-cuff) was 112±3 mm Hg. The low-dose administration of L-NAME significantly elevated BP measured on the third and sixth week of treatment vs. controls by approximately 9% and 12%, respectively. After this period, BP of L-NAME-treated rats returned to the control values. The relative left ventricular mass, heart fibrosis and collagen III/collagen I ratio were not affected by L-NAME. Similarly, there were no alterations in the cross sectional area and wall thickness/diameter ratio of the aorta and the femoral artery of L-NAME-treated rats. NO synthase activity (determined by conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline) was significantly elevated in the left ventricle and aorta of L-NAME treated rats by approximately 45 and 43%, respectively. Endothelium-dependent acetylcholine-induced vasorelaxation of the femoral artery (determined using wire myograph) was significantly increased and serotonin-induced vasoconstriction was reduced. **Conclusion:** The data suggest that chronic low-dose L-NAME treatment can increase NO production and vasorelaxation in normotensive rats without negative structural changes in the cardiovascular system.
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OW06-22

Changes of arterial pressure and *in vitro* arterial reactivity in rats subjected to chronic L-NAME and magnesium treatment

Serban D.N.[1,2], Serban I.L.[1,2,*], Hogas M.M.[1], Tucaliuc S.E.[1], Stoica B.[3], Nechifor M.[4]
[1]Dept. Physiol.; [2]Center for the Study and Therapy of Pain**; [3]Dept. Biochem.; [4]Dept. Pharmacol., Univ. Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania; dnserban@yahoo.com

Aims: Despite wide investigation of Mg^{2+} influence upon vascular smooth muscle, few studies analyse its impact on endothelium-dependent relaxation (EDR). Chronic inhibition of nitric oxide synthase (NOS) induces hypertension and Mg^{2+} load doesn't influence its development but may reduce it later. Beside this we studied the influence of chronic Mg^{2+} upon acute effects of two antihypertensives in L-NAME hypertension. **Methods:** 24 male Wistar rats were divided in 4 groups, which received (1 ml saline solution i.p. twice a day): isotonic NaCl, isotonic $MgCl_2$ 1 mmol/kg/day, and L-NAME 40 mg/kg/day, either in NaCl or in $MgCl_2$ solution. Arterial pressure and heart rate were measured with a tail-cuff sphygmomanometer. Single-dose rilmenidine (1 mg/kg i.p.) and captopril (50 mg/kg i.p.) were assesed acutely (10, 20 and 30 min after injection). Rings from aorta and mesenteric artery branches were studied by isometric myography. **Results:** L-NAME induced hypertension starting day 3, reaching in 14 days 135± 3.5 vs.104±3.8 mmHg. This is not altered by concomitant i.p. $MgCl_2$ (126±4.3 mmHg, day 14) and can be normalized acutely by i.p. rilmenidine (97±7 mmHg after 30') or captopril (104±5.3 mmHg after 30'), but this effect tends to be reduced by chronic Mg^{2+} (103±5.6 and 116±5.1 mmHg, respectively). *In vitro* data indicate that chronic Mg^{2+} promotes partial preservation of EDR in resistance arteries of L-NAME treated animals by fostering EDHF. **Conclusion:** Our combined approach brings novel evidence regarding the effects of Mg^{2+} upon EDR and the mechanisms involved.
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OW06-23

The effects of vitamin C and folic acid on the coronary circulation in isolated rat heart: different interaction with endothelial L-arginine/NO system

Jakovljevic V.L.[1], Djuric A.[2], Dacic L.[1], Azanjac A.[1], Djuric D.M.[3]
[1]Department of Physiology, Faculty of Medicine, University of Kragujevac; [2]Health Centre "Vracar", Belgrade; [3]Institute of Medical Physiology, School of Medicine, University of Belgrade, Serbia; drvladakg@yahoo.com

Aims: Vitamin C is one of the most used vitamins, without clear evidence about direct effects on coronary flow. On the other hand, folic acid, has recently gained considerable attention because of its great potential to prevent many disorders, including endothelial dysfunction. For this purpose, the aim of this study was to assess the effects of Vitamin C and Folic acid on coronary flow (CF) and nitrite outflow (NO) alone or under inhibition of nitric oxide synthase in isolated rat heart. **Methods:** The hearts of male Wistar albino rats (n=12, 8 weeks, BM 180-200 g) were perfused according to Langendorff technique at constant perfusion pressure conditions (CPP, 40-120cm H_2O). The experiments were performed during control conditions, in the presence of: a) Vitamin C (100 μM) or Vitamin C plus L-NAME (30 μM) and b) Folic acid (100 μM) or Folic acid plus L-NAME. **Results:** CF varied in autoregulatory range from 4.58±0.52 to 6.13±0.54 ml/min/g wt (50-90cm H_2O). Additionally, NO varied from 4.60±0.42 to 6.52±0.35 nmol/min/g wt and was parallel with CPP-CF curve. The autoregulatory range of CF was not significantly influenced by Vitamin C with parallel changes in NO (3.13±0.48 - 8.37±0.74 ml/min). Folic acid significantly increased CF (5.63±0.10 - 15.2±0.42 ml/min) accompanied by parallel changes in NO (2.28±0.29 - 6.66±0.50 nmol/min/g wt). L-NAME decreased Vitamin C-induced flow changes, but not folic-acid induced CF-changes. Completely opposite effects were showed on NO – Vitamin C effect was not significantly affected, while folic acid induced effects were additionally increased. **Conclusion:** The results showed that applied vitamins have different effects on isolated rat heart and opposite interaction with NO-system.

OW06-24

Interaction of nitric oxide and reactive oxygen species during development of the hypoxic pulmonary hypertension

Chovanec M., Hnilickova O., Herget J.
Department of Physiology of the Second Faculty of Medicine Charles University Prague, Czech Republic; mchovanec@seznam.cz

Aims: At the beginning of exposure to hypoxia the damage of peripheral pulmonary vessels by reactive oxygen species (ROS) caused development chronic hypoxic pulmonary hypertension (HPH). Interaction of vasodilatatory nitric oxide (NO) and vasoconstrictory ROS damaging the vessels. In our experiment with chronic hypoxic adult male rats we tested part of interaction betwen NO and ROS. **Methods:** Rats were treated with SOD mimetic -Tempol (group T) and NO/superoxide donor - Molsidomine (group M) alone and in combination (group M+T) during first 7 days of exposure to 3 weeks hypoxia. We isolated rat lungs and perfused salt solution with albumine to study haemodynamic changes of pulmonary circulation by analysis of perfusion pressure increments induced by stepwise increase flow (P/Q relationship). **Results:** The value of Intercept of P/Q relationship was significantly reduced in all groups (T, M, M+T, C) than hypoxic group without treatment (H). Thus, basal tonus of pulmonary vessels was significantly lower in all groups than group H. The value of Slope P/Q relationship in groups treated with Molsidomine (M, M+T) was significantly higher than group H. Thus, adding Molsidomine increased upstream pressure to perfusate flow. **Conclusion:** These results demonstrated potentiation of morphologic reconstruction of peripheral pulmonary vessels by Molsidomine. The damage of pulmonary vessels by ROS plays important role at the beginning of hypoxic pulmonary hypertension. Molsidomine alone or in a combination with Tempol cannot reduce development of HPH. Increased production ROS and consequently generation of peroxynitrite is the reason of that status.
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OW07-25

Is the traditional karate of benefit to young healthy men: the effects of karate on the autonomic nervous system modulation and on haemodynamics

Al-Kubati M., Fiser B., Novakova Z.
Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; mkubati7@yahoo.com

Aim: Since there are few scientific publications about Karate exercise, we decided to study the long-term effect of habitual vigorous Karate exercise on blood pressure, heart rate and their autonomic regulation by baroreflex. **Methods:** In 50 young healthy normotensive men, 25 of them were advanced Karate practitioners for 10 years, (range of 5-15 years) (KP) age 26±6 years, and 25 untrained sedentary men (S) age of 26 ± 5 years (as age matched study), we studied beat-by-beat blood pressure (SBP and DBP in mmHg), the inter-beat-interval (IBI in ms.) of the heart rate and baroreflex sensitivity (BRS in ms/mmHg) by non-invasive methods. **Results:** We found in (KP) versus (vs.) (S) that (Means ± SD): SBP 120.4 ± 12.4 vs. 117.6 ± 10.7 mmHg, p=0.32 (non-significant); DBP 69.0 ± 7.6 vs. 70.5 ± 6.4 mmHg, p=0.55 (non-significant); IBI 1024.7 ± 132.9 vs. 780.5 ± 100.0 ms, p=0.0000001; BRS 15.20 ± 8.40 vs. 7.91 ± 3.77 ms/mmHg, p=0.00014. **Conclusion:** The SBP and DBP do not differ in the Karate-practitioners compared with the sedentary men but the IBI and the BRS in the (KP) are higher than in the (S). These results support the concept that habitual vigorous Karate exercise does modulate the baroreflex regulation of blood pressure to produce normal haemodynamics during rest in healthy subjects accompanied by increasing parasympathetic activity, all of which is recommended for the well-being of anyone.

OW07-26

Psychophysio-immunological assessment of stress and stress management

Keresztes M.[1], Rudisch T.[2], Lajtos Z.[1], Ocsovszki I.[1], Tajti J.[3], Peto Z.[2], Dux L.[1]
[1]Dept. Biochem.; [2]Neuro-psychiatry Rehabilitation Unit, Psychiatry Clinic, [3]Neurology Clinic, Faculty of Medicine, University of Szeged, Hungary; margo@biochem.szote.u-szeged.hu

Aims: We aimed at the characterization of stress by analysis of granulocyte activation markers, in addition to psychological-psychophysiological examinations. **Methods:** As stress models, 11 students during exam period and 15 anxious patients were employed. Granulocyte surface activation markers (L-selectin, alfaM-beta2-integrin, CD15s and lactoferrin) were analysed by flow cytometry. To facilitate stress reduction, participants were involved in relaxation-imagery hypnosis. Psychological tests (Spielberger) and surface EMG were also done. In another study, 9 students performed relaxation training in exam period; here also surface ICAM-1 was assayed. **Results:** The onset of exams resulted in enhanced ratios of labelled cells concerning all probed markers. Particularly dramatic (cca. 5-fold, $p<0.05$) increase was observed in lactoferrin-bearing granulocytes. After hypnosis, the ratio of lactoferrin-exposing cells was considerably reduced (about to half, $p=0.001$) both in students and patients. A similar drop (by about half, $p<0.05$) was observed in CD15s-carrier cell % in patients. In our other study, in addition to lactoferrin appearance, also surface ICAM-1 showed substantial changes (stress: 4-fold /$p<0.005$/ and 3-fold /$p<0.001$/ elevation in the percentage of marker-carrier cells, respectively; relaxation: significant decline regarding especially lactoferrin-carrier granulocytes /by about half, $p<0.01$/). Concerning EMG, only patients showed reduction of muscle tension following hypnosis. No significant alterations could be demonstrated by psychological tests. **Conclusion:** Our studies indicate that stress could be associated with granulocyte activation. From the tested cell surface markers, lactoferrin appeared to be the most sensitive stress sensor. In addition, relaxation hypnosis/training seems to be effective ways to decrease the „excitement" of these activated cells.

OW07-27

Paradoxical dissociation of sympathetic activity and myocardial contractility sixty seconds preceding presyncope

Goswami N.[1], Grasser E.K.[1], Rossler A.[1], Vrecko K.[3], Hinghofer-Szalkay H.[1,2]
[1]Inst. Physiology; [2]Inst. Adaptive and Spaceflight Physiology; [3]Inst. for Physiological Chemistry, Centre for Physiological Medicine, Medical University Graz, Austria; nandu.goswami@meduni-graz.at

Aim: We studied sympathetic activity changes in relation to myocardial contractility, haemodynamic parameters and hormones in subjects undergoing orthostatic challenge. **Methods:** We used a graded orthostatic stress paradigm consisting of head – up tilt combined with lower body negative pressure up to a presyncopal end-point in 15 healthy males. Low – frequency and high-frequency oscillations were estimated from heart period and diastolic pressure values. **Results:** From supine control to presyncope, heart rate increased by 74 ± 11% ($p<0.0001$) whereas stroke index decreased by 46 ± 4% ($p<0.0001$). Thoracic impedance rose by 12 ± 1% ($p<0.0001$), total peripheral resistance index decreased by 12 ± 5% ($p= 0.03$) and end diastolic index by 33 ± 4% ($p<0.0001$). Baroreflex sensitivity decreased significantly by 62 ± 7% ($p= 0.0004$) as well as baroreflex effectiveness index by 56 ± 6% ($p= 0.0004$) but the sympathetic tone increased significantly by 39 ± 9% ($p= 0.0004$). Significant decreases in index of contractility (51 ± 4%; $p<0.0001$), acceleration index (13 ± 7%; $p= 0.02$) and left ventricular working index (27 ± 4%; $p<0.0001$) were observed. Increased plasma norepinephrine (86 ± 16%, $p= 0.0001$), epinephrine (460 ± 266%; $p= 0.06$), cortisol (10 ± 6%; $p= 0.02$), plasma renin activity (147 ± 26%; $p= 0.002$) and aldosterone (24 ± 21%; $p= 0.2$) were noted. **Conclusion:** In spite of reduction in myocardial contractility observed sympathetic activity increased sixty seconds before presyncope.

OW07-28

Corticoadrenal response to stimulation test in female patients with rheumatoid arthritis

Radikova Z.[1], Rovensky J.[2], Vlcek M.[1], Penesova A.[1], Kerlik J.[1], Vigas M.[1], Imrich R.[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]National Institute of Rheumatic Diseases, Piestany, Slovakia
Zofia.Radikova@savba.sk

Aim: Previously, we found a relative sub-responsiveness of HPA axis to hypoglycaemia in patients with rheumatoid arthritis (RA). The aim of the present study was to assess the adrenal steroidogenesis response to a minimal ACTH test in patients with rheumatoid arthritis (RA). **Methods:** Premenopausal female lean RA patients and matched healthy controls were examined. Minimal ACTH test (i.v. bolus of 1μg synthetic ACTH) was performed at 10:00 am with blood sampling every 15 min for 90 min to measure the plasma levels of cortisol, 17-OH-progesterone (17OHP), 4-androstenedione (ASD) and dehydroepiandrosterone (DHEA). **Results:** ACTH administration caused a significant increase of all steroids concentrations observed in both patients and controls ($p<0.001$). The responses of 17OHP and ASD, and the molar ratio of ASD: cortisol were significantly lower ($p<0.05$, $p<0.01$, and $p<0.05$, respectively) in a subgroup of patients treated with corticoids when compared to controls. **Conclusion:** In accord with previous study our recent results indicate a change in secretion of androgens in favour of cortisol synthesis pathway in RA patient, mainly in the subgroup receiving corticoids.

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OW08-29

Effects of olfactory ensheathing cell transplantation on cardiovascular disturbances following high spinal cord injury in rat

Kalincik T.[1], Mackay-Sim A.[2], Carrive P.[1], Waite P.M.E.[1]
[1]Neural Injury Research Unit, School of Medical Sciences, University of New South Wales, Sydney; [2]National Centre for Adult Stem Cell Research, Griffith University, Nathan, QLD, Australia; kandur@email.cz

Aim: Autonomic dysreflexia is an episodic disturbance of cardiovascular functions in subjects with spinal cord injury above the level of T6. In this study we examined the effect of transplanted olfactory ensheathing cells on cardiovascular responses in rats with spinal cord injury. **Methods:** Animals were implanted with a radio-telemetric transmitter for blood pressure monitoring. T4 transection was followed by transplantation of biodegradable gelatine sponge soaked with olfactory ensheathing cells (cell-treated, n=106) or culture medium (control group) inserted between the cut surfaces. We used colorectal distension to induce autonomic dysreflexia expressed as an increase in blood pressure and reflex drop of heart rate. **Results:** Autonomic dysreflexia was fully developed 3 weeks after the spinal cord transection. The cellular therapy did not effect the maximum changes in blood pressure and heart rate. However, the recovery of blood pressure in cell-treated rats was faster with T50 (time for the blood pressure to recover to 50% of the maximum deviation elicited by colorectal distension) significantly shorter (cell-treated: 159.7 ± 3.2 s; control: 183.8 ± 22.9 s; mean ± SD; $p<0.05$). Baseline heart rate in rats with cell transplants was higher than in control animals whereas baseline blood pressures did not differ. **Conclusion:** Transplantation of olfactory ensheathing cells at the site of a transection spinal cord injury assists in limiting the duration of the hypertension associated with autonomic dysreflexia. This could result from trophic/regenerative changes in the spinal cord above or below T4. Further histological analysis is necessary to examine structural effects of the transplanted cells on the host spinal cord.

OW08-30

Spinal interneurones involved in mediating extensor group I muscle afferent actions during fictive locomotion and scratch

Stecina K., Zhang M., Hultborn H.
Dept. Neuroscience and Pharmacology, Univ. Copenhagen, DK-2200 Copenhagen, Denmark kstecina@yahoo.com

Aim: Stimulation of group I extensor muscle afferents resets the locomotor rhythm by either prolongation of the extensor phase, or by termination of the flexor phase during fictive locomotion in the cat. The same stimuli also give rise to polysynaptic group I EPSPs in extensor motoneurones while the classical *"autogenic Ib inhibition"* of extensor motoneurones is suppressed during fictive locomotion. We aimed to identify the interneurones causing the resetting and mediating the polysynaptic group I excitation and to describe the activity of Ib inhibitory interneurones during fictive motor activity. **Methods:** In decerebrate feline preparations, extracellular interneurone recordings were collected in the L6-S1 segments prior to and during fictive (i.e. animal under paralysis) locomotion or scratch. In the absence of motor activity we examined axonal projections within the DLF and to motoneurones innervating the ipsilateral gastrocnemius and hamstring muscles and sensory-evoked activity by electrical stimulation (2-5 stimuli at 200 Hz) of hindlimb muscle afferents. Sensory-evoked activity was compared before, during and after motor activity and emerging spontaneous firing was related to the motor rhythm. **Results:** Not all of the putative resetting interneurones (n=14) showed rhythmically modulated sensory-evoked firing and spontaneous activity as expected if they were to distribute locomotor/scratch drive potentials to motoneurones. The sensory-evoked activity of the classical "*Ib interneurones*" was reduced during motor activity as expected; however some of these neurones showed rhythmic spontaneous firing during fictive scratch. **Conclusions:** Candidate resetting and Ib inhibitory interneurones include two subtypes: those with and those without spontaneous firing during fictive motor activity.

OW08-31

Analysis of NTPDase3-expression in the CNS: mapping and functional considerations

Zsarnovszky A.[1], Gyorffy A.[1], Kovacs E.G.[1], Frenyo V.L.[1], Kirley, T.[2], Belcher S.M.[2], Sotonyi P.[3]
[1]Dept. Physiol. Biochem., Szent Istvan Univ. Fac. Vet. Sci., [2]Dept. Pharmacol. Cell Biophys., Univ. Cincinnati Coll. Med., USA; [3]Dept. Anat. Histol., Szent Istvan Univ. Faculty of Veterinary. Sci., Budapest, Hungary;

Aims: Ectonucleotidases are transmembrane proteins hydrolyzing ATP necessary for intercellular signaling. Our goal was to map the distribution of ecto-nucleoside triphosphate diphosphohydrolase3 (NTPDase3) in the central nervous system and search for functional correlates using morphological and biochemical techniques. **Methods:** Antisera were raised against three distinct amino acid sequences of NTPDase3, characterized by Western blot analyses, and used to determine the localization and distribution of NTPDase3 protein in adult rat brain. Several co-localization techniques were utilized to determine the relationship of NTPDase3 to known neuronal systems. **Results:** NTPDase3-immunoreactivity (IR) was detected exclusively in neurons. IR was localized primarily to neuronal processes with prominent staining of synaptic elements. Specific perikaryal staining predominantly occurred in neurons of the lateral hypothalamus, but scattered immunoreactive neurons were also detected in the perifornical area and the oblongate medulla. High densities of IR axons and dendrites were present in midline regions of the brain and spinal cord. Scattered NTPDase3 positive fiber-like profiles were observed in the cerebral cortex, hippocampus and basal ganglia. High densities of IR punctate structures were detected in the hypothalamus and the molecular layer of the cerebellum. Co-localization studies revealed that in the lateral hypothalamus, NTPDase3 is associated with excitatory morphological profiles of orexin-A immunoreactive neurons, but is absent from GABA-ergic inhibitory systems. **Conclusion:** In the central nervous system NTPDase3 shows a neuron-specific localization. The pattern of expression and co-localization with hypocretin-1/orexin-A suggests that NTPDase3, by regulating the extracellular turnover of ATP, may modulate neuroendocrine reproductive regulatory mechanisms, feeding, sleep–wake- and other behaviours through diverse homeostatic systems.

OW08-32

Cortical spreading depression increases mitochondrial uncoupling protein-5

Viggiano E., Monda M., Viggiano A., De Luca B.
Dept Experimental Medicine, Second University of Naples, Naples, Italy
emanuela.viggiano@unina2.it

Aims: Cortical Spreading Depression (CSD) is a self propagating front of depolarization associated with a depression of the neuronal activity for several minutes and an increase of glucose and O_2 consumption. Previous studies have demonstrated that preconditioning with CSD induces tolerance to a subsequent episode of ischaemia. The mechanism of this tolerance is not clear. The aim of the present work was to evaluate the expression of the uncoupling protein 5 (UCP5) after CSD, that is a candidate neuroprotective factor. **Methods:** Unilateral CSD was induced for 15 min by application of KCl on the right cortex of 5 groups of rats. The animals were sacrificed at 15 min, 2 h, 4 h, 6 h or 24 h after CSD. The amount of UCP5 in both the CSD-treated and the contralateral cortex was evaluated by Western Blot. **Results:** There was a significant increase of UCP5 in the CSD-treated cortex at 24 h; no difference of UCP5 levels between the two cortexes was seen at earlier times. **Conclusion:** This result suggests that CSD induces the synthesis of UCP5 by 24 h. UCP5 could be involved in the neuroprotection effect of CSD, because the uncoupling proteins reduce the mitochondrial membrane potential, and consequently the production of ROS.

OTh09-33

Cardiac and pulmonary VEGF signaling in experimental dilated cardiomyopathy

Ray L.[1], Mathieu M. [1], Andrianne M.[1], Hadad I.[1], Mahmoudabady M.[1], Jespers P.[1], Motte S.[1], Peters I.R.[2], Naeije R.[1], Mc Entee K.[1]
[1]ULB, Brussels, Belgium; [2]Univ. Bristol, United Kingdom lynnray@ulb.ac.be

Aims: The pathogeny of primary dilative forms of cardiomyopathy remains unclear. Vascular endothelial growth factor (VEGF) regulates angiogenesis and vascular tone. Disruption of coordinated hypertrophy and angiogenesis contributes to the transition to heart failure (HF) while dysregulation of vascular tone and remodeling contributes to pulmonary hypertension secondary to left HF. Moreover, in the embryo, VEGF controls ventricular contractility independently of perfusion. We examined pulmonary and cardiac gene expressions of VEGF, VEGF-R1 and VEGF-R2 in a canine model of tachycardiomyopathy and related these results to functional alterations. **Methods:** Eight beagle dogs with overpacing-induced HF underwent over a 7 week period weekly haemodynamic measurements, echocardiograms and endomyocardial biopsies, post-mortem pulmonary arterial reactivity, and RTQ-PCR for mRNA quantification. Six healthy dogs served as controls. **Results:** Cardiac VEGF and VEGF-R1 progressively decreased from week 1 of pacing vs. week 0); these changes were correlated with left ventricular ejection fraction and volumes. Mean pulmonary arterial pressure increased from week 4. *In vitro* pulmonary arteries showed decreased relaxation to acetylcholine and increased hypercontraction in phenylephrine precontracted rings after antagonism of NO or prostaglandins. VEGF and VEGF-R2 were overexpressed in lung arteries vs. control. **Conclusion:** Tachycardia-induced HF is characterized by early downregulation of cardiac VEGF-VEGF-R1 and upregulation of pulmonary VEGF-VEGF-R2. Disruption of this system may represent a mechanism in the progression of non-ischaemic dilated cardiomyopathy.

OTh09-34

Diabetic cardiomyopathy is associated with impaired intracellular calcium (Ca^{2+}_i) release and sequestration

Miklos Z., Barnucz E., Duray B., Ligeti L., Ivanics T.
Inst. Human Physiol. Clin. Exp. Res., Semmelweis Univ., Budapest, Hungary

Aim: Type II diabetes is frequently associated with declining cardiac performance. The aim of our study was to reveal the Ca^{2+}_i-handling disorders potentially underlying the contractile dysfunction characterizing diabetic cardiomyopathy. **Methods:** In our study we used 6 months old hyperglycaemic "db/db" mice expressing leptin-resistant leptin receptors. Age-matched C57/BL6 mice were used as controls. Left ventricular wall dimensions and in vivo performance of the heart were assessed by echocardiography under pentobarbiturate anaesthesia. $[Ca^{2+}_i]$ changes and haemodynamic performance were investigated in isolated perfused hearts. For assessment of SERCA2a function cyclopiazonic acid (CPA), an inhibitor of the pump was infused. **Results:** In 6 months old „db/db" mice blood glucose level increased and obesity was observed. Left ventricular septal thickness was increased in diabetic hearts *(IVSd: Control: 0.12±0.01; „db/db": 0.14±0.01 mm).* $+dP/dt_{max}$ *(Control: 3611±384; „db/db": 2701±428 mmHg/s),* and $-dP/dt_{max}$ *(Control: 2535±33; „db/db": 1722±390 mmHg/s)* of isolated „db/db" hearts were reduced compared to control hearts. Analysis of the recorded Ca^{2+}_i-transients showed elevated end-diastolic Ca^{2+}_i-level *(Control: 172±89; „db/db": 409±70 nM),* reduced Ca^{2+}_i-transient amplitude *(Control: 364±97; „db/db": 218±67 nM),* and diminished rate of Ca^{2+}-release (RyR2 function) *(+dCa/dt$_{max}$: Control: 15483±3858; „db/db": 9294±2027nM/s)* and Ca^{2+}-sequestration (SERCA2a function) *(-dCa/dt$_{max}$: Control: 9434±2641; „db/db": 6217±1817 nM/s)* in the hearts of „db/db" mice. **Conclusion:** The observed defects of Ca^{2+}-releasing and -sequestering processes in the hearts of type II diabetic mice suggest expressional disorders and/or altered posttranslational modification of RyR2 and SERCA2a transporters.

OTh09-35

Deletion of PPARα results in more pronounced cardiac hypertrophy in response to chronic pressure overload

Teunissen B.E.J., Smeets P.J.H., Willemsen P.H., Brouns A.E., Janssen B. J., van der Vusse G.J., van Bilsen. M.
Department of Physiology, CARIM, Maastricht University, The Netherlands
B.Teunissen@fys.unimaas.nl

Aim: Peroxisome proliferator-activated receptor-α (PPARα) is a nuclear receptor regulating cardiac metabolism and has anti-inflammatory as well as anti-fibrotic effects. Given these properties it is anticipated that PPARα modulates cardiac remodeling. Therefore, we studied the role of PPARα in pressure-overload induced hypertrophy. **Methods and Results:** PPARα-/- (KO) and PPARα+/+ (WT) mice were subjected to transverse aortic constriction (TAC) for 28 days. Sham-operated KO and WT mice served as controls (N≥9 in all groups). KO mice had slightly lower blood pressure than WT mice (111 vs. 122 mmHg, $p<0.05$). Nevertheless, TAC resulted in more pronounced posterior and anterior left ventricular (LV) wall thickness and LV/body weight ratio in KO-TAC than in WT-TAC (LVW/BW: +37% vs. +20%, $p<0.05$). LV ejection fraction and maximal LV contractility (+dP/dtmax) were significantly lower in KO-TAC compared to sham (-19.4% and -19.2%, respectively, $p<0.05$), but not in WT-TAC. Moreover, in KO-TAC mice LV mRNA levels of hypertrophic (ANF), fibrotic (Collagen 1, MMP-2) and inflammatory (Interleunited kingdomin-6, TNF-α) genes were significantly higher relative to WT-TAC. **Conclusion:** Absence of PPARα results in a more pronounced hypertrophic growth response, indicating that PPARα attenuates pathological cardiac remodeling following pressure overload.

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OTh09-36

Effect of Atorvastatin treatment on isoproterenol-induced myocardial injury in rats

Trivedi C.J., Balaraman R., Majithiya J.B., Bothra S.B., Megraj K.V.
Pharmacy Department, Faculty of Technology and Engineering, M. S. University of Baroda, Kalabhavan, Vadodara, Gujarat, India
vinompharm@yahoo.com

Aim: Our study was aimed to find out the effects of Atorvastatin (Ator) on isoproterenol (ISO) induced myocardial infarction in rats. **Method:** Male Sprague Dawley rats (200 ± 25g) were divided in to four groups: (1) CON: received vehicle. (2) ISO-CON: received ISO (200 mg/kg, s.c.) twice at 24 hr interval. (3) ATR: received Ator (5 mg/kg, p.o.) for 21 days. (4) ISO-ATR: received Ator (5 mg/kg, p.o.) for 21 days + ISO (200 mg/kg) on 20th and 21st day at 24 hr interval. The following serum parameters like creatine phosphokinase (CK), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), lipids along with oxidative stress in heart like superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (MDA), reduced glutathione (GSH) and membrane bound enzymes like Na^+-K^+ ATPase, $Ca2^+$ ATPase and Mg^{2+} ATPase were estimated. The histopathology of heart was also carried out. **Results:** Administration of ISO caused severe cardiac damage and oxidative stress which was reflected by abnormal serum parameters like CK, GOT, LDH, TC, LDL, MDA, SOD and CAT. It also caused marked damage in membranous ATPase. Ator treatment when compared to ISO-CON group significantly increased HDL, LDL, GOT ($p< 0.01$), MDA ($p< 0.05$) & ATPase activity and significantly decreased TC, TG, LDL ($p< 0.05$), CK ($p< 0.001$), GSH, CAT and SOD activity ($p< 0.05$). The other parameters were not changed significantly by Ator. **Conclusion:** From the results we conclude that atorvastatin may attenuate isoproterenol induced myocardial injury independent of its antioxidant properties.

OTh10-37

Static and dynamic FRET studies in the cardiac I_{KS} channel complex

Haitin Y., Attali B.
Department of Physiology & Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel
haitinyo@post.tau.ac.il

Aims: KCNQ1 (Kv7.1) assembles with the KCNE1 auxiliary subunit to form the voltage-dependent cardiac I_{KS} K^+ channel. Mutations in either KCNQ1 or KCNE1 genes produce the long-QT syndrome, a life-threatening ventricular arrhythmia. Here we studied the static proximity and the voltage-dependent molecular rearrangements of I_{KS} channel subunits. **Methods:** KCNQ1 and KCNE1 were fused to ECFP and/or EYFP, expressed in *Xenopus* oocytes and simultaneous spectral analysis of the fluorescence resonance energy transfer (FRET) combined with two electrode voltage-clamp recording of K^+ currents were performed. **Results:** In the channel closed state, a strong constitutive FRET signal was observed. The static FRET signal between KCNQ1 and KCNE1 was stronger with a C-terminal truncation mutant of KCNQ1 (Δ622-676). In addition, significant static FRET signals were observed when 1:1 molar ratio of C-terminally tagged KCNQ1-CFP and KCNQ1-YFP were co-expressed. Double labeling of KCNQ1 (N- and C-termini, YFP-KCNQ1-CFP) resulted in a marked FRET signal. A clear voltage-dependent change in the FRET signal was recorded at +30 mV concomitantly with I_{KS} K^+ currents, suggesting spatial rearrangement of KCNQ1 and KCNE1 subunits during the gating process. However, no voltage-dependent FRET changes were detected between the C-termini of KCNQ1. Notably, both K^+ current and dynamic FRET changes were abolished when the D76N KCNE1 LQT5 mutant was co-expressed with KCNQ1. **Conclusion:** Channel gating is accompanied by a spatial rearrangement of the channel complex that propagates to the C-termini of both subunits. The D76N KCNE1 mutant locks the channel in the closed state and abolishes the voltage-dependent dynamic FRET signal.

OTh10-38

Spatial organization of Ca^{2+}-regulating proteins in trout ventricular myocytes

Birkedal R., Shiels H.A.
Faculty of Life Sciences, The University of Manchester, United kingdom
Rikke.Birkedal@manchester.ac.united kingdom

Aims: Trout and mammalian ventricular myocytes are different in form and function. In form, trout myocytes are long and slender and lack t-tubular invaginations of the sarcolemma. In this regard they resemble mammalian atrial and neonatal ventricular myocytes, and ventricular myocytes from all other non-mammalian species. In function, the excitation-contraction coupling of trout myocytes is more dependent on Ca^{2+}-influx across the sarcolemma via L-type Ca^{2+}-channels and reverse-mode Na^+/Ca^{2+}-exchange (NCX). The importance of Ca^{2+}-induced Ca^{2+}-release from the sarcoplasmic reticulum via ryanodine receptors (RYR) is controversial. The aim of this study was to investigate the cellular localization of the three proteins responsible for Ca^{2+} fluxes: L-type Ca^{2+}-channels, NCX and RYR. **Methods:** Trout ventricular myocytes were co-labelled with phalloidin to visualize actin and specific antibodies against L-type Ca^{2+}-channels, NCX or RYR. Cells were imaged by confocal microscopy or widefield microscopy followed by deconvolution. **Results:** Our results suggest that L-type Ca^{2+}-channels, NCX and RYR are organized in distinct, parallel bands on or near the sarcolemma. Bands of L-type Ca^{2+}-channels seemed to align with the Z-line of the sarcomeres. Bands of NCX were located between Z-lines. Bands of RYR seem to align with the M-band in the middle of the sarcomeres. **Conclusion:** This is the first study to look at the cellular organization of L-type Ca^{2+}-channels, NCX and RYR relative to the sarcomeres. Surprisingly, in trout myocytes L-type Ca^{2+}-channels and RYR did not seem to be co-localized as they are in mammalian myocytes. The functional importance of this will be discussed.

OTh10-39

Primary skeletal myocytes derived from various mouse strains exhibit different sodium current properties

Mille M., Koenig X., Uhrin P., Zebedin E., Radenkovic M., Zarrabi T., Cervenka R., Todt H., Hilber K.
Inst. Pharmacol., Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Austria; karlheinz.hilber@meduniwien.ac.at

Aims: The mouse is the preferred model organism for genetic manipulation. The fact that different mouse strains exhibit considerable distinctions in phenotype needs to be taken into account when designing studies using knock out mice. In particular, differences in the genetic background of wild type and genetically manipulated animals may introduce artificial effects independent of the genetic manipulation itself. Phenotype differences between mouse strains, for example, exist in cardiac electrophysiology. Detailed studies on ion channel expression and/or function in excitable cells originating from various mouse strains, however, are still lacking. Here, we compared voltage-gated sodium channel function between skeletal myocytes obtained from different frequently used mouse strains (C57BL/6, SV129 and FVB). **Methods:** Primary skeletal myocyte cultures were generated from neonatal and adult mice, and their sodium current properties were detected by using the whole cell patch clamp technique after defined culture time periods. **Results:** The voltage-dependencies of sodium current activation and inactivation were significantly different in skeletal myocytes isolated from various mouse strains. Differences also existed in the kinetics of fast inactivation and in the sensitivities to block by tetrodotoxin. In addition, the sodium current properties of skeletal myocytes isolated from neonatal mice differed from those derived from adult mice. **Conclusion:** The sodium current properties of primary skeletal myocytes are mouse strain-dependent. This may be explained by differences in sodium channel isoform expression. Our findings will have to be taken into account when designing studies to investigate electrophysiological phenotypes of genetically manipulated mouse models. *Supported by Austrian FWF (P19352-B11).*

OTh10-40

532 nm laser-light activates some and inhibits other neurons of snail ganglia

Reece P.J.[1], Dholakia K.[1], Cottrell G.A.[2]
[1]SUPA, Department of Physics and Astronomy; [2]School of Biology, University of St. Andrews, KY 16 SS, United Kingdom
gac@st-and.ac.united kingdom

Aims: It is known that UV and IR laser-light can stimulate populations of neurons, but the mechanism of activation is unknown. We decided to analyse effects of laser light in the mid visible range (532 nm) on individual neurons. We chose to work on specified neurons with known properties and selected an invertebrate model preparation. **Methods:** Ganglionic neurons from *Helix aspersa* were exposed in a chamber containing physiological solution. Neurons were impaled with glass microelectrodes containing 0.7M KCl, K_2SO_4 or Kacetate. Current- and voltage-clamp recordings were made. Light was applied via an 80 µm diameter optical fibre. **Results:** Neuron-specific effects on excitability were observed that resulted in each case from an increase in membrane conductance. Responses were intensity-dependent, over the range of 25mW to 1200mW, reversible and repeatable. Some neurons were depolarized and excited, with an extrapolated E_{rev} of + 60mV of the current response. Others were inhibited, with an E_{rev} close to the resting E_m. Detailed experiments on the C1 neuron, showed that inhibition resulted from an increase in Cl^- ion conductance, that occurs most probably along the axon. **Conclusions:** 532 nm light consistently excited some neurons, probably by increasing membrane permeability to Na^+ and/ or Ca^{2+} ions, but inhibited the C1 neuron by a specific action on Cl^- ion channels.

OTh11-41

Stimulation of Y2 receptor (Y2R) expression in vagal afferent neurons by cholecystokinin (CCK)

Burdyga G., deLartigue G., Morris R., Varro A., Dimaline R., Dockray G.J.
Physiological Laboratory, School of Biomedical Sciences, University of Liverpool, Liverpool, United Kingdom
gburdyga@liverpool.ac.united kingdom

Aims: Food intake is inhibited by the intestinal hormones CCK and PYY which act via vagal afferent neurons. We examined how CCK regulates the expression of Y2R, at which PYY3-36 acts, in these neurons. **Methods:** Receptor expression was studied by immunohistochemistry and qPCR of nodose ganglia from rats fasted up to 48h, or treated with CCK8, or the CCK1 receptor antagonist lorglumide. Expression of a Y2R promoter-luciferase (Y2-luc) reporter was examined in cultured vagal afferent neurons. **Results:** There was a significant five-fold decrease ($p<0.05$) of Y2R mRNA in nodose ganglia of fasted rats compared with controls. CCK8 (10 nmol, i.p.) significantly increased Y2R mRNA in fasted rats, as did re-feeding. The latter response was inhibited by lorglumide. Y2R immunoreactivity was localised to glial cells and neurons most of which (89 ± 4 %) expressed CCK1 receptors. Retrograde tracing indicated that in fasted rats there was a significant decrease in the number of Y2R-immunoreactive neurons projecting to the stomach but no change in those projecting to the proximal colon. Following transfection of Y2-luc into cultured nodose neurons, there was a 12.3±0.1-fold increase in luciferase activity in response to 10 nM CCK that was abolished by the protein kinase C inhibitor Ro-32-0432 (1.5 ± 0.4, $p<0.01$) and replicated by phorbol ester (16.2 ± 0.4 fold increase). **Conclusions:** Y2R is expressed by neurons and glial cells in nodose ganglia; expression in neurons projecting to the stomach is decreased by fasting and restored by CCK. CCK stimulates Y2R expression via a PKC dependent mechanism.

OTh11-42

Bombesin influence on bile lipid composition in rat

Moroz O., Veselsky S., Lyaschenko T., Nuryshenko N.
Subdepartment of Human and Animal Physiology of Kyiv National University Kyiv, Ukraine
olesia.moroz@gmail.com

Aim: The role of bombesin (BN) in bile formation is not clear yet. The goal of this research was to determine the effect of BN on secretion of bile lipid components. **Methods:** Investigations were conducted on white male rats 180-200 g in acute experiment. BN (Sigma, USA) in dose 1 μg/100 g b, i.v., and atropine in dose 0.2 mg/100 g, i.p. were administered. The output of bile lipids was measured by thin-layer chromatography. **Results:** BN increased the output of phospholipids (+64.2%; $p>0,05$), cholesterol (1.57 μg/g to 3.37 μg/g; $p<0.01$), its esters (0.18 μg/g to 0.43 μg/g; $p>0,05$), and free fatty acids (+70%; $p<0.05$) on the ground of decreasing of triglycerides amount. At the same time the cholate-cholesterol ratio (CCR) was reduced, the cholesterol -to its esters ratio was elevated. The phospholipids-cholesterol ratio (PCR) was diminished. The potent cholesterol crystallization index (PCCI) was not higher than 7%. Peptide administration after previously injected atropine caused the significant reduction of all biliary lipids comparatively to the administration of BN alone. It was followed by decreasing of CCR and cholesterol -to its esters ratio. But the PCR elevated (from 2.63 to 3.34). The PCCI had a tendency to grow. **Conclusions:** Obtained data show that effect of BN on phospholipid, cholesterol and its esters biliary secretion depends on the activity of M-acetylcholine receptors. After administration of BN the PCCI stays at normal rate; cytotoxic effect of bile acids is diminished due to enhancing secretion of phospholipids into bile; solubilisation properties of bile are improved.

OTh11-43

The involvement of adiponectin, leptin, and ghrelin in the development of adjuvant arthritis in rats

Stofkova A., Skurlova M.
Department of Normal, Pathological, and Clinical Physiology, Third Faculty of Medicine, Charles University in Prague, Czech Republic
andrea.stofkova@lf3.cuni.cz

Aims: Adipokines, and ghrelin are beside the regulation of metabolism and food intake involved in inflammatory processes. This study investigates whether the development of inflammation is associated with changes in adiponectin, leptin, and ghrelin in rats, and whether changes in peripheral leptin and ghrelin levels affect the expressions of their respective receptors in the hypothalamic arcuate nuclei. **Methods:** Adjuvant arthritis (AA) was induced to male Lewis rats using complete Freund's adjuvant. Food consumption and body weight were monitored for 18 days of the disease. Hormones were determined by radioimmunoassay. mRNA expression for ghrelin receptor-Ghsr and leptin receptor-OB-Rb was determined by quantitative TaqMan PCR. **Results:** Arthritic rats had decreased food intake and body weight during the whole course of the study. Plasma adiponectin was lowered on days 4, 12, and 18 of AA, ghrelin was reduced on days 12, and 18, and plasma leptin and leptin content in fat were decreased on days 12, 15, and 18. Expression of Ghsr was upregulated on days 4, 9, and 18 of AA, and expression of OB-Rb was downregulated on day 4 and upregulated on day 18. **Conclusion:** The decrease of body weight and fat loss led to lowered levels of adipokines, and their full immunomodulatory effects could not be manifested. Low leptin levels were expected to increase food intake by desinhibition of orexigenic pathway in hypothalamus. However, the inflammatory insult caused loss of appetite, resulting in low ghrelin levels and consequently in lack of food intake stimulation.

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OTh11-44

Modulatory effects of leptin on cholecystokinin-induced noradrenaline release in the hypothalamic paraventricular nucleus and plasma oxytocin levels in the female rat: a microdialysis study

Kutlu S.[1], Aydin M.[1], Ozcan M.[2], Alcin E.[1], Jezova D.[3], Yilmaz B.[4]
Firat Univ., Faculty of Medicine, Dept. [1]Physiology & [2]Biophysics, Elazig, Turkey; [3]Inst. Exp. Endocrinol., SAS, Bratislava, Slovakia; [4]Yeditepe Univ., Faculty of Medicine, Dept. Physiology, Istanbul, Turkey; skutlu@firat.edu.tr

Aim: Central noradrenaline exerts a stimulatory effect on oxytocin secretion. Administration of cholecystokinin (CCK) increases noradrenaline (NA) concentrations in supraoptic and paraventricular nuclei (PVN) and plasma levels of oxytocin. The aim of the study was to investigate effects of leptin on CCK-induced NA release in PVN and plasma oxytocin levels. **Methods:** A microdialysis probe was set into PVN of adult female rats. One h later, microdialysis samples were collected at 20min intervals for 80min. After collection of the 1st sample (control), vehicle or CCK (50mg/kg) were administered i.v. (n=8-10). In addition to CCK, 10μg leptin was infused i.c.v to additional group (n=8). Blood samples were obtained via a carotid artery cannula. NA content was determined by HPLC-ECD, plasma oxytocin by RIA. **Results:** In the 2nd interval (20-40min), NA content of PVN was significantly elevated in the CCK group compared to control values. Leptin decreased the CCK-induced rise in NA content compared to CCK administered group for 2nd and 3rd intervals. No significant differences were observed for the subsequent interval. CCK increased oxytocin levels in the 2nd and 3rd samples. Leptin infusion slightly decreased oxytocin levels in the 2nd and 3rd periods. **Conclusion:** The results indicate that CCK has stimulatory effect on NA content in the PVN which can be inhibited by leptin. CCK also elevated plasma oxytocin levels without being affected by leptin. We suggest that leptin's modulatory effect on NA release in PVN may be related to feeding behaviour rather than inhibiting oxytocin neurons. *This study was supported by TUBITAK (project No 104S486).*

OTh12-45

Seasonal encoding in mouse (*Mus musculus*) biological clock

McMahon D., Ciarleglio C.
Department of Biological Sciences, and Graduate Program in Neuroscience, Vanderbilt University, Nashville, TN, USA
douglas.g.mcmahon@vanderbilt.edu

Aims: A fundamental property of circadian rhythms is the ability to entrain to different light cycles and shape behaviour. Seasonality in both daily locomotor activity patterns and reproduction is manifest in mammals, with melatonin as an important seasonal signal controlled by the central biological clock. The purpose of this study was to examine the mechanisms of seasonal encoding by circadian pacemaker neurons in the suprachiasmatic nuclei of the hypothalamus (SCN), the brain's central biological clock. **Methods:** Transgenic mice harboring a short half-life *Per1*: GFP clock gene reporter were kept in wheel-running cages in one of three photoperiodic cycles: short-photoperiod (8:16), control (12:12) and long-photoperiod (16:8). 200u coronal slices of the SCN were taken from each mouse for time-lapse laser-scanning confocal microscopy of 4 circadian cycles *in vitro*. The organization of the mouse SCN *Per1* expression was observed at the cellular level by imaging GFP fluorescence and correlated with previous running wheel activity on the different photoperiods. **Results:** Both the waveform of *Per1* expression within individual SCN neurons and the distribution of individual neuronal peak times shaped the activity of the SCN clock network in response to photoperiod. In addition, we noted significant *"after effects"* on cellular period *in vitro* of the long and short light cycles and peak-to-peak period inconsistencies in individual neurons that suggest lability of the constituent cellular oscillators of the SCN at the molecular level. **Conclusions:** Our results suggest that both intra-neuronal molecular mechanisms and inter-neuronal coupling are involved in encoding seasonal changes by the biological clock.

OTh12-46

Season-related changes in the pineal gland activity in chickens

Majewski P., Nowak K., Joachimiak E., Skwarlo-Sonta K.
Department of Animal Physiology, Faculty of Biology, University of Warsaw, Poland
pmajew@biol.uw.edu.pl

Aim:of present study was to examine the early post-embryonic changes in gene expression and activity of two main enzymes: arylalkylamine-N-acetyltransferase (AA-NAT) and hydroxyindole O-methyltransferase (HIOMT) participating in melatonin synthesis with particular attention to the season of hatch. **Methods:** Experiments were performed in winter and summer on male Hi-Line chickens kept from the day of hatch in an artificial photoperiod L:D 12:12. On the days 2, 9 and 16 after hatch chickens were killed at ZT 11 and ZT 17 (i.e. at the end of light and in the middle of darkness). Pineal glands were dissected out and immediately frozen in liquid nitrogen. Pineal AA-NAT and HIOMT activity was assayed via the liquid biphasic diffusion method. Profiles of AA-NAT, HIOMT and housekeeping gene (TATA box binding protein - TBP) mRNA were measured using real-time RT-PCR. **Results:** AA-NAT activity and mRNA levels were elevated in darkness increased with age and were higher in summer than in winter. On the contrary, HIOMT activity and mRNA levels reached maximal values during the light phase were higher than that of AA-NAT and change with age and season. **Conclusions:** Seasonal differences in the activity and mRNA level of pineal enzymes observed in constant laboratory conditions suggest the existence of a photoperiodic memory in the chicken pineal gland. The molecular mechanism of this phenomenon is currently under investigation. *Supported by Internal BST Grant from Faculty of Biology, University of Warsaw.*

OTh12-47

Regulation of the mouse Plasminogen Activator Inhibitor-1 (PAI-1) promoter by circadian and hypoxic factors – a conserved mechanism with human

Singletary J.H., Chan D., Samani N.J., Chong N.W.
Cardiology Group, Dept. Cardiovascular Sciences, Glenfield General Hospital, Univ. Leicester, United Kingdom; jhs10@le.ac.unitedkingdom

Aims: PAI-1 is the major inhibitor of fibrinolysis and elevated plasma PAI-1 levels are associated with greater risk of myocardial infarction (MI). Circulating PAI-1 levels are under genetic control and show circadian variation, with increased levels in the morning, coinciding with the peak in risk of MI. The present study investigated the regulation of mouse and human *Pai-1* and the interplay between circadian and hypoxia-induced transcription factors. **Methods:** Fragments of the mouse and human *Pai-1* 5'-flanking regions (-707 to +108bp, -796 to +140, resp.) containing canonical E-boxes (CACGTG) and hypoxia-responsive elements (HRE, CACGTA) were cloned into Luciferase reporter vectors. Promoter activity was analysed following over-expression of factors in mouse NIH-3T3 cells. **Results:** Circadian heterodimeric factors CLOCK and BMAL1 significantly activated (2-fold) the mouse promoter via the E-box at -179 to -174bp. The HRE four bases upstream was activated by hypoxia-induced factor EPAS1 (12-fold), which was significantly augmented by BMAL1 (16-fold). Mutation of this HRE reduced activation by EPAS1 and EPAS1:BMAL1 by ~50%. E-box mutagenesis increased activation of EPAS1 and EPAS1:BMAL1 at the mouse HRE. CLOCK:BMAL1 and EPAS1 also activated the human reporter (3 and 10-fold, resp.) and addition of BMAL1 augmented activation by EPAS1 (30-fold). **Conclusions:** A conserved mechanism of *Pai-1* regulation appears to involve differential binding of circadian and hypoxic factors to the mouse and human proximal promoters. The variation in availability of circadian factors at different times of day may influence the response of *Pai-1* expression to hypoxia and contribute to the increased morning incidence of MI. *Supported by the BBSRC United Kingdom and the Coulson Trust.*

OTh12-48

Effect of altered lighting regimens and chronic mild stress on the circadian rhythms in the circadian rhythms in the rat

Jozsa R.[1], Olah A.[2], Csernus V.[1], Sandor J.[2], Muller A.[2], Cornelissen G.[3], Halberg F.[3]
[1]Dept. Anatomy, [2]Faculty of Health Sciences, University of Pecs, Hungary; [3]University of Minnesota, Minneapolis, USA; rita.jozsa@aok.pte.hu

Recent data confirmed that the chronic mild stress (CMS) model of depression has high validity. In our study, decreases in reactivity to rewards in rats following exposure to CMS were observed. The aim of the present study was to investigate the changes of the daily hormonal levels following CMS alone or in combination with various lighting schedules and to analyse the rhythm parameters. 102 adult rats of both genders were randomly distributed in the following groups: 1) controls, 2) using CMS under different lighting regimens and 3) repeated shifts of the lighting regimen without CMS. Sucrose consumption was measured weekly. The plasma corticosterone in the female CMS group kept in LD schedule was slightly decreased during the light phase compared to the control group kept in LD, and slightly elevated during the dark phase, but not in males. The observation that no statistically significant difference in circadian rhythm characteristics was found between the CMS-stressed and non-stressed animals is an indication that the load associated with lighting schedule shifts may have been harder than the CMS procedure, at least from the viewpoint of maintaining an integral circadian time structure. Mean values showed a significant reduction of testosterone of the CMS-treated male rats as compared to controls. Circadian rhythm was demonstrated for testosterone for the CMS-stressed animals but not for the non-CMS-stressed rats. In conclusion, CMS and also disturbing lighting schedule itself result in the impairment of certain hormonal rhythms and these disturbances appear in an early stage of anhedonic behaviour. *Support: ETT314/2006.*

OTh13-49

The effect of diabetes mellitus on myocardial resistance to ischaemia/ reperfusion injury and PPAR expression in the rat heart

Ravingerova T.[1], Adameova A.[2], Kelly T.[3], Antonopoulou E.[3], Matejikova J.[1], Kuzelova M.[2], Svec P.[2], Lazou A.[3]

[1]Inst Heart Res SAS; [2]Dept Pharmacol Toxicol, Fac Pharm Comenius Univ, Bratislava, Slovakia; [3]School Biol., Aristotle Univ., Thessaloniki, Greece

Aims: The study was aimed to explore the effect of diabetes mellitus (DM) on the expression of PPAR isoforms (key transcriptional regulators of lipid metabolism and energy production) and their role in myocardial ischaemia/ reperfusion injury (I/R). **Methods:** In the Langendorff-perfused hearts of rats with 5-day DM (STZ) and the controls (C) subjected to 30-min LAD occlusion and 2-h reperfusion, were measured mRNA expression (RT-PCR) of alpha, beta and gamma isoforms of PPAR before and after I/R and infarction size (IS; TTZ). **Results:** Baseline levels of PPAR isoforms were significantly increased in the DM group as compared with C. Marked down-regulation of all three isoforms following I/R in C was attenuated in diabetics, and PPAR alpha was even significantly increased. IS normalized to the area at risk (AR) size was significantly lower in DM group as compared with C (IS/AR 15.1±3.0% *vs.* 37.3±3.1%; P<0.05). **Conclusions:** Increased expression of PPAR might reflect changes in lipid metabolism induced by DM. On the other hand, maintained levels of PPAR isoforms after I/R might indicate their role in higher cardiac resistance to I/R in the acute phase of DM. *Grants UK19/2007, 2/5110/25, MVTS SR-GR 15, APVT-51-027404, GSRT- 759 5190/2005.*

Groups	PPAR alpha/GAPDH	PPAR beta/GAPDH	*PPAR gamma/GAPDH*
C	0.156±0.015	0.056±0.003	0.180±0.010
I/R	0.097±0.010†	0.028±0.010†	0.122±0.016†
DM	0.186±0.019*	0.094±0.015*	0.205±0.014*
DM+I/R	0.232±0.046*†	0.072±0.014*	0.210±0.031

*-p<0.05 vs. non-diabetics, †-p<0.05 vs. baseline

OTh13-50

Role of uncoupling proteins in heart function changes during ischaemia-reperfusion

Sagach V.F., Shymanskaya T.V., Dobrovolsky F.V., Goshovska Y.V.
Circulation Physiology Department, Bogomoletz Physiology Institute of NAS, Kyiv, Ukraine; circul@biph.kiev.ua

Aim: Heart ischaemia is well-known damaging agent, which is more evidently performed via mitochondria. Uncoupling proteins (namely UCP2 and UCP3) are the members of inner membrane transporters family that dissipating the proton gradient. Ischaemic injury and UCPs appear to be interrelated. In this study we hypothesized that UCPs are involved in pathological processes occurred in cardiac cells during ischaemia. **Methods:** In experiments on isolated rat hearts perfused under Langendorff preparation effects of ischaemia-reperfusion and blockade of UCPs with its inhibitor genipin (50 mg/L) was studied and cardiodynamic parameters were measured. Expression of UCPs was detected by reverse transcriptional polymerase chain reaction. **Results:** It was shown that postischaemic disturbances of cardiac contractility, coronary vessels tone and heart rate are accompanied with noneffective oxygen utilization by myocardial tissue. At the same time ischaemia (20 min) as well as subsequent reperfusion (40 min) increased expression of UCPs in myocardium: mRNA levels of UCP3 were significantly higher that those of UCP2, but both higher that in control. Blockade of UCPs by genipin has, in general, positive effect: increased indexes of cardiac contractility decreased oxygen cost of myocardial work and improved non-effective oxygen utilization by the heart tissue during perfusion. However, postischaemic heart disturbances after genipin administration were higher than in control. **Conclusions:** Our results demonstrate that UCPs seem to play regulatory role in cardiac activity during ischaemia-reperfusion. We suggest that uncoupling proteins (UCP2 and UCP3) are implicated in pathological mechanisms developed during ischaemia-reperfusion.

OTh13-51

Antinecrotic and antiarrhythmic effects of MAO-A inhibition in rat hearts subjected to *in situ* regional ischaemia

Ordodi V.[1], Mirica N.[1], Barglazan D.[1], Menabo R.[2], Kaludercic N.[3], Canton M[3], Cristescu A.[1], Muntean D.[1]

[1]"Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania; [2]Istituto Neuroscienze CNR; [3]Dipartimento Chimica Biologica, Universita di Padova, Padova, Italy; daninamuntean@gmail.com

Aims: Previous studies demonstrated the contribution of reactive oxygen species (ROS) produced by monoaminooxidases (MAO) to myocardial injury caused by post-ischaemic reperfusion and the protective effects afforded by irreversible MAO inhibitors. The present study was aimed at characterizing the effects of a reversible MAO-A inhibitor, moclobemid (Mocl), in the *in vivo* rat model of regional ischaemia. **Methods:** Anesthetized rats subjected to 30 min ischaemia by coronary ligation and 2 h reperfusion were randomized to receive: (i) no additional interventions (Ctrl), (ii) acute administration of 4 mg/kg Mocl IV 15 minutes before ischaemia (MoclAc) and (iii) chronic administration of 4 mg/kg IP, daily for 3 weeks (MoclChr). Occurrence of arrhythmias (ventricular premature beats, VPBs; ventricular tachycardia, VT and ventricular fibrillation, VF) was assessed along with of infarct size measurement by means of triphenyltetrazolium chloride staining. **Results:** Myocardial injury expressed as the percent of infarct to risk area ratio (I/R%) was significantly reduced by both acute and chronic Mocl treatment (1.43 ± 0.9%, 6.37 ± 2.6% and 32.19 ± 1.6% in MoclAc, MoclChr and Ctrl groups, respectively; mean ± SEM; $p < 0.05$). In addition, no arrhythmias were observed in MoclAc group (arrhythmia score 0), whereas < 30 VPBs (arrhythmia score 1) appeared during ischaemia in MoclChr group as compared to arrhythmia scores of 2 (>30 VPBs) and 3 (< three episodes of VF/VT) seen in controls. **Conclusion:** Both acute and chronic administration of Mocl elicited remarkable cardioprotection detected as reduction of infarct size and arrhythmogenicity caused by regional ischaemia-reperfusion in rat hearts.

OTh13-52

Left ventriculo-arterial uncoupling after recovery from myocardial infarction in dogs

Mathieu M.[1], El Oumeiri B.[1], Touihri K.[1], Ray L.[1], Hadad I.[1], Mahmoudabady M.[1], Meskini N.[2], Guenegou A.[2], Naeije R.[1], Brimioulle S.[1], Bartunek J.[3], Heyndrickx G.[3,4], Mc Entee K.[1]

[1]Laboratory of Physiology, Universite Libre de Bruxelles, Brussels, Belgium; [2]CARDIO3, Braine-l'Alleud; [3]Cardiovascular Center, Onze Lieve Vrouw Hospital, Aalst; [4]Universite Catholique de Louvain, Brussels, Belgium
myrielle.mathieu@ulb.ac.be

Aims: We investigated the relative contribution of systolic and diastolic function of the left ventricle and ventriculo-arterial coupling in 24 dogs after recovery from an acute myocardial infarction. **Methods:** Cardiac function was assessed using echocardiography and left heart catheterization with a conductance catheter at baseline and 11 weeks after ligation of the circumflex coronary artery and clinical recovery. **Results:** There were increases in left ventricular end-diastolic pressure (mean+/-SE 14 ± 3 mmHg at baseline vs. 23 ± 4 in heart failure) and regional systolic function (wall motion score index 1 vs. 1.8 ± 0.1), a decrease in left ventricular ejection fraction (70 ± 1 vs. 57 + 1 %) while cardiac output (2.8 ± 0.3 vs. 2.7 ± 0.2 L/min) was unchanged. Cardiac contractility was depressed (end systolic elastance (Ees) 6.1 ± 0.2 vs. 2.1 ± 0.2 mmHg/ml, preload recruitable stroke work slope 128 ± 13 vs. 72 ± 5 mmHg/ml, dP/dtmax 3677 ± 821 vs. 2570 ± 407 mmHg/sec). There were increases in time constant of relaxation (Tau 27 ± 5 vs. 38 ± 2 ms) and end diastolic volume (41 ± 5 vs. 65 ± 3 ml), while capacitance, measured with volume at pressure 15 and 20 mmHg, was unchanged. The ventriculo-arterial coupling was markedly decreased (Ees/arterial elastance 1.4 ± 0.2 vs. 0.6 ± 0.1). **Conclusion:** Recovery after acute myocardial infarction was characterized by systolic dysfunction, delayed relaxation and ventricular dilation with a severe ventriculo-arterial uncoupling and without clinical signs of overt heart failure.

OTh14-53

KCNQ1 mutation Q147R is associated with atrial fibrillation and prolonged QT-interval

Lundby A.*, Ravn L.S.*, Svendsen J.H., Olesen S.P., Schmitt N.
The Danish National Research Foundation Centre for Cardiac Arrhythmia, The Panum Institute, University of Copenhagen, Copenhagen N, Denmark
nschmitt@mfi.ku.dk

Aims: Atrial fibrillation (AF) and long-QT syndrome (LQTS) are two cardiac arrhythmia diseases that have both been related to dysfunction of the voltage-gated potassium channel subunit Kv7.1 encoded by the *KCNQ1* gene. The purpose of this study was the functional assessment of a mutation in Kv7.1 identified in a proband with permanent AF and prolonged QT-interval. We investigated if this *KCNQ1* missense mutation can form the genetic basis for both conditions in the patient simultaneously. **Methods:** We investigated the functional consequences of the novel mutation *KCNQ1* Q147R by heterologous expression of the channel and accessory subunits in *Xenopus laevis* oocytes and mammalian cells. **Results:** The Q147R mutation does not affect the biophysical properties of Kv7.1 in the absence of accessory subunits. Upon co-expression with the β-subunit KCNE1 the Q147R mutation induced a loss-of-function observed as a decrease in the current amplitude at depolarized potentials. Additionally, Q147R abolished the frequency-dependence of charge carried by Kv7.1/KCNE1 channels. Co-expression with the β-subunit KCNE2 revealed a gain-of-function for the mutant evidenced as an increase in the current amplitude at depolarized potentials. The properties of channels formed by Kv7.1 and the subunits KCNE3 and KCNE4 were unaffected by the Q147R mutation. **Conclusion:** Our data indicate that this particular mutation can form the molecular substrate for different arrhythmogenic conditions simultaneously. We suggest the mechanism to be due to heterogeneous distribution of Kv7.1 accessory subunits in the heart leading to Kv7.1 gain-of-function in the atria (AF) and Kv7.1 loss-of-function in the ventricles (QT-prolongation).

OTh14-54

Induction of Torsades de pointes after combination of claritromycin and furosemide in rat

Kmecova J.*, Mackovicova K., Vavrinec P., Ondrejkova J., Krenek P., Kyselovic J., Ochodnicky P., Klimas J.
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia; *Current address: Unit of Pharmacology and Therapeutics, University of Louvain Medical School, Brussels, Belgium; jana.kmecova@fath.ucl.ac.be

Aims: The increase of QT duration caused by drugs can induce life-threatening arrhythmias as Torsades de pointes (TdP). We studied the influence of claritromycin in combination with furosemide on ECG parameters in rats and their effect on occurence of TdP. We hypothetised, that gene rERG which encodes the expression of potassium channels and influences repolarization could be responsible for changes in QT interval and incidence of arrhythmias. **Methods:** Wistar rats were treated 7 days with claritromycin (CLA, 100mg/kg, p.o., every 12 hrs), furosemide (FUR, 100mg/kg, p.o., every 12 hrs) or both (CLA+FUR). Controls (CON) received vehiculum. 12-lead ECG was recorded in rats under Avertin anaesthesia (10 ml/kg, i.p.). We evaluated the duration of QT and RR. ECG was monitored also during beta-adrenergic stimulation (isoproterenol i.v., dose 5, 10, 15, 20, 30, 45 a 60 ng/min). Using RT-PCR, we observed the expression of gene rERG in left ventricular tissue. **Results:** The duration of QT was increased in groups in the order CON<FUR<CLA<CLA+FUR (83 ± 2 ms, 103 ± 4 ms, 111 ± 4 ms, 127 ± 5 ms, $p<0.05$). The duration of RR was increased in groups, where furosemide was administered. In CLA+FUR group during beta-adrenergic stimulation, the occurrence of TdP was noted. In this group, there was also 30% decrease in expression of rERG compared to controls ($p<0.05$). In other experimental groups, the expression of rERG did not change. **Conclusion:** The chronic administration of claritromycin and furosemide increases the duration of QT and can induce TdP. We suppose this is associated with decreased expression of rERG.

OTh14-55

Cardiomyocytes alter the sodium current properties of skeletal myocytes via a paracrine mechanism

Zebedin E., Mille M., Koenig X., Radenkovic M., Zarrabi T., Cervenka R., Todt H., Hilber K.
Inst. Pharmacol., Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Austria; karlheinz.hilber@meduniwien.ac.at

Aims: Intra-cardiac transplantation of skeletal myoblasts has emerged as a promising therapy for myocardial infarct repair and is already undergoing clinical trials. The fact that cells originating from skeletal muscle have different electrophysiological properties than cardiomyocytes, however, may considerably limit the success of this therapy and, in addition, cause side effects. Indeed, a major problem observed after myoblast transplantation is the occurrence of ventricular arrhythmias. The most often transient nature of these arrhythmias may suggest that, once transplanted into cardiac tissue, skeletal myocytes adopt more cardiac-like electrophysiological properties. **Methods:** To test if a cardiac cell environment can indeed modify electrophysiological parameters of skeletal myocytes, we treated mouse C2C12 myocytes with medium preconditioned by primary cardiocytes and compared their functional sodium current properties with those of control cells using the whole cell patch clamp technique. **Results:** This treatment significantly altered the activation and inactivation properties of sodium currents from "skeletal muscle"- to more "cardiac"-like ones. Reverse transcription PCR experiments suggest that an up-regulation of the expression of the cardiac sodium channel isoform $Na_v1.5$ versus the skeletal muscle isoform $Na_v1.4$ is responsible for the observed changes in sodium current function. **Conclusion:** Cardiomyocytes alter sodium channel isoform expression of skeletal myocytes via a paracrine mechanism. Thereby, skeletal myocytes with more cardiac-like sodium current properties are generated.
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OTh14-56

Effect of age on ion channels in the sinoatrial node

Yanni J.F., Tellez J., Boyett M.R., Dobrzynski H.
School of Medicine, University of Manchester, United Kingdom
joseph.f.yanni-gerges@postgrad.manchester.ac.uk

Aim: With age, the function of the sinoatrial node (SAN) deteriorates and the incidence of SAN dysfunction increases. The aim of the study was to investigate why. **Methods:** SAN/atrial preparations from 3-month-old (n=12) and 24-month-old (n=10) rats were compared using electrophysiology, immunohistochemistry and quantitative PCR (qPCR). **Results:** In young rats, the position of the leading pacemaker site was significantly more superior and the intrinsic heart rate was significantly faster (20%). 2 mM Cs^+ (blocker of HCN4 responsible for pacemaker current, If) caused a significantly smaller increase in cycle length in young (22 ± 5%) than in old (44 ± 10%) rats. 100 nM TTX (blocker of $Na_v1.1$ responsible for TTX-sensitive Na^+ current) caused an increase in cycle length in young rats (5 ± 2%), but a decrease in old rats (9 ± 3%); subsequent addition of 2 μM TTX (blocker of $Na_v1.5$ responsible for TTX-resistant Na^+ current) caused a significantly smaller increase in cycle length in young (11 ± 3%) than in old (37 ± 11%) rats. At protein and mRNA levels, HCN4 was present in the SAN but not atrial muscle, $Na_v1.1$ was present in both the SAN and atrial muscle, and $Na_v1.5$ was absent from the SAN but present in the atrial muscle. In young as compared to old rats, there was significantly more Nav1.1 mRNA in the SAN and significantly less $Na_v1.5$ mRNA in the atrial muscle. Furthermore, the volume of HCN4-positive/$Na_v1.5$-negative SAN tissue increased significantly (by 97%) from young to old rats. **Conclusion:** With age, changes in the electrophysiology of the SAN result from changes in ion channels.

OTh15-57

Nociceptive neurons in $Na_v1.9$ knock-out mice lack threshold change following intracellular dialysis of GTP-γ-S

Ostman J.A.R.[1], Nassar M.A.[2], Wood J.N.[2], Baker M.D.[1]
[1]Centre for Neuroscience, Institute of Cell and Molecular Science, Queen Mary University of London, London, United Kingdom; [2]Department of Biology, UCL, London, United Kingdom
m.d.baker@qmul.ac.united kingdom

Aims: GTP-γ-S has been shown to functionally up-regulate the persistent, tetrodotoxin-resistant (TTX-r) Na^+ current in small-diameter sensory neurones, and cause a change in excitability associated with more negative voltage-thresholds. We made a null-mutant mouse, in which exons 4 and 5 of *SCN11A* were replaced with a neomycin resistance cassette, and subsequently tested the hypothesis that $Na_v1.9$ was the substrate for this change in excitability. **Methods:** Sensory neurones were isolated from dorsal root ganglia of either knock-out mice or wild-type and heterozygote littermates, and maintained in culture (1-2 days). With the inclusion of 500 μM GTP-γ-S in the pipette solution, Na^+ current amplitudes and the voltage-thresholds of small-diameter (< 25 μm) neurones were measured in voltage-clamp and current-clamp, respectively, using the whole-cell patch-clamp technique. **Results:** Knock-out of $Na_v1.9$ was associated with the loss of a component of TTX-r Na^+ current (at -30 mV, $p<0.05$), operating over more negative potentials than the major TTX-r Na^+ current, $Na_v1.8$. We found no significant changes in voltage-threshold in $Na_v1.9$ knock-outs (n = 14), whereas some wild-type and heterozygote neurones showed substantial negative shifts in voltage-threshold (-13.63 ± 2.26 mV, $p<0.02$ in 3 of 19 neurones), during recordings lasting up to 30 mins with an imposed negative holding potential. **Conclusion:** These results are consistent with $Na_v1.9$ being the substrate for the persistent Na^+ current in nociceptive neurones, and the effector of the threshold-change associated with intracellular dialysis of GTP-γ-S. Functional up-regulation of $Na_v1.9$ may underlie a component of inflammatory pain.

OTh15-58

Evidence for functional expression of TRPV2 in rat DRG neurones

Babes A., Leffler A., Linte R.M., Nau C., Reeh P.
Department of Physiology, Faculty of Biology, University of Bucharest, Romania
alex@biologie.kappa.ro

Aims: Of the four heat-activated ion channels from the vanilloid-type TRP group (TRPV1-4), the least is known about TRPV2. Expressed in a variety of neuronal and non-neuronal tissues, TRPV2 is a high threshold (>52°C) heat receptor channel, blocked by ruthenium red and activated by 2-aminoethoxydiphenyl borate, and proposed to act as a sensor for intense noxious heat in mammalian sensory neurones. Our aim was to characterize TRPV2 in heterologous expression systems in terms of pharmacology and temperature dependence and to provide new evidence for functional expression of TRPV2 in rat DRG neurones in primary culture. **Methods:** Rat TRPV2 was transiently transfected into HEK293t cells and its activation by heat was investigated using the patch-clamp technique. The same method was used to record heat-activated currents in capsaicin-sensitive and capsaicin-insensitive DRG neurones from the rat. **Results:** In this study we propose a new pharmacological tool to distinguish between the heat responses of TRPV2 and the closely related channel TRPV1: the trivalent cations lanthanum and gadolinium had opposite effects on the two channels, blocking TRPV2 and sensitising TRPV1 to heat. Recordings from rat dorsal root ganglion cultures revealed that medium and large capsaicin-insensitive neurones express a heat-activated current that closely matches the temperature dependence, self-sensitisation and pharmacological properties of TRPV2 in a heterologous expression system. **Conclusion:** Taken together our results provide new evidence for a role of TRPV2 in mediating high-threshold heat responses in a subpopulation of mammalian sensory neurones.

OTh15-59

Modulation of cold sensitive TRP channels by inflammatory mediators

Ciobanu C.A., Babes A.
Department of Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, Romania
cristian@biologie.kappa.ro

Aims: TRPA1 and TRPM8 belong to the TRP ion channel family and are candidates for cold sensing. The aim of our study was to investigate the effects of bradykinin (BK) and prostaglandin E2 (PGE2) on TRPA1 and TRPM8 in mammalian dorsal root ganglion (DRG) neurons. **Methods:** The change in intracellular [Ca^{2+}] was monitored in cultured rat DRG neurons using calcium microfluorimetry. **Results:** Sensitivity to TRPM8 (cold, 18 C and menthol, 100 μM) and TRPA1 (cinnamaldehyde, CA, 100 μM) agonists was strongly coexpressed with sensitivity to inflammatory mediators BK (10 μM) and PGE2 (10 μM). Both BK and PGE2 inhibited the response to cooling in cold and menthol sensitive (CMS) DRG neurons, which are likely to express TRPM8. The effect of BK and PGE2 on CMS neurons was mediated by PKC and PKA, respectively. In control experiments, cultured DRG neurons were submitted to two consecutive applications of CA (100 μM for 3 min, at 10 min interval), while in a separate group of neurons, the second application of CA was preceded by a 1 min application of PGE2 (1 μM). CA-responsive neurons were divided into PGE2-sensitive and PGE2-insensitive. In the latter subgroup, PGE2 sensitised the response to CA by about 68% ($p = 0.02$ compared to control conditions). **Conclusions:** Our results indicate that while reducing the analgesic effect of cooling by desensitizing cold receptors, inflammatory mediators have a sensitizing action on native TRPA1. Both these effects are likely to be involved in inflammatory hyperalgesia.

OTh15-60

Enhanced nociceptive responsiveness to mustard oil in mild colitis

Mitrovic M., Holzer P.
Research Unit of Translational Neurogastroenterology, Inst. Exp. Clinical Pharmacol., Medical Univ. of Graz, Austria; ma.mitrovic@meduni-graz.at

Aims: Transient receptor potential A1 (TRPA1) channels are expressed by many afferent neurons supplying the colon and may play a role in pain signalling. This study examined whether stimulation of TRPA1 by intracolonic mustard oil (MO) causes afferent input to the spinal cord and whether this effect is changed by mild colitis. **Methods:** Female Him: OF1 mice were treated with intracolonic MO (2% in peanut oil). C-Fos expression in the superficial layers of the spinal dorsal horn (laminae I-IIo) at the level of S1 was measured immunocytochemically 1h post-treatment. Morphine (10mg/kg in saline) was administered subcutaneously 1h before the MO stimulus. Mild colitis was induced by adding dextrane sulphate sodium (DSS, 36-50kDa, 2%) to the drinking water for 1 week. The effect of DSS on stool consistency, colon length, colonic myeloperoxidase (MPO), homecage-activity, weight, feeding and drinking was monitored. **Results:** Relative to vehicle, MO increased the number of c-Fos-positive cells in laminae I-IIo, the main termination site of visceral afferent neurons, by 77.1% (t-test: $t8.784=4.851$, $p<0.05$). DSS-induced mild colitis was characterized by increased myeloperoxidase (MPO) levels, shortened colon length and loose bloody stool, whereas homecage-activity, body weight, feeding and drinking remained unchanged. Compared to control, the effect of MO to induce spinal c-Fos was enhanced after DSS pretreatment by 41.1% (t-test: $t12=3.387$, $p<0.05$). Morphine reduced the spinal c-Fos response to MO by 43.5% (t-test: $t11=-4.688$, $p<0.05$). **Conclusion:** TRPA1 stimulation by intracolonic MO induces afferent input to the spinal cord. This response is exaggerated in mice with mild colitis. This observation and the inhibition of the spinal c-Fos response by morphine reflect the nociceptive nature of MO-induced afferent input.

OTh16-61

Expression of the melatonin receptor 1 (MT1) in malignant and non-malignant human bone tumours

Svoboda M., Toma C.D., Arrich F., Assadian O., Thalhammer T.
Department of Pathophysiology, Center for Physiology and Pathophysiology; Department of Orthopaedic Surgery; Clinical Department of Hygiene and Medical Microbiology, Medical University of Vienna, Austria
Theresia.Thalhammer@meduniwien.ac.at

Aims: Bone cell differentiation and homeostasis could be influenced by melatonin receptors MT1 and MT2. **Methods:** Therefore, we investigated expression of MT1 and MT2 in human osteoblasts (hOB), osteosarcoma cell lines (HOS- and MG-63), bone marrow stromal cells (BMSC), and specimens from 30 osteosarcomas and 11 benign bone tumours using quantitative RT-PCR (values are expressed as n-fold enrichment compared to a control specimens) **Results:** MT1, but not MT2 was detectable in hOB, BMSC and bone tumour cell lines. Remarkably, high expression levels of MT1-mRNA together with low OPG-mRNA were found in both cancer cell lines, while in normal hOB and BMSC, high OPG-mRNA levels were associated with low MT1-mRNA levels. MT1-mRNA expression levels were similar in malignant (4.39 ± 4.98-fold) and benign tumours (4.64 ± 6.81-fold). This was also observed for mRNA expression levels of the osteoclast activity stimulating receptor activator of nuclear factor-κB ligand (RANKL, 7.38 ± 9.61-fold vs. 3.57 ± 3.11-fold, p=0.207) and its opponent osteoprotegerin (OPG: 23.45 ± 32.76 vs. 8.07 ± 7.23-fold, p=0.13). MT1 was also detected by Western blotting in both osteosarcoma cell lines. Double-immunofluorescence staining experiments in osteosarcoma cell lines with antibodies against MT1 and glial fibrilary acidic protein (GFAP) revealed that localization of MT1 in subcellular compartments is dependent on cellular growth conditions. **Conclusion:** High expression levels of MT1 in human bone tumours as well as in osteosarcoma cells lines and moderate levels in non-transformed hOB and BMSC suggest that MT1 participates in the regulation of human bone cell growth.

OTh16-62

Experimental chronic colitis in mice is modified by melatonin treatment

Golab M., Dudziak M., Majewski P., Skwarlo-Sonta K.
Department of Animal Physiology, Faculty of Biology, University of Warsaw, Poland
golmat@biol.uw.edu.pl

Aim: The aim of this study was to examine the immunomodulatory properties of melatonin administered to mice with experimental colitis. **Methods:** Experiment was conducted on 6-8 week-old male BALB/c mice, maintained in standard laboratory conditions, LD 12:12. Chronic colitis was induced by 1% dextran sulfate sodium (DSS) solution, administered in drinking water for 3 weeks. Mice were treated daily with melatonin (10 mg/kg b.w.) by gavage, either from the beginning of experiment or after positive occult blood tests recording. Mice were killed by CO2 asphyxia in ZT 6 and ZT 18 (i.e. mid-day and mid-night, respectively). Peritoneal cavity was flushed with PBS, peritoneal leunited kingdomocytes (PTL) were counted and their reactive oxygen species (ROS) production was measured. Colons were dissected out and used for histological tests. **Results:** In mice without colitis melatonin treatment caused cell proliferation in gut lymphatic nodules. DSS administration resulted in crypt shortening and loss with subsequent intensive mononuclear cells infiltration to the mucosa. Melatonin administration did not prevent inflammatory infiltration, but crypt loss location either in proximal or distal part of the colon depended on the length of melatonin treatment. PTL number was elevated in mice with colitis only during the light phase, and was not modified by melatonin treatment. ROS production by PTL was elevated by preventive melatonin only during the night. **Conclusion:** These preliminary results show a possible protective action of the orally administered melatonin in the gut, dependent on the length of the treatment period.

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OTh16-63

Influence of immobilization stress on mammary tumour development in rats

Kassayova M.[1], Friedmanova L.[1], Orendas P.[1], Bojkova B.[1], Bobrov N.[2], Ahlersova E.[1], Ahlers I.[1]
[1]Department of Animal Physiology, Institute of Biological and Ecological Sciences, Faculty of Science, P.J. Safarik University, Kosice; [2]Institute of Forensic Medicine, Faculty of Medicine, P.J. Safarik University, Kosice, Slovakia
monika.kassayova@upjs.sk

Aims: The relation between psychoemotional stress and mammary carcinogenesis has not been sufficiently elucidated yet. The present study is focused on the effect of prolonged immobilization stress on development of chemically induced mammary tumours in rats. **Methods:** Female Sprague-Dawley rats were injected with two intraperitoneal N-nitroso-N-methylurea (NMU) doses each per 50mg.kg^{-1} b.w. between 40-50 postnatal days. Five days after injection the rats were immobilized in special boxes three times a week for 120 minutes during 18 weeks (NMU+IMS). Melatonin (4 μg/ml of drinking water) was administered to one experimental group (NMU+IMS+MEL); application was initiated 3 days prior NMU injection and lasted until the end of the experiment. Tumour incidence, latency, frequency, average tumour volume gain and cumulative tumour gain were evaluated. **Results:** Long-term repeated immobilization of rats decreased tumour frequency per group and animal by 30 % when compared to control (NMU) group; tumour volume gain reduced by 16 % ($p<0.01$). Combination of immobilization and melatonin application decreased tumour frequency per group (by 44 %, $p<0.01$) and per animal (by 35 %); tumour volume gain increased by 35 % ($p<0.05$) and their cumulative volume markedly decreased by 74 % when compared to control. **Conclusion:** Long-term repeated immobilization stress inhibited NMU-induced mammary tumour development in female rats and this inhibiting effect of psychoemotional stress was enhanced by long-term melatonin administration.

OTh16-64

Long-term melatonin treatment slows down the development of spontaneous hypertension

Torok J., Cacanyiova S., Zemancikova A.
Institute of Normal and Pathological Physiology and Centrum of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia; Jozef.Torok@savba.sk

Aims: The aim of the study was to investigate the preventive effect of melatonin on the development of spontaneous hypertension and vascular reactivity of isolated blood vessels in young spontaneous hypertensive rats (SHRs) in early stage of hypertension development. **Methods:** For 4 weeks, melatonin (12 mg/kg/day) was added to the drinking water given to a group of 4- and 8-week-old SHRs and normotensive Wistar rats, and we compared them with groups of untreated SHRs and normotensive control Wistar rats. Blood pressure (BP) was measured by the tail-cuff technique. Rings of isolated thoracic aorta and mesenteric artery were set up for isometric tension recording. **Results:** The treatment of rats with melatonin slows down hypertension development in SHRs (BP at the end of 8th and 12th week 173.8 ± 1.3 and 123.4 ± 1.1 mmHg, respectively, compared with 191.1 ± 3.0 and 143.4 ± 1.5 mmHg in untreated SHRs, $p<0.001$), and did not significantly change BP in age-matched normotensive Wistar rats. Melatonin treatment had no significant effect either on the heart weight/body weight ratio or acetylcholine-induced relaxation in thoracic aorta. Vascular contractions to periarterial nerve stimulation and to exogenous noradrenaline were increased in SHRs. In both melatonin-treated SHR groups, contractions in the mesenteric artery induced by high frequency of stimulation were slightly inhibited. In the thoracic aorta, melatonin lowered sensitivity of vascular smooth muscle to exogenous noradrenaline. **Conclusion:** Results showed that long lasting administration of melatonin to SHRs with developing spontaneous hypertension resulted in decreased increment of BP, and had only slight effect on adrenergically induced increase of vascular tone.

OF17-65

The time-dependent effect of L-NAME on nitric oxide synthase isoform expressions in the heart and brain

Pechanova O., Kojsova S., Jendekova L.
Institute of Normal and Pathological Physiology and Centrum of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia; olga.pechanova@savba.sk

Aims: The goal of our study was to analyse a time course of L-NAME effects on eNOS, nNOS and iNOS protein expressions, NOS activity, membrane oxidative damage and blood pressure (BP), respectively. **Methods:** Adult 12-week-old male Wistar rats were divided into three groups: control, L-NAME (40 mg/kg/day) for four weeks and L-NAME (40 mg/kg/day) for seven weeks. **Results:** Both 4- and 7-week-L-NAME treatments increased BP in comparison with controls. After 4 weeks of L-NAME treatment eNOS expression in the heart increased significantly and this increase was amplified after 7 weeks of treatment. On the other hand, eNOS expression in the brain remained unchanged after 4-week-L-NAME treatment and prolonging the treatment led to significant decrease of eNOS expression in this tissue. There were no changes in protein expressions of other isoforms. NOS activity was decreased after 4 weeks of L-NAME treatment in both tissues. However, prolonging the treatment to 7 weeks increased NOS activity in the heart while NOS activity in the brain was decreased more significantly. Oxidative damage was detected in both tissues after 4-week-L-NAME treatment. Prolonging the treatment amplified the damage in the brain only. **Conclusion:** Increased expression of eNOS may be responsible for increased NOS activity and reduced oxidative damage in the heart after 7-week-L-NAME treatment. Decreased expression of eNOS led, however, to more significant decrease of NOS activity and oxidative damage in the brain. Since BP increase persisted after 7 weeks of L-NAME treatment, we hypothesised that central regulation of BP is predominant in L-NAME-induced hypertension.

OF17-66

Relationship of altered sodium transport across erythrocyte membrane and essential hypertension after blocking Na^+/K^+ pump with low temperature

Hendrixson V., Jablonskiene V., Razinkoviene L., Kucinskiene Z.
Vilnius Univ., Faculty of Medicine, Dept. Physiol., Biochemistry and Laboratory Medicine, Vilnius, Lithuania; vaiva_hendrixson@yahoo.com

Aim: Disturbances in several distinct cell membrane ion transport processes have been demonstrated in essential hypertension. Studies on altered activity of the Na^+/K^+ pump in blood cells have provided conflicting results regarding its involvement in essential hypertension. The purpose of the study was to evaluate the relationship of cellular Na^+ transport in blood cells and essential hypertension after blocking the Na^+/K^+ pump with low temperature. **Methods:** Eighty-three 60-year-old men were investigated. Blood pressure was measured according to the standard requirement. Two heparinised blood samples from normotensive (n=27) and hypertensive (n=59) men were collected to investigate Na^+ concentration in blood plasma (AVL ISE Analysator). One of the samples (blood sample I) was examined immediately and the second (blood sample II) was stored for 4 h at 1°C temperature to show altered Na^+ transport across the erythrocyte membrane by blocking the activity of the Na^+/K^+ pump with low temperature. **Results:** Systolic blood pressure of hypertensive men was 157.51±20.73, and diastolic was 93.08±9.86. Na^+ concentration in blood sample I of hypertensive men was significantly higher than it was in normotensive men (142.89±1.83 and 141.19±1.36 resp., p=0.0002). Na^+ concentration after storing the blood sample at low temperature significantly decreased in hypertensive men (135.7 ± 25.68 in blood sample II compared to 142.51±1.71 in blood sample I, p=0.04). **Conclusion:** We evaluated impaired sodium transport across the erythrocyte membrane of hypertensive men after blocking the Na^+/K^+ pump with low temperature and estimated the relationship between altered sodium transport and essential hypertension. Our results support the hypothesis that essential hypertension is a "cell membrane" disease.

OF17-67

Consistent alterations in the autonomic regulation of the cardiovascular system in young adults with positive family history for hypertension and obesity

Serafimov H.[2], Georgiev K.[2], Belova N.[1]
[1]Dept. Physiol.; [2]Undergraduate Students in Medicine, Medical University of Sofia, Sofia, Bulgaria; n_belova@abv.bg

Aims: To evaluate putative alterations in the autonomic regulation of the cardiovascular system in normotensive young adults with family history for hypertension (H+) and/or obesity (Ob+) as compared to age matched controls without family history (C). **Methods:** Heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity (BRS) were used as markers of the autonomic status. Our protocol consisted of a 5-minute orthostatic test as a sympathetic challenge with a concomitant continuous electrocardiogram recording. HRV indices in the frequency domain were assessed by means of our iCardio software using simultaneously fast Fourier and Wavelet transform. In addition we performed 5-minute non-invasive recording of blood pressure using the Finapress device and we evaluated the parameters of BPV. BRS was measured based on the 4th phase of Valsalva maneuver. **Results:** Both (H+) and (OB+) showed elevated HRV in the low frequency range as well as higher indices of autonomic balance as compared to the (C) individuals at rest. Those differences were more pronounced during orthostatic testing. They were supported by an increased BPV as well as by a decreased BRS in the subjects with family history for hypertension and obesity. The evaluated HRV alterations persisted during the follow up performed 1 year later. **Conclusion:** Our data evidence the existence of sympathetic predominance in young normotensive adults with family history for hypertension and/or obesity. The unequivocal changes in HRV, BPV and BRS prove that this battery of non-invasive methods is a reliable option for screening and could be used in primary prevention.

OF17-68

Remodeling of the saphenous vein wall and tributary vessel system after partial clipping

Nadasy G.L., Hetthessy J.R., Monos E.
Experimental Research Department and Department of Human Physiology, Semmelweis University, Budapest, Hungary
nadasy@elet2.sote.hu

Aim: We investigated the alterations of the venous wall of the main branch as well as of the related tributary system of the rat saphenous vein, after four week application of a partial stricture. **Methods:** Plastic clips restricting the outer diameter of the saphenous vein to 500 µm were surgically applied close to its confluence with the common femoral vein on the right leg. The left leg served as control. After four weeks, a segment just caudal the stricture was subjected to an in vitro microangiographic biomechanical test. In separate sets of animals, the whole superficial tributary system of the saphenous vein was carefully microprepared and studied videomicroscopically. Plastic casts of the tributary system were made, and specimens were also studied histologically. **Results:** An eutrophic wall remodeling with morphological lumen reduction was observed. An extensive collateral system developed, visibly originating from a rich network of vasa vasorum with inversed flow. **Conclusion:** The combined effect of chronically elevated pressure and reduced flow on the upstream section of the main vein branch was a morphologically reduced lumen. Development of collaterals in the venous system is complicated by the presence of valves. Blood accumulating in the main branch will be drained by a newly developed rich system of venules connected parallel, originating mostly from the vasa vasorum, and diverting blood toward long collateral veins. This newly formed portal system, however, may present a much higher haemodynamic resistance than the original main branch lumen.

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OF18-69

Global ischaemic brain injury in rats: a time course of oxidative stress in plasma

Lehotsky J., Sivonova M., Kaplan P., Durackova Z., Drgova A., Tatarkova Z., Pavlikova M., Urban P., Dobrota D.
Dept. of Medical Biochemistry, Comenius University, Jessenius Faculty of Medicine, Martin, Slovakia; Dept. of Medical Chemistry, Biochemistry and Clinical Chemistry, Comenius University, Faculty of Medicine Bratislava, Slovakia
lehotsky@jfmed.uniba.sk

Aims: Free radicals play an important role in the pathogenesis of brain injury. The purpose of this study was to evaluate the potential relationship between the peripheral oxidative stress and ischaemia/reperfusion (I/R)-induced brain. **Methods:** We quantified: (1) lymphocyte DNA damage, (2) plasma antioxidant potential and (3) uric acid levels as well as (4) a time course of peripheral oxidative stress on reperfusion period after ischaemic attack. **Results:** We observed that 15 min of ischaemia significantly increased lymphocyte DNA damage. In parallel, antioxidant potential was significantly elevated after 15 min of ischaemia, as well as after 3 h of reperfusion, when compared to pre-operative levels. A significant correlation can be found between the plasma uric acid and antioxidant potential of plasma after brain I/R. Evidence is presented by in vitro experiments that uric acid is the main component of increased antioxidant capacity seen in early reperfusion period. A significant decrease in total antioxidant potential was observed at 72 h reperfusion and this fall is correlated with the decrease of uric acid level. **Conclusions:** These results indicate a close time dependent association between brain I/R-associated oxidative stress and peripheral antioxidant defence status and/or oxidative stress in animal experiments. The study also suggests for uric acid and antioxidant capacity as an independent clinical outcome predictors in stroke patients.

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OF18-70

Oxidative stress and antioxidant supplementation in soccer players

Micle O., Micle L., Muresan M., Dorofteiu M., Matei D.
University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania
otiliamicle@rdslink.ro

Aims: Reactive oxygen species (ROS) are continuously produced as a part of metabolic processes. Intense physical activity is thought to increase the production of ROS, associated with depletion of antioxidant balance. The purpose of the study was to compare the oxidative stress in two groups of sportmen: with and without antioxidants supplemented diet. **Method:** We measured the levels of MDA (malondialdehyde), PC (protein carbonyl) and CER (ceruloplasmin) in 21 soccer players that received mixture of antioxidants (vitamin E, vitamin C, selenium, etc) in huge amounts (group A), and 30 soccer players with no antioxidant supplementation (group B). We compared them to a group of 25 blood donors that do not make systematic exercise. We mention that group A sportsmen play in the first league, but the results were poor, and group B play in the second league. **Results:** MDA concentrations were considerably increased only in a group B compared to the control ($p<0.001$). PC levels were increased on both groups, but more significant in a group B (group A $p=0.02$, group B $p<0.001$). CER levels were decreased in a group A ($p<0.01$). Both soccer ball teams had an oxidative stress. In a group A it is reflected by the high concentration of CP and reduced levels of CER. Group B showed considerably increased levels of MDA and PC, but the concentration of CER is similar to the control group. **Conclusion:** Exercise produces an imbalance between ROS and antioxidants. Antioxidant supplementation reduced oxidative stress, but did not significantly improve the sport performance of the team.

OF18-71

Tempol increases rat upper airway muscle force

Skelly J.R.[1], Carberry J.[1], Bradford A.[2], O'Halloran K.D.[1]
[1]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland; [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland.
ken.ohalloran@ucd.ie

Aims: Upper airway muscle dysfunction is implicated in obstructive sleep apnoea (OSA). Agents that improve respiratory muscle performance may be useful as an adjunct therapy. The aim of this study was to examine the effects of superoxide scavengers on rat pharyngeal dilator muscle contractile properties. **Methods:** Adult male Wistar rats were killed humanely and isometric contractile properties of isolated strips of sternohyoid muscle were examined in aerated physiological salt solution at 35°C in vitro. Muscles were incubated in tissue baths under hyperoxic ($95\%O_2/5\%CO_2$) or hypoxic ($95\%N_2/5\%CO_2$) conditions in the absence (control) or presence of the antioxidants: Tiron (10mM) or Tempol (10mM). Force-frequency relationship was determined in response to supra-maximal stimulation (10-100Hz, 300msec). **Results:** Under hyperoxic conditions, both Tiron and Tempol significantly increased sternohyoid muscle force and caused a left-shift in the force-frequency relationship. Thus peak force was 17 ± 2, $22 \pm 2^*$ $27 \pm 1^*$ N/cm² for control (n=9), Tiron (n=8) and Tempol-incubated (n=9) muscles (*$p<0.01$ ANOVA). The EF50 ie stimulus frequency producing 50% of peak force was 66 ± 4Hz for control, $56 \pm 4^*$ for Tiron and $49 \pm 2^*$ for Tempol, $p<0.01$ ANOVA. Sternohyoid muscle force was significantly lower in hypoxia compared to hyperoxia for all groups. Tempol-incubated muscles generated significantly more force than control muscles in hypoxia at stimulus frequencies ranging from 60-100Hz. **Conclusions:** This study illustrates that superoxide scavengers increase upper airway muscle force and protect against hypoxia-induced decreases in muscle performance. We conclude that antioxidant therapy may be beneficial in the treatment of OSA.

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OF18-72

Is melatonin a natural brain antioxidant?

Cheng H.M., Pang B.H., Khalifah S.
Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia
chenghm@ummc.edu.my

Aims: Antioxidants, both endogenous and dietary are involved in mechanisms to ensure cellular heath. We chose 3 established antioxidants, ascorbic acid, glutathione, melatonin to compare their protective effects in a DNA damage test by Comet assay. **Methods:** The target cells were human lymphocytes incubated with hydrogen peroxide to induce a baseline DNA injury. **Results:** We found that antioxidant property was dependent on the dose of the chemical used. At high concentrations, both dietary ascorbic acid and glutathione alone produced considerable DNA damage to the cells. This pro-oxidant effect was minimal when cells were treated with melatonin, a natural hormone released from the pineal gland. **Conclusion:** The importance of dose-response relationships is highlighted when investigating any beneficial antioxidant effects in vitro. Also, the concentration of antioxidant used in bench assays ought to be matched with prescribed dose for dietary supplementation, assuming that absorption is adequate. Melatonin secretion is unique in that it peaks during sleep. A cellular protective effect for this nocturnal hormone could reflect its role in repair and regeneration during the period of daily rest. Melatonin may be a natural antioxidant produced by the brain.

OF19-73

Selected hormones in plasma and brain-derived neurotrophic factor in the hippocampus of rats kept under enriched environment

Bakos J.[1*], Ondicova K.[1*], Steinbusch H.W.M.[2], Jezova D.[1]
*equally contributed
[1]Inst. Exp. Endocr., Slovak Acad. Sci., Bratislava, Slovakia; [2]Brain & Behaviour Inst., Maastricht Univ., Netherlands; ueenjbks@savba.sk

Aims: Several physiological functions, including stress hormone release are gender dependent. The aim of this study was to test the hypothesis that environmental enrichment used as a model of increased brain plasticity, induces gender-dependent effects on neuroendocrine activity in rats. **Methods:** Wistar rats were housed under standard conditions or in big cages with repeated changing of environmental objects (3 times a week) for 6 weeks. Male and female rats were housed separately. Blood and tissues for hormone and/or brain derived neurotrophic factor (BDNF) analyses were samples following decapitation. **Results:** Plasma corticosterone and the weight of adrenal glands were higher in female compared to male rats. Though corticosterone levels were not modified by housing in enriched environment, plasma ACTH increased significantly ($p<0.05$) in male but not in female rats exposed to environmental enrichment. Oxytocin concentrations in plasma were higher in female than in male rats ($p<0.001$). Oxytocin content in the posterior pituitary was not modified by any experimental condition. As expected, concentrations of BDNF in the hippocampus were significantly increased by exposure to enriched environment. Moreover, BDNF levels were higher in female than in male hippocampus and the influence of environmental enrichment was significantly more intensive in females than in males ($p<0.001$). **Conclusion:** Increase in neural plasticity as well as changes in neuroendocrine functions induced by housing the rats in enriched environment seem to be different in males and females.
The study was supported by grants of APVV LPP-0194-0 and VEGA 5064.

OF19-74

Concerning the different role of the central and peripheral AMPA/ kainate glutamate receptors in regulation of basal gastric acid secretion in rats

Falalyeyeva T., Beregova T., Shtanova L.
Kyiv National Taras Shevchenco University, Pharmaco-Physiology Department, Kyiv, Ukraine
tfalalyeyeva@mail.ru

Aim: To investigate the influence of antagonist of AMPA/kainate glutamate receptors IEM 1751 on basal GAS in rats with intact nervous system (INS) and after the bilateral vagotomy. **Methods:** The study was carried out in acute experiments in 46 white rats under urethane anesthesia (1.10 g/kg, intraperitoneally (i.p.)). The animals were divided into two groups: I - rats with INS; II - rats after surgical bilateral vagotomy. GAS was investigated by method of isolated stomach perfusion by Ghosh and Shild. We studied the influence of antagonist of central and peripheral AMPA/kainate glutamate receptors IEM 1751 (5 mg/kg, i.p.) on basal GAS. The nicotinic acetylcholine receptors were blocked by pentamini (3.2 mg/kg, i.p.). **Results:** In rats with INS IEM 1751 reduced spontaneous GAS by 44%, $P<0.01$. The basal GAS was inhibited by the bilateral vagotomy by 29%, $p<0.05$. However in rats after vagotomy IEM 1751 enhanced the total acid secretion by 217%, $p<0.001$. In these rats pentamini removed the excitatory action of IEM 1751 on basal GAS. **Conclusion:** These results indicate that peripheral AMPA/kainate glutamate receptors realize their effects through vagal cholinergic neurotransmission in the ENS. Probably the peripheral AMPA/ kainate glutamate receptors are located on preganglionic cholinergic nerves prejunctionally or on interneurones of ENS. Thus we supposed that endogenous glutamate acting on AMPA/ kainate glutamate receptors in CNS intensify basal GAS but in ENS suppressed basal GAS. So the central and peripheral AMPA/kainate glutamate receptors play different role in regulation of basal GAS.

OF19-75

Lanthanides induce neurotransmitter release from the vesicular pool in presynaptic brain endings

Waseem T.V., Kolos V.A., Lopatina L.P., Fedorovich S.V.
Institute of Biophysics and Cell Engineering NAS, Minsk, Belarus
tanya.waseem@gmail.com

Aim: Neurotransmitter (NT) release in neurons is coupled with calcium influx into the presynaptic terminals. In some cases, action of osmotic shock, lanthanides or ruthenium red, calcium-independent exocytosis might be registered. The mechanism of calcium-independent exocytosis remains unexplored. **Methods:** We have investigated action of lanthanides on exocytosis and D-[^{3}H]Aspartic acid efflux in isolated presynaptic endings (synaptosomes). Exocytosis was determined using fluorescence probe Acridine Orange (AO). Sodium concentration ($[Na^+]_i$) was monitored by fluorescent dye Sodium Green and SBFI-AM. **Results:** Application of 300mkM $GdCl_3$ increases spontaneous release of D-[^{3}H]Aspartic acid. Experiments with AO reveal $GdCl_3$ and $La(NO_3)_3$ induce rapid dose-dependent increase of AO fluorescence intensity indicating exocytosis. Gadolinium blocks calcium channels in neurons. It was shown that P/Q type of calcium channels are raft associated in presynaptic membranes. Gd^{3+}-induced exocytosis registered using AO was Ca^{2+}-independent, similar results were obtained with D-[^{3}H]Aspartic acid. Pretreatment of synaptosomes with methyl-betta-cyclodextrin, compound destroying lipid rafts, didn't affect $GdCl_3$ and $La(NO_3)_3$ induced D-[^{3}H]Aspartic acid release. Do lanthanides influence any systems downstream Ca^{2+}-sensor? We established that $GdCl_3$ and $La(NO_3)_3$ induced an increase of $[Na^+]_i$. Enhancement of $[Na^+]_i$ was not mediated neither by Na^+ entry via VD channels, as this effect was not prevented by tetrodotoxin, a specific blocker of VD channels nor by lipid rafts. **Conclusions:** Lanthanides induce Ca^{2+}-independent vesicular release of neurotransmitters via mechanisms common for polyvalent cation.

OF19-76

Effects of risperidone on vigilance and EEG power spectra

Katai Z.[1,3], Kitka T.[1,3], Gyongyosi N.[1], Bagdy G.[1,2,3]
[1]Laboratory of Neurochemistry and Experimental Medicine, National Institute of Psychiatry and Neurology, Budapest; [2]Group of Neuropsychopharmacology, Hungarian Academy of Sciences and Semmelweis University, Budapest; [3]Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Budapest, Hungary
bag13638@mail.iif.hu

Aims: Risperidone, an atypical antipsychotic agent with high affinity to several monoamine receptors including serotonin 5-HT2 and dopamine D2 receptors, was studied for its effects on vigilance states and EEG power spectra. **Methods:** Polysomnographic recordings (EEG, EMG, motor activity) were performed in freely moving male Sprague-Dawley rats for 2 h after treatment with risperidone (0.03, 0.1 or 0.5 mg/kg i.p.) at light onset. Active and passive wake (AW, PW), light and deep slow wave sleep (SWS-1, SWS-2) and paradoxical sleep (PS) were studied. **Results:** All doses decreased the time spent in PS. The highest dose of risperidone significantly increased and decreased the cumulative time of PW and SWS-2, respectively. The lowest dose of risperidone increased the EEG power at 4 Hz during PW, SWS-1 and SWS-2, furthermore, decreased the beta (13-30Hz) band during PW. An increase was noticeable in EEG power density at the 3-4 Hz and 6-8 Hz frequency ranges in SWS-1, and there was also an increase in the 2-5 Hz frequency ranges in SWS-2. Interestingly, 0.1 mg/kg dose of risperidone took effect only in SWS-1 (an increase at 7 Hz), and the highest dose had no effects on EEG power spectra. **Conclusion:** Sleep pattern and EEG power spectra were affected most effectively by highest and lowest doses of risperidone, respectively. The strongest effects of this drug were a decrease in time spent in PS and an elevated EEG power at 4 Hz.
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OF20-77

Role of solubile guanylyl cyclase $\alpha_1\beta_1$ ($sGC\alpha_1\beta_1$) isoform in mice corpus cavernosum smooth muscle relaxation

Nimmegeers S., Sips P., Buys E., Brouckaert P., Van de Voorde J.
Department of Physiology and Physiopathology and Department for Molecular Biomedical Research, Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, Ghent, Belgium
johan.vandevoorde@UGent.be

Aims: Soluble guanylyl cyclase (sGC) is, as major effectors molecule for NO, an interesting therapeutic target for the treatment of erectile dysfunction. Therefore, we assessed the functional importance of the predominant soluble guanylyl cyclase (sGC) $\alpha_1\beta_1$ isoform in corpus cavernosum (CC) relaxation. **Methods:** CC isolated from male $sGC\alpha_1^{-/-}$ mice and wild type littermates were mounted in organ baths for measurement of agonist- or electrical field stimulation (EFS)-induced tension responses. **Results:** The endothelium-dependent relaxation to acetylcholine (ACh) or bradykinin (BK) and the neurogenic response to electrical field stimulation (EFS) were nearly abolished in the $sGC\alpha_1^{-/-}$ CC. The relaxing influence of exogenous NO (from sodium nitroprusside (SNP) and NO-gas) was also significantly decreased in the $sGC\alpha_1^{-/-}$ mice. The remaining relaxation seen in the $sGC\alpha_1^{-/-}$ mice with exogenous NO, was strongly but not completely inhibited by the sGC-inhibitor ODQ. In the preparations of the $sGC\alpha_1^{-/-}$ mice, the response to BAY 41-2272 (NO-independent sGC-activator) and to T-1032 (phosphodiesterase type 5 inhibitor) were also significantly reduced. The specificity of the impairment of the sGC-related responses was demostrated by the similar forskolin (adenylyl cyclase activator)-and 8 pCPT-cGMP (cGMP-analogue)-induced responses. **Conclusion:** Our findings indicate the involvement of an sGC isoform with the α_1-subunit in NO-induced CC smooth muscle relaxation. However, the remaining relaxing influence of exogenous NO in the $sGC\alpha_1^{-/-}$ mice, suggests the contribution of (an) additional pathway(s).

OF20-78

Hypoxia affects individual types of IP3 receptors in neuronal and cardiac cells

Krizanova O.[1], Holotnakova T. [2], Zahradnikova A.[1], Pastorekova S.[2], Jurkovicova D.[1]
[1]Institute of Molecular Physiology and Genetics; [2]Institute of Virology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia
olga.krizanova@savba.sk

Aims: Hypoxia is a state of reduced oxygen supply to the tissue below physiological levels despite adequate perfusion of the tissue by blood. The purpose of the study was to test, whether hypoxia can affect levels of individual IP3 receptors in neuronal and cardiac cells. **Methods:** We compared mRNA levels of Type 1 and 2 IP3 receptors by reverse transcription and polymerase chain reaction (RT-PCR) and also protein levels by Western blot and immunofluorescence. Five mice were subjected to hypoxia and compared to 5 control mice. Moreover, hypoxia was tested on primary culture of cerebellar granular cells and also on H962 cardiomyocyte culture. **Results:** We observed significant increase in both, type 1 and 2 IP3 receptors in all tested cells on mRNA and protein level after 24 hours exposure to 4% hypoxia. Nevertheless, while maximal increase in type 1 IP3R occurs after 24 hours, type 2 IP3R mRNA was maximally increased after 3 hours. **Conclusion:** We have shown that hypoxia significantly increases types 1 and 2 IP3 receptors in neuronal and cardiac cells. Physiological relevance of this observation remains to be elucidated; nevertheless, involvement of IP3Rs in remodelling of at least neuronal cells is probable.

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OF20-79

Role of individual S/T's at the GIRK1 and GIRK4 subunits in heterologous facilitation as well as in trafficking of the channel complex

Treiber F., Rosker C., Fritz R., Mullner C., Steinecker B., Schreibmayer W.
Molecular Physiology Laboratory, Institute for Biophysics, Medical University of Graz, Graz, Austria; wolfgang.schreibmayer@meduni-graz.at

Aims: It has been shown previously that G-Protein dependent inwardly rectifying K^+-channels (GIRK's), are regulated by cAMP dependent proteinkinase (PKA) and by proteinphosphatase 2A (PP2A; "heterologous facilitation"). Both the GIRK1 and the GIRK4 subunit have been identified as prominent targets for PKA phosphorylation and the structural determinants on the channel subunits were identified. Aim of the current study was to investigate the roles of the individual subunits in the heterologous facilitation of IK+ACh and in membrane trafficking of the channel complex. **Methods:** Wild type and phosphorylation deficient GIRK1 and GIRK4 subunits were heterologously expressed in oocytes of *Xenopus laevis* and the effect of PKA phosphorylation on agonist induced as well as on basal currents was quatitated for different subunit compositions. Membrane trafficking of GFP tagged subunits, following activation and deactivation of PKA was studied by confocal microscopy. **Results:** It was found, that phosphorylation of the GIRK4 subunit is most important for the instantaneous increase in the K^+ current observed (heterologous facilitation), while GIRK1 subunit phosphorylation contributes little. Instead phosphorylation of the GIRK1 subunit turned out to be crucial for membrane localization and trafficking of the channel complex: it was found, that channel complexes containing WT GIRK1 get internalized 1-2 h after cytosolic injection of cAMP or Sp-cAMPS. In turn cytosolic injection of the PKA antagonist Rp-cAMPS produced a marked increase in surface localization. **Conclusion:** These results demonstrate the importance of individual S/T's on both the GIRK1 and the GIRK4 subunit in regulation of channel activation as well as in membrane trafficking.

OF20-80

Possible role of beta3-adrenoceptors in the hyperthermic effects of MDMA in the mouse

Bexis S., Docherty J.R.
Department of Physiology, Royal College of Surgeons in Ireland, 123 St. Stephen's Green, Dublin 2, Ireland; sbexis@rcsi.ie

Aims: In studies of the beta3-adrenoceptor antagonist SR59230A, it has been reported that the hyperthermia to MDMA involves beta3- in addition to alpha1-adrenoceptors in rats (Sprague et al., 2004). In this study, we wished to investigate the actions of SR59230A on temperature responses to MDMA in mice. **Methods:** C57-BL/6 WT mice (22-30g male) were implanted under ether anaesthesia with intra-abdominal temperature probes (DSI). After 14 days, temperature was recorded in freely moving mice by telemetry. Drugs were injected subcutaneously. Statistical comparisons were carried out using ANOVA with post tests, as appropriate. **Results:** MDMA (20 mg/kg) produced a significant hyperthermia beginning at approximately 100 min after injection. Prazosin (0.1mg/kg) or SR59230A (5 mg/kg), or the combination of prazosin and SR59230A, produced essentially similar effects: the response to MDMA was altered from a monophasic hyperthermia to a biphasic hypothermia followed by hyperthermia (e.g. MDMA produced initial decreases of 1.94 ± 0.45°C and 1.96 ± 0.31°C in the presence of prazosin or SR59230A, respectively). Selective alpha1A- or alpha1D-adrenoceptor antagonists when given alone did not affect the hyperthermia to MDMA, but in combination acted like prazosin, revealing an initial hypothermia to MDMA. However, SR59230A also has potency as an antagonist at alpha1A-, alpha1B- and alpha1D-adrenoceptors in functional and ligand binding studies *in vitro*. **Conclusion:** The beta3-adrenoceptor antagonist SR59230A reduced hyperthermic actions of MDMA in mice, but we cannot rule out alpha1-adrenoceptor antagonist actions.

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OF21-81

Superoxide dismutase mimetic tempol inhibits hypoxic pulmonary vasoconstriction in rats independently on either nitric oxide production or basal tone of pulmonary vessels

Snorek M., Hodyc D., Brtnicky T., Herget J.
Department of Physiology, Charles University in Prague, 2nd Medical School, Prague, Czech Republic
snorek@iol.cz

Aims: Hypoxic pulmonary vasoconstriction (HPV) is regulated by changes in the production of and interactions among reactive oxygen species (ROS). There is a controversy as to whether HPV is mediated by an increase or a decrease in ROS production. Also, the role of nitric oxide (NO) in HPV remains unclear. We investigated whether the inhibition of HPV by the antioxidant tempol was dependent on the concentration of NO, and how its effect was influenced by increased basal pulmonary vascular tone. **Methods:** In isolated rat lungs we measured vasoconstrictor responses to acute ventilatory hypoxia before and after administration of tempol during perfusion with or without L-NAME (experiment A) and after increase of basal vascular tone in the perfused lungs by increasing the potassium ion concentration in the perfusate by 15 mmol/l (experiment B). **Results:** Administration of tempol significantly decreased vasoconstriction response to acute hypoxic challenge both in controls (89% less) and in L-NAME group (100% less), the vasoconstrictor response did not differ significantly between these groups. The administration of tempol in experiment B significantly decreased HPV both in K^+ treated (96% less) and in K^+ non-treated group (68% less), the vasoconstrictor response did not differ significantly between these groups. **Conclusion:** Our results indicate that inhibition of HPV by the SOD mimetic tempol does not depend on either NO production or basal tone of the pulmonary vessels. The paper presenting complete results of this study has been accepted for publication in the Journal by The Physiological Society and Blackwell Publishing.

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OF21-82

Reactive oxygene species in arterial pulmonary hypertension

Micle L.[1], Muresan M.[1], Micle O.[1], Maghiar F.[1], Matei D.[1], Cheregi C.[1], Marta M.[2], Neagoe C.[1], Pop M.[2], Dorofteiu M.[1]
[1]Faculty of Medicine and Pharmaceutics, University of Oradea; [2]Pneumophthisiology Hospital Oradea, Romania
lianamicle@rdslink.ro

Aim: The purpose of the study was to follow the manner in which sulfizol influences the arterial pulmonary pressure and reactive oxygen species (ROS) production. **Methods:** Our study was performed on a group of patients, which were diagnosed with arterial pulmonary hypertension using the echocardiographic method. The patients were divided in 2 groups: one group, which received sulfizol administrated orally for 3 days, 3x1g/day and another group, which was not treated with sulfizol. There are researches sustaining that sulfizol blocks the ET1 receptors. Before and after the treatment, all the patients were echocardiographically examined and simultaneously blood sampled and malondialdehyde (MDA), carbonylated proteins (CP) and ceruloplasmin concentrations in serum assessed. **Results:** There are no significant changes of the parameters echocardiographically measured in both group of patients, before and after the treatment. The patients, compared with the reference group of healthy subjects, have considerably increased concentrations of MDA and CP ($p<0.001$), thus demonstrating the presence of the oxidative stress in patients. After 3 days of treatment, the MDA and CP concentrations reduce no significantly at both groups of patients ($p>0.1$). The same situation is noticed regarding ceruloplasmin levels before and after the treatment. **Conclusions:** The arterial pulmonary hypertension is associated with a marked oxidative stress demonstrated by elevated values of MDA and CP. A 3 days treatment with sulfizol argues for a possible action of this drug on endothelin receptors.

OF21-83

The association of catecholamines response to stress stimulus with metabolic risk factors in young men with early stage of non-treated hypertension

Penesova A.[1], Radikova Z.[1], Cizmarova E.[2], Kvetnansky R.[1], Blazicek P.[3], Vlcek M.[1], Koska J.[1]
[1]Inst. Exp. Endocrinol., SAS; [2]Pediatric Cardiology Out-patient Clinic Karlova Ves; [3]Dept. Clin. Biochem., Hospital of Ministry of Defence, Bratislava, Slovakia ; adela.penesova@savba.sk

Increased activity of sympathetic nervous system (SNS) was observed in early stage of hypertension onset. The **aim** of our study was to assess the relationship between catecholamines response to stress stimulus and metabolic risk factors in young males with early-diagnosed non-treated hypertension grade 1 (HT) and normotensive controls (NT). **Methods:** Insulin tolerance test (ITT) and oral glucose tolerance test (oGTT) were performed in 21 HT male subjects and in 19 NT males. Plasma levels of glucose, insulin, adrenaline, noradrenaline (NA), plasma renin activity (PRA), total and HDL cholesterol, triglycerides (TG), and PAI-1 were determined. Insulin resistance index (IR) HOMA and indices of insulin sensitivity (ISIs) Matsuda, Cederholm and Gutt were calculated. **Results:** HT subjects had higher levels of baseline NA, insulin and PAI-1 ($p<0.05$) and higher levels of NA during ITT ($p<0.05$). HT patients had higher IR HOMA ($p<0.01$) and lower ISIs ($p<0.001$). Baseline concentrations of adrenaline correlated negatively with ISI Matsuda ($r=-0.361$, $p<0.05$), ISI Cederholm ($r=-0.354$, $p<0.05$) and ISI Gutt ($r=-0.429$, $p<0.01$), with HDL cholesterol ($r=-0.336$, $p<0.05$) and positively with PRA ($r=0.499$, $p<0.01$). Baseline concentrations of NA correlated positively with basal plasma PAI-1 levels ($r=0.433$, $p<0.01$) and plasma insulin in 2nd h of oGTT ($r=0.397$, $p<0.05$) and negatively with ISI Gutt ($r=-0.385$, $p<0.05$). **Conclusions:** The early stage of onset of hypertension (in normal weight young patients) is associated with sympathetic overactivity. Negative correlation with lower insulin sensitivity and HDL cholesterol suggests SNS contribution to metabolic cardiovascular risk factors.

OF21-84

Role of the cholinergic system in blood pressure reactions to nicotine

Michailov M.C., Neu E., Welscher U., Schulz G., Foltinova J.
Inst. Umweltmedizin c/o ICSD e.V. Muenchen-Paris-Sofia/Kazanlak & Univ. Erl.-Nuernberg; Inst. Histol., Univ. Bratislava, Slovakia
m_michailov@hotmail.com

Introduction: Nicotine is a classical ganglion-stimulating/blocking agent. Presently will be given a summary of earlier and recent data about Nicotine-effect on rat blood pressure (Front. Rad. Ther. Oncol. 31, 22-35/1997; Proc. Rad. Res. I., 434/1995; Strahlenther. 151, 549-554/1976). **Method:** Registration by Statham element on Rikadenki recorder (see introd.). **Results:** Nicotine (5-170 µg/kg), similar to mercaptoethylguanidine (MEG: 200-400 mg/kg), transformed acetylcholine-depressor (ACH: 1-2 µg/kg) into a biphasic depressor/pressor reaction (dR/pR). Nicotine potentate MEG-effect: This inhibitor of Ca^{++}-independent isoform of NO-synthase augmented also pR of non- (AHR-602, MCN-A-343: 1-100 mg/kg) and nicotinic-like (DMPP: 15-120 µg/kg) ganglion-stimulating agents and of vasopressin (5-100 mU/kg) in normal and spinal rats. MEG blocked dR after peripheral, but transformed it into dR/pR after central vagal electrical stimulation (CVS: 55 Hz, 2 ms, 5 s, 5 V), whereby vasopressin antagonized this effect (also ACH-dR). MEG inverted serotoninergic dR (5-HT-liberation from thrombocytes by nicotine) into pR. **Conclusion:** Nicotine excites/inhibits (dose-dependently) via nicotinic cholinergic receptors CNS-structures/preganglionic cholinergic sympathetic neurons, responsible for cardio-vascular regulation leading to biphasic CVS-/ACH-dR/pR as well as to sensitisation of central adrenergic and cholinergic (+vasopressin) neurons and of sympathicus (presynaptical neurons) to non- and nicotine-like ganglion-stimulating-agents. Nicotine-effects incl. complex interaction of hormonal/drug-factors could induce non-calculable vasoconstriction leading to cerebral apoplexy and cardial angiospasms.

OF22-85

Diabetes is associated with alteration of calcium release from the endoplasmic reticulum of submandibular acinar cells

Kopach O.[1], Voitenko N.[1], Fedirko N.[2]
[1]Bogomoletz Institute of Physiology, Kiev; [2]Franko National University, Lviv, Ukraine
kopach@biph.kiev.ua

Aims: Xerostomia and polydipsia are widespread complications of diabetes mellitus which are associated with impaired functioning of salivary glands; however their cellular mechanisms are not determined yet. We studied the changes in free Ca^{2+} concentration in the lumen of endoplasmic reticulum ($[Ca^{2+}]$ ER) of submandibular acinar cells under diabetic conditions. **Methods:** Diabetes was induced by a single i.p. injection of streptozotocin (60 mg/kg b.w.). Imaging of $[Ca^{2+}]$ ER was performed using mag-fura-2/AM in beta-escin-permeabilized acinar cells. Concentration of free calcium in intracellular solution ($[Ca^{2+}]_i$) was clamped at the 30 nM and 100 nM. **Results:** We have found the increase of the InsP3-induced calcium release and passive calcium leak from the ER under diabetic conditions. It was shown: i) rise in the amplitude of InsP3-induced $[Ca^{2+}]$ ER decrease by 48 ± 8% ($p<0.05$, n=11) at the $[Ca^{2+}]_i$=100 nM; ii) increase of 3 mcM InsP3-induced $[Ca^{2+}]$ER decrease by 58 ± 11% ($p<0.05$, n=19) at the $[Ca^{2+}]_i$=30 nM; iii) dramatic prolongation of the half-time of agonist-induced $[Ca^{2+}]$ ER transient by 107 ± 12% ($p<0.01$, n=13) under diabetic conditions. We have also found that puromycin (0.5 mM), that removes nascent polypeptides from the ER-ribosome translocon pores, caused significant increase of the passive Ca^{2+} leak from the ER under the diabetes (0.094 ± 0.022 in diabetes vs. 0.068 ± 0.013 in control). We suggest that diabetes is accompanied with rise in sensitive of InsP3-receptors and increase in passive calcium leak from the ER under diabetic conditions in submandibular acinar cells.
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OF22-86

Aspects of electrical instability in obese chronic myocardial infarction patients

Mozos I., Hancu M., Chiulan C., Gorun C.
University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania
ioanamozos@umft.ro

Aims: Chronic myocardial infarction (CMI) is associated with electrical instability. Obesity increases ventricular variability indices and the incidence of abnormal signal averaged electrocardiograms (SAECG). The aim of this study was to assess the influence of obesity on electrical instability in CMI patients. **Methods:** 32 chronic myocardial infarction patients, 14 obese (body mass index-BMI: 33 ± 3 kg/m^2) and 18 normal weighted (BMI: 26 ± 2 kg/m^2), underwent SAECG (to detect late ventricular potentials-LVP) and 12-lead ECG (assessing ventricular activity variability indices: QT and QRS dispersion, as the differences between maximal and minimal duration of the ECG intervals). **Results:** QRSdc (heart rate corrected QRS dispersion) was 32 ± 27 ms in obese, and 17 ± 12 ms in normal weighted CMI patients. QTdc (heart rate corrected QT dispersion) was 35 ± 23 ms in obese and 33 ± 25 ms in the normal weighted group. SAECG parameters were: SA-QRS (SAECG QRS duration): 127±28 ms, LAS40 (Low Amplitude Signal): 61 ± 40 ms and RMS40 (Root Mean Square): 20±14μV. QRSdc correlated with BMI (r=0.604) and was significantly increased in obese (32 ± 27 ms) compared to normal weighted CMI patients (17 ± 12 ms) (p=0.0027). The mean values for QTdc were higher in the obese (28.4 ms) compared to the normal weighted patients (26.75). Late ventricular potentials were present in 71% of the obese patients and 56% of the normal weighted CMI patients. SAECG parameters also correlated with BMI: r (SA-QRS, BMI): 0.5, r(LAS40, BMI):0.45. **Conclusion:** The presence of obesity in CMI affects intraventricular conduction, increasing ventricular arrhythmia risk. There exists a BMI associated increase in CMI patients' sudden death risk.

OF22-87

Role of Connexin43 during ischemic preconditioning in normal and hyperlipidaemic rat hearts

Gorbe A., Shultz R., Csont T., Ferdinandy P.
Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged, Hungary
aniko@biochem.szote.u-szeged.hu

Aims: The loss of early preconditioning was subsequently confirmed by our group in hearts isolated from rats exposed to dietary cholesterol. Information exchange via Connexin43 (Cx43) gap junctions located mainly in the intercalated disc in the hearts, could be involved as an effector molecule in ischemic preconditioning (IP). The aim of the study was to detect expression and intracellular migration of Cx43 during IP in hyperlipidemic rats. **Methods:** Male Wistar rats were fed laboratory chow enriched with 2% cholesterol or standard chow for 12 weeks and after diet period hearts were isolated and perfused according to Langendorf. IP protocol (3X5 min ischaemia/ reperfusion) or 10 min perfusion was applied, which was followed by 30 min global ischaemia and 5 min reperfusion. Cx43 expression was detected by immunohistochemistry or western blotting (WB) in total hearts and by WB in isolated mitochondria. **Results:** Cx43 expression did not change significantly in hyperlipidaemia. Cx43 content of mitochondria significantly increased ($p<0.05$) due to ischaemia and reduced ($p<0.05$) following PC in both groups. Intracellular migration of connexins from intercalated discs was observed in hyperlipidaemic hearts, which further decreased ($p<0.05$) after IP. **Conclusion:** Intracellular migration of Cx43 was detected in hyperlipidaemic hearts, and altered intracellular pattern of connexins after IP, which could be involved in lost preconditioning in cholesterol-fed rats.

OF22-88

Symbolic dynamics analysis of heart rate control in young patients with diabetes mellitus type 1

Javorka M., Trunkvalterova Z., Tonhajzerova I., Javorkova J., Javorka K.
Institute of Physiology, Comenius University, Jessenius Faculty of Medicine; Paediatric Clinic; Martin Teaching University Hospital, Martin, Slovakia
MJavorka@lefa.sk

Aims: Cardiovascular dysregulation and autonomic neuropathy are common complications of diabetes mellitus (DM). Modern sensitive methods, including heart rate variability (HRV) analysis, can detect autonomic nervous system dysregulation even in early phases of DM. **Methods** of nonlinear dynamics are increasingly applied to HRV analysis to improve the description and classification of cardiac dysregulation during different pathological conditions. In contrast to other nonlinear methods, investigation of symbolic patterns after coarse graining of the dynamics – symbolic dynamics analysis – can be applied also to relatively short (few hundreds of heart beats) recordings. The aim of this study was to ascertain which of the HRV symbolic dynamics measures are different in young patients with DM compared to control group. **Methods:** We investigated patients with type 1 DM (17, 10 females, 7 males) aged 12.9-31.5 years (mean+/-SEM: 22.4+/-1.0 years) and 17 age and gender matched subjects. The length of R-R intervals was measured using telemetric system. Several parameters based on 4 symbols encoding were used for quantification of heart rate variability and complexity. Results: Our results suggest slightly reduced complexity (expressed by marginally nonsignificantly reduced number of "forbidden words") even in young diabetic patients pointing out to another aspect of heart rate dysregulation in this group. In addition we have found qualitative difference in distribution of symbolic words expressed by parameter "wpsum02". **Conclusion:** We conclude that parameters of symbolic dynamics could be used for quantification of information hidden in heart rate time series in patients with dysregulation.

OF23-89

Mechanisms of angiotensin II–mediated induction of the expression of CYP11B2 in human and rat adrenocortical cells

Szekeres M. [1], Cserzo M. [1], Supeki K. [1], Szidonya L. [1], Turu G. [1], Hazay M. [1], Chaplin T. [2], Clark A.J.L. [2], Hunyady L. [1]
[1]Dept. Physiol., Semmelweis Univ., Faculty of Medicine, Budapest, Hungary; [2]Dept. Endocrinol., Barts & The London, Queen Mary School of Medicine, London, United Kingdom; szekeres@puskin.sote.hu

Aims: Angiotensin II (AngII) activates several signaling pathways mediating physiological and pathophysiological responses in different cell types and organs, such as vasoconstriction, cell-proliferation, inflammation and atherosclerosis. It is known, that aldosterone synthase (CYP11B2) is regulated via calmodulin kinases (CAMK) and transcription factors (e.g. Nurr1/NGFIB). Our purpose was to investigate AngII-induced transcriptional effects and signaling mechanisms in human and rat adrenocortical cells. **Methods:** Human adrenocortical cells (H295R) were subjected to microarray analysis. 2-hour AngII (1μM) stimulation upregulated more than 200 genes. Some genes of steroidogenesis and transcription factors were evaluated by real-time PCR. To investigate AngII-induced signalling mechanisms, we applied specific inhibitors: MEK inhibitor (PD98059, 20μM), PKC inhibitor (BIM1, 3μM), CAMK inhibitor (KN93, 10μM). **Results:** In H295R cells AngII induced expression of transcription factors Nurr1 (NR4A2) and NGFIB (NR4A1) by around 25- and 10-fold, respectively. CYP11B2, was induced by 5-fold. In rat glomerulosa cells, AngII induced 4-fold stimulation of the expression of CYP11B2, whereas transcription factor NGFIB was more activated than Nurr1 (11- over 2-fold). PD98059 and KN93 reduced AngII-induced activation of both Nurr1 and CYP11B2 in H295R cells such as NGFIB in rat cells. Activation of rat CYP11B2 was reduced by KN93. **Conclusion:** Angiotensin II-induced activation of CYP11B2 involves MAP-kinases and CAMK in humans and CAMK in rats. In rat glomerulosa cells NGFIB may have a dominant role over Nurr1. *Supported by Grants OTKA T49851 and Jedlik Anyos 1/010/2005.*

OF23-90

Insulin-sensitizing effect of chronic AT1 blockade in adipose tissue of rats

Zorad S.[2], Tybitanclova K.[2], Dou J.[2], Benicky J.[1], Hutanu D.[1], Zhou, J.[1], Saavedra J.M.[1],
[1]Section on Pharmacology, NIMH, Bethesda, MD, USA; [2] Inst. Exp. Endocrinology, SAS, Bratislava, Slovakia; stefan.zorad@savba.sk

Aim: Recent data suggest that Angiotensin II receptor type 1 (AT1) antagonists besides lowering blood pressure reduce obesity-related metabolic disturbances. In order to verify the possible insulin-sensitizing effects of AT1 receptor blockade in adipose tissue we treated male Wistar Kyoto rats with Candesartan cilexetil (Cc; 10 mg/kg/day) for 18 weeks. **Methods:** Adipocyte cell size was determined by light microscopy after colagenase digestion of adipose tissue. Circulated levels of hormones in serum were determined by RIA. Gene expression of adipokines and RAS components in adipose tissue were evaluated by real-time PCR. AT1 and AT2 receptor protein levels were determined by immunoblot. **Results:** Significant loss in body weight without change in food intake was observed at the end of the experiment. In epididymal and retroperitoneal adipose tissue significant decrease in mass was noticed due to hypotrophy. Serum leptin was decreased and serum adiponectin was increased in treated rats. Cc lowered leptin and TNFα expression and elevated adiponectin, fatty acid synthase and PPARγ mRNA. In addition, Cc treatment resulted in the increase of epididymal AT2 receptor gene and protein expression. **Conclusion:** We propose that Cc modulates adipokines production and release and regulates fat tissue cellularity. These effects might be the consequence of local AT1 receptor inhibition and/or AT2 receptor stimulation, probably involving PPARγ activation. Increased adiponectin and PPARγ together with decreased TNFα suggest insulin-sensitising action of long-lasting AT1 receptor blockade. *Supported by DIRP, NIMH and by VEGA 2/5090/25.*

OF23-91

Local generation of angiotensin in isolated rat pulmonary vessels.

Gurzu B.[1], Petrescu B.C.[2], Dumitriu I. L.[1], Mihai A.C.[1], Slatineanu S.M.[1]
[1]Faculty of Medicine, Department of Physiology; [2]Faculty of Bioengineering, Department of Ophthalmology, University of Medicine and Pharmacy "Gr. T Popa" Iasi, Romania
bgurzu@yahoo.com

Aims: The renin angiotensin system plays important roles in pathogenesis of various pulmonary diseases as pulmonary hypertension and lung fibrosis. The contractile effects of angiotensinogen (Aogen) and its metabolisation pathways were studied on rat pulmonary artery (RPA) and vein (RPV) rings with or without endothelium. **Methods:** We quantified Aogen – induced contractions in the presence or in the absence of pepstatin A (a renin inhibitor, 10 μM), captopril (an ACE inhibitor, 10 μM), chymostatin (a chymase inhibitor, 10 μM), amastatin (an aminopeptidase-A and -M inhibitor) or losartan (a specific AT1 blocker, 10 μM). **Results:** On all rings, Aogen-induced contractions were only reduced by pepstatin A (with two thirds on RPA and one third on RPV) or captopril (with a half on RPA and at least 35% for RPV), amplified by amastatin (with 27.96 ± 5.86% on RPA and 11,72 ± 7.03% for RPV) and totally prevented by losartan. The Aogen contractile effects were powerfully on bronchi obtained from ovalbumin-sensitized rats (after challenge) but these differences were blocked by chimostatin, which did not significantly modify Aogen-induced contractions on normal rats. The enzymes effects were not influenced by the presence or absence of intact endothelium. **Conclusion:** It is suggested that pulmonary vessels possess a local renin-angiotensin system and there is the possibility of angiotensin production within the vessel walls using various and physiological circumstances – dependent enzymatic pathways.

OF23-92

Renal function in patients treated with ACE inhibitors

Tirlea F., Burta O., Burta L., Avram G., Gak A.
Faculty of Medicine and Pharmacy, Oradea, Romania
ftirlea@yahoo.com

Aim: Angiotensin converting enzyme (ACE) inhibitors are largely used for hypertension and heart failure; they are also renoprotective, slowing the progression of renal diseases towards renal failure by reducing proteinuria. We evaluated their effect on the renal function. **Material and methods:** The study included 30 patients with chronic glomerular diseases, with proteinuria, haematuria and hypertension. They were divided in 3 groups, each of them receiving either 10 mg of Enalapril, 2 mg of Trandolapril or 20 mg of Fosinopril for 30 days. We evaluated the glomerular filtration rate with Cockroft-Gault formula, the changes in serum creatinine, proteinuria, blood pressure in the beginning, at 15 and 30 days of therapy. **Results:** GFR had a mean decrease of 17.4% (from 101.6 to 83.9 ml/min). The decrease was constant in Enalapril patients; in Fosinopril patients, GFR increased at 15 days, but decreased at 30 days; in Trandolapril patients, GFR decreased at 15 days and had a slight increase after one month. Serum creatinine increased, but the values were not statistically significant ($p<0.05$). In Enalapril patients, it had a slow, constant elevation; with Fosipril, it decreases at 15 days and normalizes at 30 days; with Trandolapril, it rises at 15 days and is maintained after 30 days. Proteinuria decreased with 43% and the blood pressure decreased from 154.4/94 to 143.9/84.2 mmHg after one month of ACE inhibitors. **Conclusion:** The glomerular filtration rate and serum creatinine remain in the physiological limits during the therapy with ACE inhibitors. The changes in their values are not statistically significant.

OF24-93

Expression of activin A and myostatin systems in experimental dilated cardiomyopathy

Mahmoudabady M., Ray L., Mathieu M., Dewachter C., Hadad I., Jespers P., Naeije R., Mc Entee K.
Laboratory of Physiology, Faculty of Medicine, Free University of Brussels, Brussels, Belgium; maryam.mamoudabadi@ulb.ac.be

Aims: Activin A (ActA) and myostatin (MSTN) are members of the transforming growth factor-β superfamily. They have negative effects on tissue growth, are expressed in myocardium and upregulated after myocardial infarction. Pathogenic mechanisms of dilated cardiomyopathy are not clearly understood. We studied the time course expression of cardiac MSTN and ActA systems together with markers of the apoptotic process in a canine model of tachycardiomyopathy. **Methods:** Endomyocardial biopsies were weekly taken from 15 dogs during development (7 weeks) of tachycardiomyopathy. Cardiac gene expression of MSTN, ActA, their receptors and modulating ligands together with Bax, Bcl-2, caspases 9 and 3 were assessed by real-time quantification PCR. **Results:** MSTN, activin receptor I, follistatin, activin receptor interacting protein 1, Bax, Bcl-2 and caspase 9 stayed unchanged. ActA increased with a four fold overexpression in overt heart failure (4.1 ± 1.6 wk 7 vs. 1 ± 0.2 wk 0). A 2 fold decrease of activin receptor IIA (0.6 ± 0.09 vs. 1 ± 0.12), IIB (0.5 ± 0.05 vs. 1 ± 0.09) and activin receptor interacting protein 2 (0.5 ± 0.09 vs. 1 ± 0.11) was observed. Caspase 3 increased (2.1 ± 0.37 vs. 1 ± 0.16) and was correlated with ActA, but, at immunohistochemistry, apoptototic nuclei were not increased compared to controls. **Conclusion:** The activin system may play a role in the development of experimental dilated cardiomyopathy more than myostatin and pro-apoptotic signalling.

OF24-94

Augmentation of interstitial cell responses to ATP and low pH stimulation by cell adhesion

Sui G-P., Wu C., Fry C.
Department of Physiology, RF&UCL Medical School, Rowland Hill St, London, United Kingdom; c.fry@ucl.ac.united kingdom

Aim: Bladder suburothelial interstitial cells generate Ca^{2+} transients and Cl^- currents on exposure to exogenous agonists. It is hypothesised these cells modulate sensations arising from bladder filling. Because of their structural network characteristics we examined if cell-pair formation modulates their responses. **Methods:** Guinea-pigs (300-500g) were killed by cervical dislocation according to UK Home Office guidelines and the bladder removed. Mucosa was separated from detrusor and digested in a collagenase-containing solution to generate isolated interstitial cells. Cells were loaded with Fura2, superfused with a CO_2/HCO^{3-} or HEPES-buffered solution and voltage-clamped (Cs-filling solution). Data are mean ± SD; differences were examined with Student's t-tests ($p<0.05$). **Results:** ATP elicited intracellular Ca^{2+} transients and inward currents at -60mV (25.8±18.7pA.pF^{-1}, n=29). When a cell touched firmly against a second, the response was significantly augmented by 180±58% (paired *t*-test). However, cell capacitance was unaffected (13.6±6.8 vs. 12.9±6.9pF). Similar responses were observed on exposure to low pH solutions. Augmentation was absent when interstitial cells touched a detrusor myocyte. Cell pair formation also lowered the threshold response to ATP about ten-fold. With glivec (30μM) the response was not augmented by cell-pair formation (94±50% of control, n=24). **Conclusion:** Responses of suburothelial interstitial cells to exogenous agonists are augmented by touching another cell and formation of cells into a tight network increases their functionality. Glivec deregulates tyrosine-receptor kinase activity that in turn modulates pathways also modified by adherens junction formation. Identification of such pathways will help to understand this augmentation. *We thank the Wellcome Trust for support.*

OF24-95

Ca-dependence of stretch-activated vesical channels

Welscher U., Foltin V., Wirth M., Neu E., Michailov M.C., Seidenbusch W.
Inst. Umweltmedizin c/o ICSD e.V. Muenchen-Paris-Sofia/Kazanlak & Univ. Erl.-Nuernberg; Inst. Physik, Univ. Bratislava; Inst. Exp.Physik, Univ. Innsbruck; eva_neu15@hotmail.com

Aim: On example of vesical-preparations (guinea-pig) will be given recent/earlier results concerning Ca-dependence of motor/electrical activities [Biomed.Techn. 39, 312-3/1994; Biophys. & Mol.Biol. 65/1, 170/1996; Proc. IUPS 14 (Budapest), 2428/1980; 17 (Helsinki) 529/1989; Faseb J. 19/4, A585/2005; Eur.J.Physiol. 443, S334/2002; 420, R99/1992; 419, R98/1991]. **Method:** Recording of motor/isometric and electrical/intracellular activity (see introd.). **Results:** Spontaneous phasic detrusor-contractions (SPC): Detrusor-amplitudes (4.1±2.4mN=100%) after 3x$CaCl_2$ (normal 2.1 mM=1x, McEwen-solution) 101/111% (3/50 mN), frequency (4.0 ± 0.7/min=100%) 66.9/72.7% (3 mN/50 mN). Tonic trigonum-contractions (STC): Amplitudes (17.2 ± 11.6 mN=100%) changed after 3x$CaCl_2$ 82.5/133.6% (3/50 mN), frequency 65.8/18.7% (3/50 mN). After changed [$CaCl_2$] (0.1 to 3x) appeared different motor patterns incl. detrusor-SPC/STC (similar to trigonum). Ca-effect on electrical action potentials of vesical myocytes: Not only spike (S) activity was transformed into a burst-plateau (BP) one by stretch (3 to 80 mN), but also [$CaCl_2$]-reduction (0.5-0.1x) induced BP with increased frequency (reciprocal proportional to [$CaCl_2$]). Electro-induced detrusor-contractions (10Hz, 0.3ms, 3s) are strongly increased after stretch: Ca-increase had augmentory effects (110/130% 3/50 mN) (total n=50). **Conclusion:** Ca^{2+} participates essentially in electrical S-generation and S-transformation into BP probably via Ca-activated K-stretch-dependent channels: SPC could be related to electrical S- and STC to BP-activity. Further pharmacophysiological investigations could help for better pharmacological and electrotherapy of urinary bladder (incontinence, overactive bladder, etc.).

OF24-96

Preconditioning by phenylephrine is dependent on PKC and K_{ATP} channel activation in adult rat isolated ventricular myocytes

Turrell H.E.[1], Dickens M.[2], Standen N.B.[1]
[1]Departments of Cell Physiology and Pharmacology; [2]Biochemistry, University of Leicester, United Kingdom; HET5@le.ac.united kingdom.

Aims: Pretreatment with the α_1-adrenoceptor agonist phenylephrine (PE) has been shown to protect cardiac tissue from a subsequent period of ischaemia. This phenomenon, known as pharmacological preconditioning, decreases infarct size and increases the functional recovery of the intact heart. The present study investigated the role of protein kinase C (PKC) and ATP-sensitive K^+ (K_{ATP}) channels in PE-dependent preconditioning of isolated myocytes. **Methods:** Isolated adult rat ventricular myocytes were maintained at 35-37°C, stimulated at 1 Hz, and exposed to metabolic inhibition and reperfusion. Cells were pretreated with a 10 minute perfusion of substrate-free Tyrode solution containing PE (5 μM), the PKC inhibitor chelerythrine (5 μM), PE + chelerythrine, the K_{ATP} inhibitor glibenclamide (10 μM) or PE + glibenclamide. Contractions were recorded using video-microscopy and stored on DVD for later analysis. Recovery of contractile function and the times to contractile failure and rigor were used as indicators of protection. **Results:** Phenylephrine pre-treatment caused a significant increase in the percentage of cell recovering contractile function compared to Tyrode-pretreated controls (43.8 ± 5.4% vs. 18.8 ± 2.3%, mean ± SEM). This effect was abrogated by chelerythrine or glibenclamide (14.2 ± 5.4% and 8.2 ± 6.9% respectively). However, glibenclamide also decreased recovery in the absence of phenylephrine (3.2 ± 2.2%). Substrate-free Tyrode solution alone increased functional recovery compared to control in 2 of 6 experiments, however average recovery was not significantly different from control. **Conclusion:** Preconditioning of isolated cardiac myocytes by phenylephrine involves the activation of PKC and K_{ATP} channels.

Poster Presentations

PW01-1

Electrostatic interaction between the pore and the voltage sensors in the voltage-gated Na^+ channel

Cervenka R., Zarrabi T., Koenig X., Zebedin E., Hilber K., Todt H.
Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Vienna, Austria
hannes.todt@meduniwien.ac.at

Aims: Voltage gated ion channels consist of an ion conducting central pore, several gates and voltage sensors. In the voltage-gated Na channel the central pore is believed to be lined by the S6 segments of all four domains, which may contain the activation and inactivation gates. The voltage sensors extend radially from the central pore axis. We sought to investigate a possible electrostatic coupling of the permeation pathway to the voltage sensors. **Methods:** A critical residue within the putative selectivity filter of the rNav1.4 channel, K1237 was replaced by the negatively charged glutamate (K1237E). The constructs were expressed in *Xenopus laevis* oocytes and studied by means of the two electrode voltage clamp technique. **Results:** In K1237E the midpoint of fast inactivation (V05) was shifted to the hyperpolarized direction relative to wild-type (-60 ± 13 vs. -47 ± 11 mV, n=6, P<0.01). Such shift is consistent with an electrostatic bias on the voltage-sensors by the introduced negative charge in the outer vestibule. Furthermore, we generated serial replacements by cysteines of 16 residues in the domain IV-S6 segment. These mutations generated inconsistent changes of V05. However, when these mutations were combined with the K1237E mutation, V05 was shifted to more negative values in all but one double mutants, irrespective of the direction and the amount of shift produced by the single S6 mutation (mean shift = -13 ± 5 mV). **Conclusion:** The pore of the voltage-gated Na channel is electrostatically coupled to the voltage sensor for fast inactivation.

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PW01-2

A possible mechanism of the regulation of Ca^{2+} release

Fedorenko O., Marchenko S.
Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine, Kiev, Ukraine
fea80@ukr.net

Aims: We have reported previously that the nuclear envelope of pyramidal neurons of hippocampus contained multiple IP_3-activated Ca^{2+} channels (IP_3Rs) and large conductance cationic channels (LCC). The aim of this study was to investigate effects of membrane potential on properties of these channels. **Methods:** Single ion channels were recorded with the patch – clamp technique from the inner membrane of nuclei isolated from pyramidal neurons of CA1 region of rat hippocampus. **Results:** Channel activity of the IP_3Rs was potential – dependent. The probability of the open state (P_o) was much higher at positive potentials in the perinuclear space and decreased at negative potentials with the complete blockage at potentials below – 80 mV. P_o of the LCC channels depended on the membrane potential too. At positive potentials they were almost all time open ($P_o > 0.8$). At negative potentials the activity of these channels significantly decreased with complete block of the channels by potentials ≤ -40 mV. **Conclusion:** These results demonstrate that the activity of IP_3Rs and LCC is inhibited by negative potential in the perinuclear space. Ca^{2+} release from intracellular stores is accompanied by large transfer of electric charge which results in development of negative potential in its lumen. This may prevent any further Ca^{2+} release by the voltage – dependent inhibition of IP_3 receptors. The voltage – dependent inhibition of LCC channels may enhance this effect by facilitating the negative shift of the potential. So we suggest that these two types of ion channels operate in concert to take part in the regulation of Ca^{2+} signal duration.

PW01-3

Chronic electrical stimulation affects calcium channel kinetics in skeletal myocytes

Koenig X., Radenkovic M., Zebedin E., Mille M., Zarrabi T., Cervenka R., Todt H., Hilber K.
Institute of Pharmacology, Center for Biomolecular Medicine and Pharmacology, Medical University of Vienna, Vienna, Austria
karlheinz.hilber@meduniwien.ac.at

Aims: Skeletal myocytes exhibit a remarkable capacity to adapt their properties in response to altered functional demands. Such adaptations can be triggered in vitro, e.g., by electrical stimulation of cultured myocytes. Whereas changes in the expression of contractile proteins and metabolic enzymes in response to electrical stimulation are well understood, very little is known about possible adaptations in the electrophysiological properties of skeletal myocytes. In this study, we investigated the effects of electrical stimulation on the properties of currents through voltage-gated calcium channels of skeletal myocytes. **Methods:** Mouse C2C12 skeletal myocytes were electrically stimulated continuously for up to two weeks at a frequency of one Hertz. Thereafter, their barium current properties were detected using the whole cell patch clamp technique, and compared with those of control cells. **Results:** Electrical stimulation of skeletal myocytes significantly speeded the activation kinetics of their calcium channels. In addition, channel activation seemed to consist of a fast and a slow component in many electrically stimulated myocytes, whereas in control cells, the slow component predominated. **Conclusion:** Chronic electrical stimulation alters the properties of currents through voltage-gated calcium channels in skeletal myocytes. This could be explained by an up-regulation of the cardiac isoform of the L-type calcium channel in response to electrical stimulation.

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PW01-4

Effect of luminal Ca^{2+} on the sensitivity of the cardiac ryanodine receptor to ATP

Tencerova B., Gaburjakova J., Zahradnikova A., Gaburjakova M.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia
barbora.tencerova@savba.sk

Aims: Ryanodine receptor (RyR) is a calcium-activated, calcium-permeable channel of the sarcoplasmic reticulum (SR) that mediates excitation-contraction coupling in cardiac muscle cells. There is growing evidence that Ca^{2+} in the lumen of the SR can be effectively involved in the different aspects of RYR channel regulation. In this study, we tested whether luminal Ca^{2+} exerts any effect on the sensitivity of the RYR channel to adenosine triphosphate (ATP) – a well known activator of the RYR channel with potential physiological importance. **Methods:** RyR channels isolated from the rat heart were reconstituted into a planar lipid membrane. Single-channel currents were recorded under asymmetric conditions with either luminal Ca^{2+}, luminal Ba^{2+} or a mixture of Ca^{2+}/Ba^{2+}. Luminal Ba^{2+} mimicked the situation when no Ca^{2+} is present on the luminal face of the channel. **Results:** In the absence of luminal Ca^{2+}, the channels were only marginally activated by ATP ($P_{max} = 0.01$ at [ATP] = 10 mM). In the presence of luminal Ca^{2+}, ATP induced dose-dependent activation of the channel. The maximum open probability increased, while the EC_{50} of ATP activation decreased with elevating luminal Ca^{2+} concentration from 1 mM to 53 mM (Pmax ~ 0.025, EC50 ~ 7.5 mM at 1 mM luminal Ca^{2+}; P0 ~ 0.019, P_{max} ~ 0.89, EC_{50} ~ 0.5 mM at 53 mM luminal Ca^{2+}). **Conclusion:** The potency and efficacy of ATP as activator of the cardiac RYR channel at diastolic Ca^{2+} concentrations is dramatically modulated by the luminal Ca^{2+} concentration.

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PW01-5

Ion conductances related to shaping the repetitive firing in rat retinal ganglion cells

Kolodin Y.O., Veselovskaia N.N., Veselovsky N.S., Fedulova S.A.
International Center for Molecular Physiology, NAS of Ukraine, Kiev, Ukraine; ykolodin@biph.kiev.ua

Aims and **methods**: Intrinsic firing properties of retinal ganglion cells (RGCs) of adult rat were studied by the whole cell patch-clamp technique in retinal flat-mounted preparations. In response to 500-ms depolarizing current step the majority (93.4%) of the examined RGCs displayed tonic firing that lasted for the duration of depolarization period. Only 6.6% of the RGCs displayed transient firing accommodated during the stimulus. In addition, 60.7% of the examined RGCs displayed sustained high-frequency firing with the steady-state firing frequency over 50Hz. Ionic conductances underlying excitability in tonically firing neurons were studied by applications of selective pharmacological blockers. **Results:** Application of TTX (1μM) caused reversible disappearance of action potentials (AP) in response to stimulus. Suppression of Ca^{2+} influx through voltage-activated Ca^{2+} channels by 200μM Cd^{2+} lead to increase of steady-state firing frequency, and to increase of single AP repolarization rate, without abolishing the basic pattern of tonic firing. Role of different types of voltage-gated potassium channels were studied using the application of the respective blockers. It was found that potassium conductance highly sensitive to external TEA (1mM) or 4-AP (200μM) is responsible for fast repolarization and after-hyperpolarization of a single AP, providing the cells with the ability for high-frequency firing. Conductances, sensitive to other blockers of voltage-gated potassium channels (α-DTX and CTX) did not play such a role. The known specificity of these drugs and the fast-spiking phenotype of majority of the cells strongly suggested that this 4-AP and TEA-sensitive conductance is mediated by Kv3 potassium channels. **Conclusion:** Thus, in our cells, the Na^+ and Kv3-like K^+ currents generate a basic firing pattern, while Ca^{2+} and Ca^{2+}-dependent conductances stabilize tonic firing, efficiently regulate discharge frequency.

PW01-6

Inhibitory effect of glibenclamide on mitochondrial chloride channels from the rat heart

Kominkova V., Ondrias K.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic
kominkova@savba.sk

Aims: Glibenclamide is a sulfonylurea drug that is widely used for the treatment of non-insulin-dependent diabetes mellitus. It was reported that this drug inhibited cystic fibrosis transmembrane regulator (CFTR) Cl^- channels in epithelial and cardiac cells. Aim of our study was to test whether glibenclamide can inhibit also intracellular chloride channels. **Methods:** The lipid bilayer technique was used to examine the effects of glibenclamide on the activity of mitochondrial chloride channels. Single channel activity was measured after reconstitution of the mitochondrial membrane vesicles isolated from the rat heart. **Results:** Conductance of the observed chloride channels was in the range of 90-150 pS and single channel amplitude at 0 mV was 2.6 -3.7 pA (using 250/50 mM KCl cis/trans solutions). Open probability of the chloride channels was in the range of 0.7-0.9. Glibenclamide reversibly inhibited the high chloride channel activity in the concentration range of 100-150 μM in a voltage-dependent manner. The inhibition effect of glibenclamide was pronounced in the presence of 2 mM ATP. **Conclusion:** Sulfonylurea glibenclamide, blocker of ATP-dependent potassium channel, can also inhibit mitochondrial chloride channels. The obtained results may contribute to understanding mechanisms of its cardiovascular effects: decrease in ischemic preconditioning or anti-arrhythmic effect during the ischemic period.

PW01-7

Store-operated calcium entry in sensory DRG neurons

Korol S., Korol T.
Department of General Physiology of Nervous System, Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine, Kiev, Ukraine
itisnew@ukr.net

Aims: It is known that store-operated calcium influx is the main route of Ca^{2+} entry in non-excitable cells. Recently this type of Ca^{2+} entry was revealed in neurones. But there are few works dedicated to store-operated Ca^{2+} entry (SOCE) in neuronal cells. The aim of this study was to discover SOCE in sensory DRG neurons mediated by caffeine-sensitive Ca^{2+} stores. **Methods:** Current-voltage relationships have been recorded in control conditions and after applying caffeine by means of patch clamp technique from rat acute isolated sensory DRG neurons. **Results:** Difference between these two relationships turned out to be similar to current-voltage relationship of store-operated Ca^{2+} channels. It has region of inward rectifying and very positive reversal potential (>+70 mV). After applying the blocker of store-operated Ca^{2+} channels 2-APB, this difference disappeared. **Conclusion:** These results demonstrated that sensory DRG neurons needs in one else mechanism of Ca^{2+} entry besides the voltage-operated one. We conclude that SOCE is one of the constituents of calcium signaling in sensory DRG neurons.

PW01-8

Low amplitude in late Ca^{2+} spikes is a result of decreased calcium release flux

Zahradnikova A., jr., Polakova E., Pavelkova J., Zahradnik I., Zahradnikova A.
Inst. of Molecular Physiology and Genetics, SAS, Bratislava, Slovakia; umfgsasa@savba.sk

Aims: The extent and synchrony of calcium release from individual calcium release sites in cardiac myocytes varies considerably. Our aim was to compare latencies and amplitudes of calcium release events to reveal possible common determinants. **Methods:** Local calcium release events (Ca-spikes) were evoked by calcium currents in voltage-clamped isolated rat ventricular myocytes. The cells were excited by a 70-ms depolarization from -50 to 0 mV. Ca-spikes were measured using 0.1mM fluo-3 as the calcium indicator and 1 mM EGTA to limit calcium diffusion, and compared with simulated Ca-spikes [1]. Three-dimensional convolution of fluo-3 concentration with a Gaussian kernel was used for simulation of Ca-spike images. Both the measured and simulated local calcium release events were analyzed as previously described [1]. **Results:** The amplitude as well as kinetic parameters of simulated Ca-spikes were strongly dependent on the distance of the event from the focal plane. In measured early calcium release events, the relationships between their fluorescence amplitude, time-to-peak, and duration was consistent with uniform calcium release flux amplitude, while in the case of the late calcium release events these relationships could be explained only if assuming a decreased calcium release flux amplitude. **Conclusion:** These results indicate that late Ca-spikes have decreased calcium release flux, either because the presence of inactivated ryanodine receptors, or due to locally lower sarcoplasmic reticulum calcium contents. *Support: APVT-51-031104, EU Contract No. LSHM-CT-2005-018833/EUGeneHeart, NIH-FIRCA-R03-TW-05543.*

1. Zahradnikova A., jr. et al., J Physiol. 578: 677-691, 2007.

PW02-9

Effects of chronic hypoxia on diaphragm muscle contractile and endurance properties

McMorrow C.[1], Bradford A.[2], O'Halloran K.D.[1]
[1]UCD School of Medicine and Medical Science, University College Dublin, Ireland; [2]Dept. of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland; ken.ohalloran@ucd.ie

Aims: Chronic hypoxia (CH) is common in respiratory disease and occurs in healthy individuals at altitude. CH alters skeletal muscle structure and oxidative capacity. The aim of this study was to examine the effects of CH on diaphragm muscle contractile function. **Methods:** Adult male Wistar rats were exposed to hypobaric hypoxia (barometric pressure = 380mmHg) for 6 weeks (n=6). Age-matched control rats (n=6) were housed in normobaric conditions. At the end of the treatment periods, isometric contractile properties of isolated strips of diaphragm muscle were measured in tissue baths under hyperoxic ($95\%O_2/5\%CO_2$) or hypoxic ($95\%N_2/5\%CO_2$) conditions. Force-frequency relationship was determined in response to supra-maximal stimulation (10-100Hz, 300msec). Fatigue was assessed in response to repeated tetanic contractions (40Hz, 300msec) every 2 sec for 5 minutes. **Results:** CH exposure was confirmed by evidence of elevated haematocrit and right ventricular hypertrophy compared to control rats. Peak tetanic force at 100Hz was reduced following CH but this did not achieve statistical significance (20.0±2.4 vs. 14.2±1.8 N/cm^2, control vs. hypoxia). The fatigue index (ie ratio of force at 5 min of fatigue to initial force) was significantly altered by CH (32.5±4.8 vs. 51.7±3.2*; % of initial force, control vs. CH, $P<0.05$). Under *in vitro* hypoxic conditions diaphragm muscle force was significantly reduced and muscle endurance was significantly lower than muscles in hyperoxia for both control and CH groups. However, the magnitude of diaphragm fatigue was less in hypoxia for CH muscles. **Conclusions:** CH causes functional plasticity in diaphragm muscle. CH reduces specific force but increases muscle endurance and hypoxic tolerance. These changes may be potentially adaptive in circumstances of prolonged hypoxaemia.

PW02-10

Effect of perinatal anoxia on exploratory behaviour of rat offspring

Dubovicky M.[1], Mach M.[1], Brucknerova I.[2], Ujhazy E.[1]
[1]Institute of Experimental Pharmacology SAS; [2]1st Child Clinic, School of Medicine, Comenius University, Bratislava, Slovakia
exfadubm@savba.sk

Aim: Hyperactivity is considered a typical manifestation of functional injury of the brain after peri- and neonatal hypoxia/ischaemia. However, in our previous experiments, we found inhibition of motor activity of the rats subjected to neonatal anoxia tested in a one-day session. In the present study we evaluated the effect of perinatal anoxia on the intensity of exploratory behaviour of the rat offspring in repeated testing in the open field. **Methods:** Female pregnant rats were sacrificed on day 20 of gestation. The uteruses were placed in 37°C water bath for 20 min. After the anoxic insult the pups were adopted by foster mothers. On day 28 and 180 of age, exploratory behaviour of the rats (intensity of motor activity and rearings) was tested in the repeated open field test (5 consecutive days, 10 min session). **Results:** After weaning, the study showed different courses of habituation of intensity of motor activity and rearings in anoxic animals compared to controls. There was a decrease of motor activity on day 1 of testing and an increase on days 3, 4 and 5 in the anoxic group compared to controls (sum of the activity counts on days 3, 4 and 5 for anoxic group: 1848 ± 169, for controls: 1279 ± 150, $p < 0.05$). **Conclusions:** The study revealed the need of a complex approach to the evaluation of motor activity after hypoxic- ischemic insults. Single testing can conceal the phenomenon of hyperactivity, which can be detected during repeated testing of the animals in a novel environment.
Study was supported by the grant VEGA No. 2/5052/27

PW02-11

Antenatally stressed rat dams: their pups survival, development and behaviour

Dunaeva T.Y., Graf A.V., Trofimova L.K., Sokolova N.A.
Lomonosov Moscow State University, Biology Faculty, Department of Human & Animal Physiology, Moscow, Russia
conniephys@gmail.com

Aims: Prenatal hypoxia is considered to be one of the main pathologies of pregnancy. It is not only one of the main reasons of different diseases of newborns and prepuberty, but can also disturb reproduction. The purpose of the study was to test the hypothesis that acute prenatal hypoxic stress influences the second generation of white rat pups. **Methods:** Female rats survived acute prenatal hypoxia in early organogenesis. We investigated their descendants' survival, development and behaviour. On the 22nd, 36th and 57th days of life pups were tested in the hole board. Each animal was placed in the field and its behavioural characteristics were visually registered during following 4 minutes. **Results:** The pups of prenatally stressed dams were born immature, and males remained behind in physical development up to the 57th day of life. By the 57th day of life a 20% mortality enhance was observed in experimental group compared to control. We observed that on the 22nd day of life the pups of prenatally stressed dams showed decreased horizontal motor activity and rearing, and on the 57th day of life only male pups showed increased horizontal motor activity and rearing. **Conclusion:** According to our results, acute prenatal hypoxic stress keeps it influence by at least the second generation of white rats and disturbs survival, development and behaviour.

PW02-12

Effect of prenatal hypoxia on the development of CNS and cardiovascular system

Graf A.V., Maslova M.V., Trofimova L.K., Dunaeva T.Y., Sokolova N.A., Goncharenko E.N.
Lomonosov Moscow State University, Moscow, Russia
stasy_gr@pochta.ru

Aims: The lack of oxygen is considered to be one of the main interfering factors and the reason of different diseases of newborns and prepuberty. **Methods:** Pregnant rat females were exposed to acute hypoxia on the 9–10th day of gestation (the beginning of organogenesis). We evaluated the acute hypoxia influence on the cardiomyocytes contractility in newborn rats, ECG, behavioural activity and biogenic amines levels in brain were investigated in grown – up descendants. **Results:** It was shown that newborn rats, survived prenatal hypoxia, were born with lower birth weight compared to control and remained behind in physical development up to at least the 60th day of life. Antenatal stress led to noticeable changes of chronotropic index and NE-induced chronotropic effects in hypoxic group in comparison with control one. Thus, the baseline cardiomyocytes contractility rate was increased by 50-60% while NE-induced enhancement of contraction was significantly lower in animals survived antenatal hypoxia. The increase of heart rate and activation of sympathetic system were observed in adult rats survived prenatal stress. The significant differences in heart rhythm have been attributed to alterations in brainstem development. The enhancement of NE- and DA- levels both in brain stem and cerebral cortex were observed in experimental males whereas females demonstrated the significant increase of 5 HT and DA in brain stem only. The behaviour changes following prenatal hypoxia were registered only in females demonstrated the decrease of both locomotion and rearing. **Conclusion:** The prenatal hypoxic stress contributes to the development of severe neurological and cardiovascular impairments in grown-up descendants.

PW02-13

Phytotherapeutical alternatives in preventing oxidative stress disorders due to thyroid gland dysfunction: experimental data

Joanta A.[1], Sarlea S.[1], Decea N.[1], Miclaus V.[2], Bojan M.[1], Clichici S.[1], Socaciu C.[2], Moldovan R.[1]
[1]University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Romania; [2]University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania; adelaelena@yahoo.com

Aims: Increased oxidative stress have been described previously in models of hyperthyroidism and in human subjects with Basedow disease. In this study, the influence of a diet enriched in soy and sea buckthorne on reactive oxygen and nitrogen species and antioxidant defence was explored. Soy products are attractive because of their beneficial effects on chronic diseases such as cardiovascular diseases, atherosclerosis, and type II diabetes. Also, polyunsaturated fatty acids, having an antioxidant effect, were found in high concentration in *Hippophaea rhamnoides* (sea buckthorne). **Methods:** Hyperthyroidism was elicited by intraperitoneally L-Thyroxin administration in white, male, Wistar rats. Animals' diet was enriched in *Hippophaea rhamnoides* and soy. Oxidative stress markers (lipid peroxides, carbonyl proteins), nitrites and antioxidant defence (thiols groups, hydrogen donor ability) were assessed from blood and from some target tissues of the thyroid hormones: thyroid gland, hepatic tissue and myocardium. **Results:** The diet enriched in *Hippophaea rhamnoides* and soy led to a significant decrease in oxidative stress markers and nitrites and to an increase in antioxidant defence of the analysed tissues, soy being more efficiently. The most relevant results were noticed in hepatic tissue showing its involvement in reactive oxygen and nitrogen species production when is subjected to an excess in thyroid hormones. Biochemical markers are correlated with histological studies of the analysed tissues. **Conclusion:** Present data suggest that *Hippophaea rhamnoides* and soy decrease tissue susceptibility to oxidative insult generated through an excess in thyroid hormones. A new approach regarding fytotherapeutical alternatives in thyroid gland dysfunction has to be considered.

PW02-14

Antenatal intermittent hypoxia changed balance of GABA-ergic system in rat brain

Trofimova L.K.[1], Goncharenko E.N.[2], Bayzhymanov A.A.[2], Graf A.V.[1], Dunaeva T.Y.[1], Sokolova N.A.[1]
[1]Department of Human and Animal Physiology; [2]Department of Biophysics, Biological Faculty, Moscow State University named by Lomonosov M.V., Moscow, Russia; akapi@yandex.ru

Aims: Hypoxia is one of the most widespread pathologies during pregnancy that leads to different dysfunctions in postnatal period of offspring's life. We investigated whether intermittent normobaric hypoxia modelled during intrauterine development (period of early organogenesis) influences on the level of brain GABA-ergic system components in postpubertal rats. **Methods:** The white rats' females were subjected to intermittent hypobaric hypoxia on the 9th and 10th days of pregnancy: 5 min of hypoxia (10.5% O_2) – 5 min of normoxia, 2 hours/day. Contents of GABA and glutamate-decarboxylase were determined by fluorescent analysis while GABA-transaminase was determined by spectrophotometric analysis in the tissue of whole brain in 60 days-old rats. Males and females of offspring were investigated separately. Control groups consisted of pups, which mothers were not subjected to hypoxia during pregnancy. **Results:** GABA-transaminase content was 21% more in experimental males than in control ($p<0.05$). Content of GABA and glutamate-decarboxylase were not different from control group. At the same time females contained indistinguishable from control amounts of glutamate-decarboxylase and GABA-transaminase, but GABA content was 12% less than in control group ($p<0.05$). **Conclusion:** Antenatal intermittent hypoxia partly altered balance of components of GABA-ergic system in rat's brain. Changes were different in male and female offspring. These alterations can be markers of inhibitory processes modifications and metabolic disturbances in brain tissue caused by antenatal hypoxia.

PW02-15

Exploratory behaviour of stressed rats in the elevated alley Suok test under the quercetin administration

Tubaltseva I., Lozova V., Tukalenko E., Makarchuk M.
The department of Human and Animals' Physiology, Biological Faculty of Taras Shevchenko National University of Kyiv, Kyiv, Ukraine; ini-t@ukr.net

Aim: The treatment with antioxidants can improve behaviour and mental state of a chronically stressed organism via preventing stress-related oxidative damages. We assessed whether pre-stress administration of bioflavonoid Quercetin improves behavioural outcome in the animal model. **Methods:** The chronic stress was induced by electric footshock during 40 min (0.8 mA, 10s per shock, 22-25s intervals between pulses). The stressing was administered once per two days during 2 weeks. The Quercetin (100 mg kg(-1)) was fed daily for 2 weeks before the stress exposure. Four groups of Male Wistar rats 5 months old (n=9 each one) were used for this experiment. Stressed and Quercitine administrated (SQ) group was exposed to both treatments. Stressed (S) group and Quercitine administrated (Q) group were exposed only to one of the treatments. Control group was intact. We used elevated alley Suok test (Kalueff et al. 2005) on the 4th day for all the groups. All data were analyzed by nonparametric Mann-Whitney U-test (threshold significance was $p=0.05$); results are presented as median (including lower quartile and upper quartile). **Results:** S group has the number of lookdown reactions lower (2 (2 and 4)) than in control group (8 (3 and 13)). The parameter was recovered in the SQ group (6 (4 and 9)) compared to the S group. In the SQ and Q groups the number of lookdown reactions did not significantly differ form the control. **Conclusion:** The administration of an antioxidant attenuates stress-related inhibition of exploratory activity, but other achieved behavioural data need further analysis.

PW03-16

Susceptibility to ischaemia-induced arrhythmias and the effect of mitochondrial K_{ATP} channels activation and antioxidant treatment in the diabetic rat heart

Matejikova J., Ondrejcakova M., Pancza D., Blazickova I., Formankova I., Ravingerova T.
Institute of Heart Research, Slovak Acad. Sci., Bratislava, Slovakia

Aims: Opening of mitochondrial K_{ATP} channels (mK_{ATP}) interacting with free radicals is suggested as a key element in antiischaemic protection in the normal heart, while its role in diabetes mellitus (DM) is unclear. The aims were to characterise ischaemia-induced arrhythmias and effects of N-acetylcysteine (NAC) and mK_{ATP} opener diazoxide (D) in the diabetic rat heart. **Methods:** Seven days after streptozotocin injection, Langendorff-perfused hearts were subjected to 30min occlusion of the LAD coronary artery with or without 15min treatment with D (50μM) or NAC (4mM). **Results:** Total number of ventricular premature beats (VPB) was lower in the DM group. Similarly, number of episodes and duration of ventricular tachycardia (VT) were reduced to 6.1±3.7 and 1.8±0.6s (from 12.1±2.4 and 3.4±0.5s, resp., in non-DM controls; $P<0.05$). Both, D and NAC were antiarrhythmic in non-DM group. In the diabetics, D further suppressed arrhythmias (VT 0.6±0.4 and 0.4±0.2s; $P<0.05$ vs. non-treated DM), while NAC did not modify arrrhythmogenesis (VT 6.2±2.6 and 2.8±1.2s; $P>0.05$ vs. non-treated DM). **Conclusions:** Early period of DM is associated with enhanced resistance to ischaemia-induced arrhythmias facilitated by mK_{ATP} opening. DM might induce adaptive processes in the myocardium that reduce susceptibility to antioxidant treatment. *Grants VEGA SR 2/5110/25, APVT 51-027404.*

Groups	VPB (total number)		
	C	D	NAC
Non-diabetic	538 ± 58	168 ± 22*	290 ± 52*
Diabetic	224 ± 53*	71 ± 24*†	207 ± 51*

* – $P<0.05$ vs. non-diabetics controls; † – $P<0.05$ vs. non-treated diabetics

PW03-17

Effect of continuous light exposure of rats on cardiac morphology, NO-synthase and response to ischaemia-reperfusion

Paulis L.[1,4], Vazan R.[2,5], Janega P.[3,4], Zicha J.[7], Pechanova O.[4], Simko F.[1,6], Styk J.[5]
[1]Inst. Pathophysiol., [2]Dept. Physiol., [3]Dept. Pathol., School Med., Comenius Univ., Bratislava; [4]Inst. Normal and Pathol. Physiol., [5]Inst. for Heart Research, SAS, Bratislava; [6]3rd Clinic of Medicine, School Med., Bratislava, Slovakia; [7]Inst. Physiol., Academy of Sciences of the Czech Rep., Prague, Czech Republic; ludo@lfuk.sk

Aims: The investigation of factors modulating cardiac susceptibility to ischaemia-reperfusion (I/R) remains a current topic of experimental cardiology. Although it is known that continuous light exposure influences cardiovascular system, its effects on cardiac I/R sensitivity, morphology and NO-synthase (NOS) have been less intensively investigated. **Methods:** Two groups of male adult Wistar rats were investigated (n=15 each): controls exposed to normal light/dark cycle and rats exposed to continuous light for 4 weeks. Perfused isolated hearts (Langendorff tech.) were exposed to 25min global ischaemia and subsequent 30min reperfusion. The recovery of functional parameters (coronary flow, left ventricular developed pressure, and contractility and relaxation index) during reperfusion as well as the incidence, severity and duration of arrhythmias during the first 10 minutes of reperfusion were determined. Non-perfused hearts were used for NOS activity and (endothelial and inducible isoform) expression, conjugated dienes (CD) concentration, fibrosis and collagen I/III ratio determination. **Results:** The hearts from rats exposed to continuous light showed more rapid recovery of functional parameters, but the incidence, duration and severity of reperfusion arrhythmias were higher in this group. The NOS activity was attenuated and collagen I/III ratio increased, while the level of fibrosis, concentration of CD and NOS expressions remained unaffected by light exposure. **Conclusion:** Continuous light exposure of rats modified myocardial structure and cardiac response to I/R. This effect could be at least partially mediated by the simultaneously observed attenuation of NOS activity. *(GUK 29/2007, APVT 51-027404, 51-018004, VEGA 1/3429/06, 2/6148/26, 2/5110/25, SP 51/0280900/0280901)*

PW03-18

Oxidative stress and myocardium, plasma antioxidant status in aged rats

Sivonova M., Tatarkova Z., Dobrota D., Pavlikova M., Kaplan P.
Dept. Med. Biochem., Comenius Univ., Jessenius Faculty of Medicine, Martin, Slovakia

Aims: To provide better insight into the role of oxidative stress during aging, we studied the relationship between the antioxidant potential of plasma and heart homogenate and several oxidative stress parameters, the macromolecules damage (lipid peroxidation, uric acid, total sulfhydryl group content and oxidative damage to DNA), in young (6 months), old (15 months) and senescent (26 months) male Wistar rats. **Methods:** The antioxidant capacity of heart homogenate and plasma was determined by R-phycoerythrin based TRAP method. The content of total sulfhydryl groups and degree of lipid peroxidation in plasma and heart homogenate was determined spectrophotometricaly. DNA single strand breaks were measured using alkaline comet assay. **Results:** The antioxidant capacity was significantly decreased in plasma and myocardium of old and senescent rats, whereas plasma level of uric acid was elevated in 26 months old rats. Age-related decline in plasma and heart antioxidant capacity was accompanied by a significant loss in total sulfhydryl group content, increased lipid peroxidation and higher DNA damage in lymphocytes. Correlations between TRAP and oxidative damage to lipids, proteins and DNA suggest that the decline in antioxidant status may play an important role in age-related accumulation of cell damage caused by reactive oxygen species. **Conclusions:** Our study demonstrates an age-related decrease in the total antioxidant capacity of rat plasma and myocardium and provides the indication that this decrease may be involved in the mechanisms of free radical-induced damage to lipids, proteins and DNA during aging. *Supported by grants UK/38/2005, VEGA 1/2263/05 and APVT-51-027404.*

PW03-19

Effect of *Momordica cymbalaria* Fenzl on myocardial injury induced by isoproterenol

Koneri R.[1,2], Hariprasad[2], Megraj V.K.[1], Balaraman R.[1]
[1]Pharmacy Dept., Faculty of Technology & Engineering, M S Univ. of Baroda, Vadodara, Gujarat, India; [2]Dept. Pharmacol., Visveswarapuram Inst. of Pharmaceutical Sciences, K R Road, Bangalore, Karnataka, India; rajukoneri@rediffmail.com

Aims: The present study was aimed to elucidate the effect of *Momordica cymbalaria* (MC) on isoproterenol (ISO) induced cardiac damage. **Methods:** Male Wistar rats were divided into Group I: control (distilled water p.o), Group II: ISO (60mg/kg, s.c.), Group III and IV rats were treated with ethanolic extract of roots of MC, 250 and 500mg/kg p.o. respectively for 45 days followed by ISO (60mg/kg, s.c.). Twelve hours after second injection of ISO, animals were sacrificed, blood and hearts were collected. The serum was used for determination of myocardial infarction marker enzymes, lipid parameters, and uric acid. Hearts were used for histopathological studies and for the assay of oxidative stress parameters. **Results:** When compared to ISO administered group, the pre-treatment with ethanol extract at 250 and 500mg/kg for 45 days significantly prevented the elevation of serum marker enzymes like lactate dehydrogenase ($P<0.01$), creatinine kinase-MB fraction ($P<0.001$), aspartate transaminase ($P<0.001$), alanine transaminase ($P<0.001$), alkaline phosphatase ($P<0.001$) and the alterations in the oxidative stress markers ($P<0.05$) like lipid peroxidase activity, glutathione activity, catalase and superoxide dismutase. The serum lipid levels were also elevated by ISO, but the treatment with the extract significantly prevented the elevation of total cholesterol ($P<0.001$), triglycerides ($P<0.001$), low density lipoproteins ($P<0.001$) and increased the high density lipoproteins ($P<0.001$). The cardiac sections also revealed the protective effect of the extract. **Conclusion:** From the results it can be concluded that the ethanol extract of MC offers protection against ISO induced myocardial injury and the effect was more prominent at 500mg/kg.

PW03-20

Effects of transient cerebral ischaemia on the heart mitochondria in the rat

Horecky J.[1], Kuricova M.[1], Liskova A.[1], Neubauerova E.[1], Tulinska J.[1], Vancova O.[2]
[1]SPTEC, Slovak Medical Univ.; [2]Pharmacobiochemical Lab., Comenius Univ., Faculty of Med., Bratislava, Slovakia; jaromir.horecky@szu.sk

Aims: Cerebral ischaemia/reperfusion (I/R) injury results in mitochondrial dysfunction, decreased ATP production, inflammation and increased production of reactive oxygen species. The aim of our study was to examine biochemical changes in brain and their effects on heart mitochondria in a rat model of I/R injury. **Methods:** Transient cerebral ischaemia was induced by our minimally invasive transmanubrial approach for the occlusion of brachiocephalic trunk and left common carotid artery. After 8-days of reperfusion, brain and heart were removed for polarographic mitochondrial respiratory chain analyses. Endogenous antioxidants were determined by HPLC, markers of brain inflammation by ELISA. Immunophenotypic analysis of peripheral blood leukocytes and expression of adhesion molecules were examined by flow cytometry. **Results:** After 20min of ischaemia and 8-days of reperfusion, there was decrease in the respiratory control ratio (RCR, $P<0.001$), decrease in the oxygen uptake stimulated by ADP (QO2S3, $P<0.001$) and decrease in the rate of ATP production (OPR, $P<0.001$) of the brain and heart mitochondria. Concentration of the α-tocopherol decreased by 31% and concentration of proinflammatory cytokine TNF-α increased by 38% ($P<0.01$) in brain mitochondria. Expression of adhesion molecule CD11b increased by 26% ($P<0.001$) and expression of marker MHC II by 21% ($P<0.05$) in peripheral blood lymphocytes. **Conclusion:** The results demonstrated mitochondrial dysfunction and inflammatory reactions in rat brain after acute ischaemia and reperfusion. We suppose that inflammatory toxic products and immune response might contribute to the mitochondrial dysfunction detected in the heart as a secondary target organ. *Supported by APVV-21-022004.*

PW03-21

The effect of insulin and antivenom on electrocardiogram of rabbit envenomed by *Hemiscorpious lepturus*

Fatemi Tabatabaei S.R.[1], Razi Jalali M.[2], Ghodrati M.
[1]Department of Physiology, [2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran

Aims: The scorpion toxins have important effects on autonomic nervous system and heart by their action on ion channels. This experiment was designed to study the electrocardiographic changes of *Hemiscorpious lepturus* toxin, and the protective role of insulin and antivenom therapy. **Methods:** Thirty six male New Zealand white rabbits with an average weight of 2000± 200g were divided randomly into A (control), B, C and D groups, and each group divided into three subgroups. The subgroups 1, 2 and 3 in all four groups were injected with 1, 2 and 3 µg/g (SC) of *Hemiscorpious lepturus* venom respectively. Insulin (0.25 IU/kg, IM), antivenom (2ml/ BW, IM) and insulin plus antivenom were administered to groups B, C and D respectively, 20min after envenoming. The electrocardiogram was taken before and after 15, 60, and 180 minutes of envenoming. **Results:** The distinguished electrocardiographic disorders were ST segment depression, reduced T wave amplitude, notch in R wave, increase of QRS complex duration, and tachycardia. The incidence of the disorders was greater in group A, and they were lower in B, C, and D groups. The electrocardiographic disorders were not lower in group D in comparison with groups B and C. **Conclusion:** Most of the disorders, especially ST segment depression and T wave changes can be related to myocardial ischemia. We suggest that treatment of the envenomed rabbits with insulin and antivenom reduces the electrocardiographic changes but the combined treatment has no any more benefit.

PW04-22

Behavioural and electrophysiological effects of manganese given to rats intranasally in different chemical forms

Sarkozi, L., Szabo, K., Hornyik, T., Horvath, E., Szabo, A.
Department of Public Health, University of Szeged Faculty of Medicine, Szeged, Hungary
sarkozi@puhe.szote.u-szeged.hu

Aims: The pathophysiological effects of environmental pollutants are determined by chemical and physical characteristics. Nanoparticles, in the submicron range, are a major part of air pollutants, and their effects can be distinct from those of larger particles. In this work, manganese was given to rats in nanoparticle and dissolved form, and the effects on open field behaviour and cortical electric activity were observed and compared. **Methods:** Male Wistar rats received intranasal instillation of dissolved $MnCl_2$ or nanosuspension MnO_2 (both 40 µl, equal to 2.53 mg Mn per rat) once, or for 3 and 6 weeks every workday. Five days after single administration, or at the end of the subacute treatment period, the rats had a 10 min session in an open field box, were then anesthetized with urethane, the left hemisphere was exposed, and spontaneous and stimulus-evoked activity was recorded from the primary somatosensory, visual and auditory area. **Results:** In the open field, the rats treated once had reduced horizontal and vertical motility, while local motility and immobility was increased, and solute Mn had stronger effect. After 3-6 weeks, the effect of nanoparticle Mn was increased and the behavioural changes were more expressed. For all treated rats, the increase of the somatosensory evoked potential with increasing stimulation frequency was less than in the controls. The latency of the visual evoked response was lengthened more by nanoparticle than by solute Mn. **Conclusion:** The effects of Mn in nanoparticle vs. dissolved form were qualitatively similar. The quantitative differences may indicate differences in absorption and kinetics.

PW04-23

Behavioural and endocrine effects of chronic treatment with aldosterone in rats

Hlavacova N., Jezova D.
Institute of Experimental Endocrinology, Laboratory of Pharmacological Neuroendocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
natasa.hlavacova@savba.sk

Aims: Aldosterone is the last component of the renin-angiotensin-aldosterone system inducing its peripheral effects via mineralocorticoid receptors (MR). Brain MR are mainly occupied by glucocorticoids. Recent studies indicate that manipulations with MR in the brain are associated with behavioural changes. We have investigated anxiety-like behaviour and levels of selected hormones in response to chronic aldosterone treatment. **Methods:** Rats were implanted subcutaneously with osmotic minipumps and treated with aldosterone or vehicle for two weeks. Elevated plus-maze test was used to measure anxiety-like behaviour and simultaneously, as a mild stressor to evaluate hormone responses. **Results:** As measured by daily water intake, aldosterone treatment was found to increase water consumption ($p<0.05$). Aldosterone-treated animals entered significantly less often ($p<0.05$) and spent less time ($p<0.05$) in the open arms of the elevated plus-maze. General locomotor activity was unchanged. Aldosterone treatment affected significantly also risk assessment behaviour. Basal plasma corticosterone and adrenocorticotropic hormone levels as well as their responses during stress remained unchanged after aldosterone treatment. **Conclusions:** The present findings indicate an anxiogenic profile of aldosterone as demonstrated by alterations in several indicators of anxiety-like behaviour measured in the elevated plus-maze test. Chronic treatment with aldosterone failed to modify basal or stress-induced activity of the hypothalamic-pituitary-adrenocortical axis. Presented data suggest that the mechanisms of behavioural effects induced by aldosterone might be, at least partly, mediated via its effects on central mineralocorticoid receptors or by its non-genomic actions in the brain.
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PW04-24

Nonlethal doses of ionizing radiation effects upon instrumental behaviour of rats

Tukalenko E.V., Varetsky V.V., Rakochi A.G., Makarchuk M.J., Dmitrieva I.R.
Scientific Center for Radiation Medicine of Academy of Medical Sciences of Ukraine, Kyiv National Taras Shevchenko University, Kyiv, Ukraine
eugen_tuk@ukr.net

Aim: of this study was to find the dose-effect relationship of ionizing radiation effects at nonlethal dose range upon conditioned instrumental behaviour of rats. **Methods:** The instrumental activity in shuttle box of white rats was studied after single total exposure to gamma-radiation (^{60}Co) in the dose range of 0.05 to 7 Gy. The rats' performance was evaluated with negative painful enforcement (414 animals) weekly during two months after the exposure. **Results:** The results of instrumental behaviour studies showed complicated non-linear dose dependence with three characteristic intervals. The first of them (0.05 to 1.0 Gy), according to the shuttle box behaviour, is characterized by a decrease of the number of conditioned responses, accompanied by their latency increase; a decrease of acquired learning skills stability. The second dose-dependence interval obtained in the shuttle boxes corresponds to the dosage range from 1.0 to approximately 3.0-4.0 Gy. It is characterized by an elevated level of conditioned activity of the rats: increase in the number of conditioned responses, their latency shortening, and increase in stereotyped behaviour stability. The third interval of the dose-dependence function is characterized by a decrease of all behaviour indices, and it corresponds to doses above 4.0-5.0 Gy. Statistical model of dose-effect relations of conditioned responses numbers changes may be presented as function: $Y=1.3401*X^3-18.546X^2+65.252X+69.291$. **Conclusions:** Studies of the instrumental activity of the rats after exposure to ionizing radiation showed complicated nonlinear dependence of the behavioural indices on the exposure dose.

PW04-25

Impact of α2-adrenoceptor stimulation on the activity of oxytocin and tyrosine hydroxylase neurons in the supraoptic nucleus of vasopressin deficient Brattleboro rats

Bundzikova J.[1], Pirnik Z.[1], Zelena D.[2], Mikkelsen J.D.[3], Kiss A.[1]
[1]Lab. Functional Neuromorphology, Inst. Exp. Endocrinology, SAS, Bratislava, Slovakia; [2]Inst. Exp. Medicine HAS, Budapest, Hungary; [3]Dept. of Translational Neurobiology, NeuroSearch A/S, Ballerup, Denmark; ueenkiss@savba.sk

Aims: Agonists of α2-adrenoceptors in the CNS inhibit vasopressinergic (AVP) and stimulate oxytocinergic (OXY) neurons in the hypothalamic supraoptic nucleus (SON). We studied the impact of xylazine (XYL), an α2-adrenoceptor agonist, on the activity of OXY and tyrosine hydroxylase (TH) synthesizing neurons in SON of control Long Evans rats (+/+), heterozygous (di/+), and AVP-deficient homozygous (di/di) Brattleboro rats. **Methods:** The animals were injected with XYL (10 mg/kg, i.p.) and 90 min later sacrificed by transcardial perfusion with fixative. Activity of OXY and TH neurons was signalized by the presence of Fos protein visualised by dual immunohistochemistry. Co-labelings were analyzed on 30μm thick coronal sections using computerized light microscope. **Results:** In +/+ rats no TH immunoreactive perikarya occurred but +/+ rats showed 4% activated OXY neurons. In these rats XYL raised the amount of Fos/ OXY cells up to 67%. In di/+ rats 18% of OXY cells was activated and XYL increased their number up to 60%. No TH neurons occurred in the SON of saline di/+ animals, however, 1 from 6 animals injected with XYL displayed TH immunoreactivity with 25% of Fos co-labelings. Di/di rats displayed many activated OXY (61%) as well as TH (35%) producing neurons. XYL potentiated the number of Fos/OXY neurons up to 83% and lowered the number of activated TH neurons to 5%. **Conclusion:** The present data indicate that stimulation of α2-adrenoceptors activates OXY and inhibits TH neurons in the SON of Brattleboro rats. *Supported by VEGA 2/7003/7.*

PW04-26

Differences in stimulatory effect of antipsychotics on the oxytocinergic population of neurons in the hypothalamic paraventricular nucleus (PVN)

Kiss A.[1], Bundzikova J.[1], Pirnik Z.[1], Mikkelsen J.D.[2]
[1]Lab. Functional Neuromorphology, Inst. Exp. Endocrinology, SAS, Bratislava, Slovakia; [2]Dept. of Translational Neurobiology, NeuroSearch A/S, Ballerup, Denmark; ueenkiss@savba.sk

Aims: Acute administration of antipsychotics induces peripheral release of OXY. This motivated us to reveal how intensively may respond the OXY-ergic neurons in the PVN to pharmacologically differently acting antipsychotic drugs and whether the activated OXY neurons exhibit spatial distribution differences in the PVN. **Methods:** Wistar male rats received a single injection (i.p.) of haloperidol (1mg/kg), clozapine (30mg/kg), olanzapine (30mg/kg), risperidone (2mg/kg), vehicle (5 % chremophor), and saline. Sixty min later, the animals were perfused with fixative. Fos/OXY co-stainings were analyzed by dual immunohistochemistry in 4 PVN subdivisions: anterior (Ant), middle (Mid), dorsal cap (Dc), and periventricular (Pev) ones, using computerized light microscope. **Results:** Most apparent activation of OXY cells was induced by clozapine, i.e. 27.4%, 23.9%, 34.7%, and 26.7% of in Ant, Mid, Dc, and Pev, respectively. The second most effective drug was olanzapine indicating for 12.1%, 10.4%, 18.7%, and 25.3% of co-localizations in Ant, Mid, Dc, and Pev, respectively. Around 2% of Fos/OXY cells were stimulated by haloperidol and risperidone treatments. Naive controls and vehicle treated rats did not show Fos/OXY colocalisations. **Conclusion:** The present data indicate for the existence of substantial differences in the stimulatory effect of the tested antipsychotics on the quantity of PVN oxytocinergic cells with the preferential action of the atypicals clozapine over olanzapine and little effects of haloperidol and risperidone. Spatial distribution distinctions of activated OXY cells in the PVN were less pronounced. *Supported by VEGA 2/7003/7.*

PW04-27

Partial serotonergic lesion attenuates REM sleep reduction by citalopram

Kitka T.[1,4], Katai Z.[1,4], Kirilly E.[1,4], Balogh B.[1], Renoir T.[2], Lanfumey L.[2], Hamon M.[2], Kantor S.[1], Bagdy G.[1,3,4]
[1]Lab. Neurochem. Exp. Med., Nat. Inst. Psychiat.&Neurol., Budapest; [2]Fac. Med. P.&M. Curie, Univ. P.&M. Curie, Paris, France; [3]Group Neuropsychopharmacol., HAS & Semmelweis Univ.; [4]Semmelweis Univ., Fac. Med., Dept. Pharmacol. Pharmacother., Budapest, Hungary

Aims: Selective serotonin reuptake inhibitors (SSRIs) are extensively used for the treatment of depression and anxiety disorders. In addition, ecstasy (3, 4-methylenedioxymethamphetamine, MDMA) users frequently suffer from depression and are often treated by SSRIs. Effects of citalopram on vigilance states were studied after serotonergic damage by MDMA treatment. **Methods:** Seven-week-old male Dark Agouti rats received an injection of MDMA (15mg/kg, i.p.) or vehicle. Three weeks later, citalopram (2.5mg/kg, i.p.) or vehicle was administered, and 24h polysomnographic recordings (EEG, EMG, motor activity) were performed. Active and passive wakefulness, light and deep slow wave sleep, rapid eye movement (REM) sleep and REM sleep latency were calculated by sleep stating. We labeled serotonergic fibers with 5-HT transporter (5-HTT) immunohistochemistry. The functional state of 5-HT_{1A} receptors was assessed by quantifying [^{35}S]GTP-Y-S-binding in response to stimulation by 5-carboxamido-tryptamine (5-CT). **Results:** 5-HTT density decreased by 30-40% in several hippocampal and hypothalamic but not brainstem regions after MDMA treatment. 5-CT-evoked [^{35}S] GTP-Y-S-binding was reduced in hippocampus CA1 area but not dorsal raphe nucleus. MDMA treatment reduced REM sleep latency and increased the amount of REM at the beginning of sleep. Citalopram-induced decrease in REM had a shorter duration in MDMA-treated animals compared to controls. **Conclusion:** Our findings suggest that reduction of PS is a useful measure of inhibition of 5-HT reuptake. These effects of SSRIs are likely altered in ecstasy users. *Supported by: 5th FP, QLG3-CT-2002-00809 and 6th FP, LSHM-CT-2004-503474 of the E.C., HRF Grant D-048502.*

PW04-28

The comparative investigation of cerebellar and olivary neurons baseline activity under vibration

Sahakyan S.G., Grigoryan G.Y.
Department of Human and Animals Physiology, Yerevan State University, Yerevan, Republic of Armenia; sus_sah@yahoo.com

Aims: The cerebellum and inferior olive provide transformation and integration of diverse influences, in particular vibration. This is necessary for the adequate control of motor activity. Therefore, a comparative study of baseline activity of cerebellar and olivary neurons in the dynamics of vibration influence was conducted. **Methods:** In acute experiments on Nembutal-anesthetized albino rats, extracellular recording of baseline activity of neurons in the fastigial nucleus of cerebellum and inferior olive were performed in norm and after exposure to whole – body vibration for 5, 10 and 15 days. The distributions of neurons were analyzed in terms of the regularity and dynamic of spike flows along with the mean spike frequency and the coefficient of variation of interspike intervals. **Results:** The significant increase of average frequency of neurons' impulsation in both fastigial nucleus and inferior olive were observed at 5th day of influence. Starting from 10th day of exposure there was a gradual transformation of the rhythm of fastigial neuron activity, which by 15th day turned into a sharp decrease, along with significant increase in the coefficient of variation. We have revealed also drastic decrease in quantity of neurons with group activity and mean frequency of impulsation of olivary neurons on day 10 of influence that stayed in the same level on 15th day, along with an increase in the coefficient of variation. **Conclusion:** These results testify to weakness of vibration stimulative influence on the neuronal activity of these structures and initiation of adaptive processes occurring at 10th day of action of factor.

PW04-29

The role of GABAergic inhibition on kainic acid-induced persistent gamma oscillations in the rat basolateral amygdala *in vitro*

Randall F.E., Whittington M.A., Cunningham M.O.
School of Neurology, Neurobiology and Psychiatry, Newcastle University, UK
mark.cunningham@ncl.ac.uk

Aims: Oscillatory activity has been observed in the basolateral amygdala (BLA) *in vivo* during emotional arousal and is thought to have an important role in emotional processing. GABAergic interneurons are known to have a critical role in the generation and maintenance of network oscillations. Using an *in vitro* model of gamma oscillations in the BLA, we have investigated the role of GABAergic inhibition in this activity. **Methods:** Oscillations were induced in coronal slices (450μm) of rat BLA from male Wistar rats by bath application of kainic acid (400nM). Compounds known to affect $GABA_A$ receptor-mediated inhibition; Gabazine (1μM), Pentobarbital (30μM) and Diazepam (1μM), were applied to stable, persistent oscillations. **Results:** Intracellular recordings during oscillations revealed that principal cells in the BLA receive gamma frequency IPSPs in phase with the field oscillation. Blockade of $GABA_A$ receptors by Gabazine completely abolished the oscillatory activity. Pentobarbital significantly reduced the frequency but not the power of the oscillation (control v. Pentobarbital; frequency 32 ± 2 v. 26 ± 1 Hz, P=<0.05; power 23 ± 9 v. 2 ± 1 μV^2/Hz, P>0.05, n=5). Diazepam had no significant effect on the frequency or power of the oscillation (control v. Diazepam; frequency 34 ± 1 v. 33 ± 1 Hz, P>0.05; power 135 ± 84 v. 96 ± 73 μV^2/Hz, P>0.05, n=5). **Conclusion:** BLA oscillations are subject to modulation at the barbiturate site on $GABA_A$ receptors but not the benzodiazepine site. These results demonstrate that $GABA_A$ receptor-mediated inhibition is important in network oscillations in the BLA.

PW04-30

The analysis of functional connectivity of the brain during mental rotation using EEG coherence

Cimrova B., Riecansky I.
Institute of Normal and Pathological Physiology, Department of Brain Physiology, Slovak Academy of Sciences, Bratislava, Slovakia
baaaa@pobox.sk

Aims: EEG coherence is a measure of synchronization between corresponding frequency components of two signals. The aim of this study was to consider activity and functional coupling of distributed neuronal assemblies in the brain during mental rotation – a visuo-spatial cognitive function based on imaginary manipulation with the object. **Methods:** EEG was recorded during a mental rotation of 2D alphanumeric characters in 14 healthy undergraduates. EEG coherence was computed in the interval 500 ms before subject's response and compared for stimulus angular displacement 120° (mental rotation) and 0° (control task without mental rotation). Pearson's correlation coefficients were used to investigate the relationship between EEG coherence and reaction time. **Results:** During mental rotation a widespread increase in EEG coherence was observed in the delta and beta 2 frequency bands. Fronto-temporal coherence in the delta band was negatively correlated with reaction time – the increase in coherence was higher in subjects who solved the task more quickly. In contrast, correlation in the beta 2 band was positive – higher parieto-temporal coherence was associated with longer reaction time. In other frequency bands the changes were less pronounced and less consistent. **Conclusions:** Our results indicate that synchronization of neuronal activity in the delta band could be specifically related to mental rotation process. Synchronization in the beta band, on the other hand, seems to be related to effort exerted to solve the task.

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PW04-31

Human cognitive that related to memory reduces spectral power of alpha-range of electroencephalogram

Sivash M.
Kyiv National Taras Shevchenko University, Kyiv, Ukraine
marchantia@ukr.net

Aims: Such cognitive reactions, as attention and memory cause depression of low-frequency subrange of alpha-rhythm. The purpose of the investigation was to check up the changes of dominant frequency and spectral power of alpha range during the praxis concerned with cognitive activity and memory processes. **Methods:** By the method of electro-encephalography (EEG) were inspected 32 volunteers in age from 18 to 24 years. We registered their EEG in a state of vigil at implementation of cognitive exercises of related to memory. **Results:** Sharply expressed alterations of rhythmic structure of alpha range do not take place, although there was its depression – sufficiently clear sentinel oppression in reply to the cognitive exercises. It shows up in the substantial decline of spectral power of alpha-rhythm during memorizing of words, during their maintenance in memory, and in transition of inspected person to afterwork rest relative to starting data. **Conclusion:** During activating of brain, which is related to the mechanisms of memory, does not take place complete disappearance of alpha-rhythm. Dominant frequency of range of alpha does not change here. There is sufficiently the substantial decline of spectral power of dominant frequency of alpha range during realization of such cognitive functions as memory and attention.

PW04-32

Correlation between free saliva testosterone levels, polymorphisms of testosterone metabolism genes and cognitive abilities in different groups of individuals

Prazmariova E.[1], Holesova Z.[3], Minarik G.[4], Celec P.[2], Ficek A.[4], Kelemenova S.[1], Ostatnikova D.[1]
[1]Dept. Physiol.; [2]Dept. Pathophysiol., Fac. Med.; [3]Dept. Genetics; [4]Dept. Mol. Biol., Fac. Nat. Sci., Comenius Univ., Bratislava, Slovakia;

Aim: Aim of the study was to observe correlations between free saliva testosterone levels, polymorphisms of testosterone metabolism genes and cognitive abilities in different groups of individuals. Various groups of patients were used in this project: gifted children, elderly hypogonadic patients, patients with prostate cancer and individuals with autistic phenotype features. **Methods:** Effects of testosterone could be influenced by several types of polymorphisms in genes, whose products participate on metabolism of steroid hormones, e.g.: androgen receptor gene (AR), aromatase gene (CYP19), 5α-reductase gene (SRD5A2), estrogen receptor gene (ESR1) and gene for sexual hormone binding protein. DNA samples were isolated from buccal cells in saliva and subsequently DNA was amplified by PCR. The CYP19 C^{1558}-T and SRD5A2 A49T polymorphisms were determined by RFLP analysis and the AR $(CAG)_n$ polymorphism was determined by fragment analysis with fluorescently labeled primer. Salivary testosterone levels were measured with radioimmunoassay. Cognitive abilities were assessed via standard psychological tests – coefficient of mental rotation (CMR) and coefficient of spatial abilities (CSA). **Results:** We found significant correlation between polymorphism A49T of SRD5A2 gene and CMR, as well as CSA in the group of gifted children. Polymorphisms of AR and CYP19 genes did not correlate with cognitive abilities. Gifted children of both sexes had lower levels of testosterone in comparison with their peers in common population. **Conclusions:** Based on the hypothesis that intelligence and spatial abilities are influenced by genetic as well as epigenetic factors, it is not surprising that certain correlation between previously mentioned polymorphisms and cognitive abilities exist.

PW05-33

Effect of resveratrol on glucose- and swelling-induced insulin secretion

Bacova Z., Orecna M., Hafko R., Strbak V.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; zuzana.bacova@savba.sk

Aims: Experimental data suggest that resveratrol (3´5´4´ trihydroxystilbene, a natural phytoalexin), has a beneficial role in prevention of diabetes and alleviates diabetic complications. Resveratrol substantially restricts glucose-induced insulin secretion from freshly isolated rat pancreatic islets. **Methods:** To characterize its action, effect of 50 μmol/l resveratrol on 15 mmol/l glucose-, 30% hypotonic medium- and 80 mmol/l ethanol-induced insulin release from freshly isolated pancreatic islets and two rat insulinoma cell lines INS-1 and INS-1E in Ca^{2+} containing medium was compared. **Results:** Resveratrol significantly decreased glucose-induced insulin release from pancreatic islets and both cell lines. Interestingly, hypotonicity-induced insulin secretion from pancreatic islets was even more pronounced in presence of resveratrol. While hypotonicity alone inhibits insulin release from INS-1E cell line, in the presence of resveratrol hypotonicity induced distinct release of insulin. Resveratrol abolished ethanol-induced insulin release from all tested systems. PDBu (PKC activator) and forskolin (adenylate cyclase activator) partially restored glucose-stimulated insulin secretion in the presence of resveratrol. **Conclusion:** There is substantial difference in signaling for glucose- and hypotonicity-induced insulin secretion, resveratrol has opposite effect on two pathways. Effect of resveratrol on ethanol-induced insulin secretion resembled that on glucose. It is likely that resveratrol does not involve effect on PKC and cAMP system.

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PW05-34

Effects of aromatase inhibitor letrazole on femur fracture in female rats: a biomechanical study

Aydin M.[1], Alcin E.[2], Kelestemur M.H.[3], Kutlu S.[2], Yilmaz B.[4]
[1]Mustafa Kemal University, Faculty of Medicine, Department of Physiology, Hatay, Turkey; [2]Firat University, Faculty of Medicine, Department of Physiology; [3]Engineering Faculty, Department of Metallurgical and Materials Engineering, Elazig, Turkey; [4]Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey; draydinmehmet@yahoo.com

Aim: Aromatase inhibitors (AI) are used in treatment of early and advanced breast cancer. Low estradiol levels in women are associated with decreased bone mineral density and increased fracture risk. In this study, we aimed to investigate effects of an AI, letrazole, on femur fracture and serum estradiol levels. **Methods:** Total 40 adult Sprague-Dawley female rats were divided into five groups (n=8). Controls received saline alone. Letrazole was administered to the animals in the second and third groups by daily oral gavage at 0.2 and 1 mg/kg doses, respectively, for six weeks. Another group of letrazole (1mg/kg)-treated rats were allowed to recovery for two weeks. The last group of rats was ovariectomized. At the end, all animals were decapitated and the femur rapidly removed. Serum estradiol levels were determined by ELISA. A universal testing machine was used for determination of biomechanical properties (bending characteristics, stiffness and toughness) on the femur samples. **Results:** Serum estradiol levels were significantly reduced by letrazole in a dose-dependent manner ($p<0.01$) which returned to control values following two weeks of recovery ($p<0.05$). Following three point bending tests, it was observed that a slight increase ($p<0.05$) in the strength of samples occurred for the letrazole-treated rats depending on given doses with respect to those of control group, however, a significant increase ($p<0.01$) in the brittleness and even stiffness was observed. **Conclusion:** The present findings suggest that long-term use of letrazole may increase risk of osteoporosis and fracture. It is advised that bone mineral density should be measured in patients taking AI and anti-resorptive therapy be commenced if osteoporosis is already present.

PW05-35

Metabotropic hormonal factors react to propylene glycol supplementation in peripartum dairy cows

Gyorffy A.[1], Keresztes M.[2], Faigl V.[2], Frenyo V.L.[1], Kulcsar M.[2], Gaal T.[3], Huszenicza G.[2], Zsarnovszky A.[1], Bartha T.[1]
[1]Szent Istvan Univ. Fac. Veterinary Sci. Department of Physiology and Biochemistry; [2]SzIU FVS Department and Clinics of Obstetrics and Reproduction; [3]SzIU FVS Department and Clinic of Internal Medicine, Budapest, Hungary; andrea.gyorffy@gmail.com

Aims: In peripartum dairy cows energy intake does not cover energy demands; therefore, a so-called Negative Energy Balance (NEB) evolves. Excessive NEB leads to reproductive and metabolic disorders. We aimed at investigating the effects of propylene glycol (PG) on the metabotropic hormone system. **Methods:** Experiments were conducted on Holstein cows (n_1= 49). The feed of experimental animals was supplemented with pulverous PG for 3 weeks peripartum. Blood samples were collected once a week for determination of plasma T4 and T3 concentrations. Tissue gene expression was investigated from liver biopsy samples (n_2=16; samples obtained on d 7-10 postpartum) by means of real-time PCR and analyzed by statistical methods. **Results:** In the treated group, plasma concentrations of triiodothyronine (T3) and thyroxine (T4) were significantly ($P< 0.001$) higher than those found in controls. PG supplementation significantly increased the gene expression of type I deiodinase (2.4-fold increase; $P= 0.001$) and that of the short-form of leptin receptor (11.9-fold increase; $P= 0.001$). The gene expression of leptin and its long-form receptor did not change significantly. **Conclusion:** Our results indicate that PG increases the basal metabolic rate by facilitating the conversion of increased levels of T4 to T3. This idea is supported by the observed increase in DIO1 mRNA expression. Increased Ob-Ra mRNA levels suggest that leptin effects on energy homeostasis and reproduction are simultaneously also modulated.

PW05-36

Hypotonicity, ethanol and urea affect insulin secretion by different mechanisms

Hafko R.[1], Orecna M.[1], Bacova Z.[1], Chorvat D.[2], Strbak V.[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]International Laser Center, Bratislava, Slovakia
roman.hafko@savba.sk

Aim: Permeants and hypotonicity are believed to induce exocytosis via the same mechanism – cell swelling. This study was performed to compare their effect on insulin release from rat insulinoma cell lines INS-1 and INS-1E. **Methods:** Static incubations experiments were performed on all three experimental systems (30% hypotonicity, ethanol and urea). **Results:** In Ca^{2+} containing medium hypotonicity induced insulin release from pancreatic islets and INS-1 cells but inhibited insulin secretion from cell line INS-1E. Noradrenalin inhibits glucose-induced but not hypotonicity (swelling)-induced insulin release from islets and INS-1 cells. Ethanol and urea (in isosmolar medium) induced distinct cell swelling in both tumour cell lines. Ethanol (in 40, 80 and 160 mmol/l concentration) in isosmotic medium stimulated insulin secretion from both cell types. Ethanol stimulation was abolished by 1 μmol/l noradrenaline. In contrary to previous data our result show suppressive effect of urea (in 40, 80 and 160 mmol/l in isosmolar medium) on insulin secretion from INS-1 and INS-1E cells. **Conclusion:** Mechanism of ethanol-induced insulin secretion in these cell lines differs from that induced by hypotonicity; it was not mediated by its permeant effect. Signaling pathway for ethanol stimulation shares noradrenaline sensitive step(s) with glucose-induced insulin secretion. The inhibiting effect of urea in tumour cell lines should utilize different mechanism.

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PW05-37

Spatial performance in a view of aromatase and GnRH expression in testosterone and cyproterone acetate treated rats

Hodosy J., Gardlik R., Behuliak M., Palffy R., Celec P., Ostatnikova D.
Institute of Physiology and Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
hodosy@gmail.com

Aim: To evaluate the effect of testosterone levels and spatial performance in rats during adulthood and to assess whether testosterone affects spatial performance itself or via its metabolite estradiol. **Methods:** In 40 male rats divided into 4 groups (control, testosterone, cyproterone acetate, testosterone+cyproterone acetate) spatial memory and learning was tested in Morris water maze during 5 consecutive days. Latency times were assessed by video tracking AnyMaze™ software. Frontal cortex and hypothalamus were stored in trizol for RNA isolation and RT-PCR was performed to assess RNA expression of genes for aromatase and GnRH. Testosterone levels were determined. **Results:** All groups improved during 5 days ($p<0.02$), on the third and the fourth day, there were no significant differences in latency times between the groups. During the first and the second day, however, control and combined groups reached shorter latency times in comparison to testosterone and cyproterone acetate, groups ($p<0.02$ and $p<0.04$, respectively). In hypothalamus, aromatase expression was the highest in the control group ($p<0.003$), and in cortex, testosterone group showed the highest expression rate of aromatase ($p<0.01$). There were no significant differences among groups in cortex regarding GnRH, however, in hypothalamus, control group reached the highest expression ($p<0.001$). **Conclusion:** The results suggest that aromatization of testosterone may play a role in spatial memory and learning in adult rat males, however, it seems that for working memory non-genomic effects of androgens should be considered.

PW05-38

Hormonal response to stress in rat strains with different susceptibility to immunologic challenge

Macho L.[1], Rovensky J.[2], Kvetnansky R.[1], Radikova Z.[1], Zorad S.[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]National Institute of Rheumatic Diseases, Piestany, Slovakia; ueenlaco@savba.sk

Aims: Different response of Lewis and Fischer 344 rat strains to induction of inflammation could be affected not only by activity of hypothalamo-pituitary-adrenal axis but also by the changes in plasma levels of hormones with immunoregulatory action. The study was aimed to compare the changes in plasma hormone levels involved in the response to stress exposure in both rat strains. **Methods:** Adult rat males of Lewis (LEW) and Fischer 344 (FIS) strain (8 animals/group) were exposed to restrain stress for 2 hours. Blood samples were collected (5, 20 and 120 min of stress) from cannulated tail artery. Control animals were without exposure to stressor. Corticosterone (CS), testosterone (TE), dehydroepiandrosterone (DHEA), 17β-estradiol (ES) and progesterone (PGS) were determined by radioimmunoassay, epinephrine (E) and norepinephrine (NE) levels by radioenzymatic method. **Results:** The levels of plasma CS (LEW: 21.3±0.7, FIS: 39.9±3.2 μg/100ml; $p<0.05$), E (LEW: 450±50, FIS 2050±650 pg/ml in 20 min stress; $p<0.05$) and NE levels after stress exposure were significantly higher in FIS as compared to LEW rats. Decrease of TE levels was noted after exposure to stress. However, the difference of TE concentration in control and stressed rats were higher in LEW rats (0.90 in FIS and 3.50 ng/ml in LEW, $p<0.05$). No strain differences were observed in DHEA levels. Lower levels of ES (FIS: 37±2 pg/ml, LEW: 49±5 pg/ml; $p<0.05$) and higher values of PGS plasma levels (FIS: 3.11±0.80 ng/ml, LEW: 0.29±0.12 ng/ml; $p<0.05$) were noted in FIS rats after stress. **Conclusion:** These results demonstrated the differences in the response of CS, E, NE, and gonadal steroids after exposure to stress in LEW and FIS rats with lower levels of hormones with anti-inflammatory action in LEW rats.
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PW05-39

Effect of short and long-term exposure of rats to hypergravity 2G on gene expression of catecholamine biosynthetic enzymes in the adrenal medulla

Petrak J.[1], Jurani M.[2], Baranovska M.[2], Hapalla I.[2], Frollo I.[3], Buckendahl P.[4], Kvetnansky R.[1]
[1]Inst. Exp. Endocr., SAS, Bratislava; [2]Inst. Anim. Biochem. Genetics, SAS, Ivanka pri Dunaji, Slovakia; [3]Inst. of Measurement Science, SAS, Bratislava, Slovakia; [4]Rutgers University, Piscataway, New Jersey, USA;

Aims: The enzymes responsible for catecholamine synthesis are tyrosine hydroxylase(TH), dopamine-β-hydroxylase(DBH) and phenylethanolamine-N-methyltransferase (PNMT). Different stressors variably activate increased synthesis of these enzymes. The present experiment tested the hypothesis that hypergravity (HPG) is a stimulus for catecholaminergic enzymes synthesis in the adrenal medulla (AM), the main source of adrenaline secretion in response to stressful stimuli. **Methods:** Six groups of 8 male Sprague Dawley rats each were centrifuged at 2G for 0, 6, 24, 72 or 144 hours. Rats were killed by decapitation after the indicated duration of HPG. AM was removed and frozen in liquid nitrogen. mRNA from AM was isolated and quantified by RT-PCR to determine gene expression for TH, DBH and PNMT. **Results:** mRNA levels for TH and PNMT after 6, 24 or 72 hours of HPG were significantly increased compared with absolute control ($p<0.01$). After 144 hours, levels of TH and PNMT mRNA had fallen significantly ($p<0.05$) compared to 72 hours and were no longer increased relative to absolute control. DBH mRNA levels were significantly increased after 24 hours HPG ($p<0.05$) and 72 hours ($p<0.01$) compared with absolute control. After 144 hours, DBH mRNA levels fell and were not significantly different from any of the other four groups. **Conclusion:** From our results, it is clear that short term HPG was an intensive stressor that activated TH, DBH and PNMT enzyme gene expression in the adrenal medulla. Longer term HPG for 144 hours decreased these enzymes gene expression, suggesting adaptative mechanisms probably involving adrenergic feedback.

PW05-40

Short-term exposure to somatostatin or muscarinic agonists regulates acetylcholine-evoked ^{3}H-MPP$^+$ release from bovine adrenal chromaffin cells

Ribeiro L., Martel F., Azevedo I.
Department of Biochemistry, Faculty of Medicine, University of Porto, Portugal; lribeiro@med.up.pt

Aim: To investigate the effect of a short-term exposure to somatostatin (SS), its receptors (SSTR) selective agonists as well as muscarinic receptors agonists upon acetylcholine-induced release of ^{3}H-MPP$^+$ from bovine adrenal medullary cells. **Results:** Acetylcholine (ACh, 100, 500 μM) was found to increase the release of ^{3}H-MPP$^+$ by these cells (to 175 and 171% of basal release, respectively). ACh-elicited ^{3}H-MPP$^+$ release was significantly reduced by hexamethonium (100 μM) and atropine (100 μM), selective nicotinic and muscarinic antagonists, respectively. Previous exposure to any of two muscarinic agonists, oxotremorine or pilocarpine, led to a significant reduction of ^{3}H-MPP$^+$ release in response to 100 μM ACh, to about a maximum of 51% and 78% of control, respectively. Somatostatin (SS, 0.01-0.1 μM), previously applied to the preparation, depressed ACh-elicited ^{3}H-MPP$^+$ release by 25-27%, but only when a 500 μM ACh concentration was used. The inhibition exerted by SS upon ACh-evoked ^{3}H-MPP$^+$ release appeared to be mediated by its SSTR: (1) SSTR2, 3 and 4 subtype agonists mimicked the effects seen with SS, and (2) the SSTR non-selective antagonists, cyclo-SS, counteracted the SS inhibitory effect. When SS was tested in the presence of any of the muscarinic agonists, oxotremorine or pilocarpine, its inhibitory effect on 500 μM ACh-induced ^{3}H-MPP$^+$ release was no longer detectable. **Conclusion:** The similar effect of short-term exposure to SS and muscarinic agonists over ACh-induced release of ^{3}H-MPP$^+$, as well as the loss of effect of SS by the presence of the muscarinic agonists, suggest that these compounds may share signaling pathways.

PW05-41

Pinopsin in the pineal gland of the turkey

Prusik M., Lewczuk B., Bulc M., Przybylska-Gornowicz B.
Division of Histology, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland
mprusik@gmail.com

Aims: The avian pineal is directly photosensitive due to the presence of photopigments, among which pinopsin seems to be one of the most important and responsible for acute influence of light on the pinealocyte activity. Up till now, pinopsin has been detected in the chicken and the Japanese quail. The purpose of our study was to check the presence and distribution of pinopsin in the pineals of domestic turkeys at various stages of postembryonic life and as in cultured pinealocytes. **Methods:** Pineals of 1-day-, 2-, 14-, 22- and 56-week-old turkeys as well as monolayer cultures of turkey pineal cells were fixed and subjected to immunohistochemical staining with primary antibody against pinopsin. **Results:** The pinopsin-immunoreactivity was observed in apical processes of rudimentary-receptor pinealocytes limiting the follicular lumen and in short processes originating from secretory pinealocytes laying in outer layer of the follicular wall in the pineals of turkeys from 1 day to 1 year old. The number of secretory pinealocytes with pinopsin-positive processes increased with age. In monolayer cultures, the positive staining for pinopsin was observed in pinealocytes. It was especially prominent in short cytoplasmic processes originating from cytoplasmic pools of cells. **Conclusions:** Pinopsin is present in turkey pineal glands during the whole period of postembryonic life and shows very specific distribution.

PW05-42

The pineal concretions in the turkey as a result of collagen mediated calcification

Przybylska-Gornowicz B., Lewczuk B., Prusik M., Bulc M.
Division of Histology, Department of Functional Morphology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland
przybyl@uwm.edu.pl

Aims: The calcification is a well known phenomenon of the pineal gland in mammals and completely unknown in birds. In the present work we analyzed the pineal concretions in the turkey in relation to their internal composition. **Methods:** The studies were performed on the pineals collected from 56-week-old domestic turkeys (*Meleagris gallopavo*). We employed standard morphological methods as well as the alizarin red S procedure and the potassium pyroantimonate method for localization of calcium at light and electron microscopy levels. **Results:** In light microscopy the calcified concretions with diameters from 300 μm to 2 mm and in number from 3 to 6 were observed in the parenchyma of all examined pineals. The concretions were stained red with the alizarin S method and showed the presence of collagen fibers. In electron microscopy the concretions were composed mainly of the collagen fibers and calcium deposits. Their central part showed typical appearance of calcified hard tissue and contained some osteocyte-like cells. **Conclusion:** The formation of the pineal concretions in turkey is a process associated with the mineralization of collagen fibers. It is completely different from the mechanism responsible for the formation of concretions in the mammalian pineal gland. The question arises if the process is typical for all birds or it is species specific.

PW05-43

Different expression of IL-6 mRNA in male and female rat adrenal gland during chronic inflammation

Skurlova M., Stofkova A.
Dept. of Normal, Clinical, and Pathol. Physiology, 3rd Faculty of Medicine, Charles University Prague, Czech Republic; avolruks@hotmail.com

Aims: Proinflammatory cytokine IL-6, is produced beside immune cells in adenopituitary, and adrenal gland where it exerts local regulatory function. Adjuvant arthritis (AA) in the rat is a chronic inflammation associated with stimulation of the hypothalamic-pituitary-adrenal axis (HPA), via pro-inflammatory cytokines. These are under negative control of circulating corticosterone (CORT). Purpose of this study was to describe the relationship between activity of HPA axis, and mRNA expression of IL-6 in adenopituitaries (AP), and adrenals in chronic phase of AA in male and female rats. **Methods:** To seven week-old, Long Evans males and females, randomly in cycle, AA was induced by injection of complete Freund's adjuvant. Control rats received vehicle. On day 23 of AA rats were decapitated. In plasma, adrenocorticotropin (ACTH) and CORT were measured by radioimmunoassay, AP, and adrenals were extracted for mRNA. The mRMA expressions for proopiomelanocortin (POMC), and IL-6 in AP and IL-6 in adrenals, were estimated by quantitative real-time PCR using TaqMan probes and primers. Paw oedema was estimated volumometricaly. **Results:** The significant activation of HPA-axis (plasma CORT) was the same in both sexes. IL-6 mRNA expression was significantly elevated in male and female AP, and female adrenals, however in male adrenals it was significantly inhibited. **Conclusion:** Chronic activation of HPA (namely enhanced CORT levels) during AA is not sufficient to inhibit IL-6 production in AP, or adrenals in females. The sex difference in adrenal response can not be ascribed only to the action of CORT, and warrants further study. *Supported: VZ 002162018, and GACR 305/ 06/ 0427.*

PW05-44

Evaluation of endocrine disruptive effects of cypermethrin and trifluralin in male rats by Hershberger assay

Yilmaz B.[1], Bulmus O.[2], Sandal S.[2], Sahin Z.[2], Yildiz S.[3], Kelestimur H.[2]
[1]Yeditepe Univ., Faculty of Medicine, Dept. of Physiology, Istanbul; [2]Firat Univ., Faculty of Medicine, Dept. of Physiology, Elazig; [3]Kafkas Univ., Faculty of Veterinary Medicine, Dept. of Physiology, Kars, Turkey

Aim: Cypermethrin and trifluralin are widely used synthetic pyrethroid insecticide and herbicide, respectively. We have investigated their effects on serum levels of testosterone, luteinising hormone (LH) and follicle stimulating hormone (FSH) levels and weights of androgen-sensitive tissues in peri-pubertal male rats using Hershberger assay. **Methods:** Male Sprague-Dawley rats (42-day old) were used. First group was sham-orchidectomized. The remaining rats were orchidectomized and then divided into five groups: Group II (orchidectomy), Group III (testosterone propionate, TP, 0.5 mg/kg/day sc), Group IV (TP+flutamide, reference anti-androgen), Group V (TP+Cypermethrin; 20 mg/kg/day, oral gavage) and Group VI (TP+Trifluralin, 15 mg/kg/day, oral gavage). All drugs were administered for 10 days, and animals decapitated 24h after the last injections. Prostate gland, seminal vesicles and m. levator ani (mLA) were weighed out. Serum levels of testosterone, LH and FSH were determined by ELISA. **Results:** Serum levels of testosterone were significantly reduced in orchidectomy and flutamide groups ($p<0.001$). Serum LH and FSH levels were significantly increased by both trifluralin and cypermethrin ($p<0.05$). Flutamide significantly increased serum gonadotrophin levels and decreased weights of androgen sensitive tissues compared to sham or TP groups ($p<0.01$). Weights of prostate ($p<0.05$; except cypermethrin group), seminal vesicles ($p<0.01$) and mLA ($p<0.01$) were significantly reduced by both pesticides. **Conclusion:** These findings indicate that both cypermethrin and trifluralin produced anti-androgenic effects in pre-pubertal rat model using Hershberger assay. We suggest that these pesticides may cause endocrine disruptive effects. *Supported by TUBITAK (project # 104-T-240).*

PW06-45

Effects of diuretic-induced hypovolemia/isosmotic dehydration on cardiorespiratory responses to experimental hyperthermia

Brozmanova A.[1], Jochem J.[2], Javorka K.[1], Zila I.[1]
[1]Department of Physiology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia; [2]Department of Physiology, Silesian Medical University, Katowice-Zabrze, Poland
abrozmanova@jfmed.uniba.sk

Aims: Under conditions of heat stress and hyperosmotic dehydration, both animals and humans reduce thermoregulatory evaporation and regulate deep body temperature at elevated levels. Hyperosmotic dehydration attenuates the heat stress-induced cutaneous vasodilatation. However, little is known about the effects of hypovolemia/isosmotic dehydration on cardiorespiratory responses to hyperthermia. **Methods:** Therefore, cardiorespiratory responses to hyperthermia during isosmotic dehydration/hypovolemia were studied in 17 anaesthetized adult rabbits divided into two groups: normovolemic group (NV; n = 10) and hypovolemic group (HV; n = 7). In the HV group, hypovolemia (16% decrease in plasma volume) was induced by furosemide administration (5 mg/kg i. v.). **Results:** During hyperthermia (the rise in body temperature /BT/ to 42°C by a gradual body surface heating), the HV rabbits had the breathing frequency lower ($P < 0.05$) than the NV animals. The panting was absent in the HV rabbits at the BT of 42°C, unlike the NV animals. From cardiovascular variables, the vasoconstrictor response in visceral (mesenteric) region during hyperthermia was attenuated ($P<0.05$), whereas the heat stress-induced cutaneous vasodilatation was not significantly influenced by hypovolemia. **Conclusion:** The lower frequency of breathing, thus lower respiratory evaporative heat loss during exogenous hyperthermia in dehydrated animals are present not only during hyperosmotic dehydration, but they occur also under conditions of furosemide-induced isosmotic dehydration/hypovolemia.

PW06-46

Influence of chest irradiation on cough response in guinea pigs

Javorkova N.[1], Brozmanova M.[1], Hajtmanova E.[2], Zamecnik L.[2], Bartos V.[3], Plank L.[3], Javorka M.[4], Hanacek J.[1], Tatar M.[1]
[1]Institute of Pathological Physiology, Jessenius Faculty of Medicine, Comenius University; [2]Centre of Oncology – Department of Radiotherapy and Oncology, Martin Teaching Hospital; [3]Institute of Pathology, Jessenius Faculty of Medicine; [4]Institute of Physiology, Jessenius Faculty of Medicine, Comenius University; Martin, Slovakia
javorkova@lefa.sk

Aims: Radiotherapy of tumours in the chest and neck regions may have serious pulmonary side-effects. Inflammation is an essential manifestation of radiation-induced injury and it may progress up to irreversible pulmonary fibrosis. To prevent such complications it would be useful to have simple non-invasive sensitive method for monitoring the course of airway and lung post-irradiation inflammation. We suppose that cough reaction intensity (CRI) could be the method that will be able to detect early postirradiation changes in airways. **Methods:** Guinea pigs (Trick strain, n=32) were used in the study. Animals were divided into two subgroups – non-treated (NT) group (n=14; M=7, F=7) were submitted to sham chest irradiation; animals of treated (XRT) group (n=18; M=9, F=9) were exposed to single dose of gamma rays. Cough was provoked by citric acid aerosol (CA, increasing concentrations). CRI testing was performed two days before sham/real chest irradiation, on 1st, 3rd, 10th, 15th, 21st and 28th days following irradiation. CRI was quantified by counting the number of coughs induced by all used CA concentrations. **Results:** Significantly higher values of CRI were found in animals of XRT group on 10th and 21st days when compared to animals of NT group. Significant increase of CRI was also found within the XRT group on the 10th day after irradiation when compared to pre-irradiation values of CRI. **Conclusion:** We conclude that CRI testing could provide a sensitive marker of post-irradiation changes in airways.

PW06-47

Respiratory plasticity in animal models of sleep apnoea

O'Halloran K.D.[1], Bradford A.[2]
[1]UCD School of Medicine and Medical Science, University College Dublin, Dublin 4, Ireland. [2]Department of Physiology and Medical Physics, Royal College of Surgeons in Ireland, Dublin 2, Ireland; ken.ohalloran@ucd.ie

Aims: Sleep-disordered breathing is extremely common and is characterized by intermittent hypoxia/asphyxia due to recurrent apnoea during sleep. We sought to determine the effects of chronic intermittent hypoxia (CIH) and asphyxia (CIA) on respiratory control in conscious animals. **Methods:** Adult male Wistar rats were exposed to either 1) alternating periods of air and hypoxia, twice per minute, 8 hours a day for 5 weeks (CIH-treated, n=16) or 2) alternating periods of air and asphyxia, twice per minute, 8 hours a day for 5 weeks (CIA-treated, n=16). Control rats (n=32) were exposed to an air/air cycle under identical experimental conditions. At the end of the study, ventilation during normoxia and acute hypoxia [$FiO2=0.12$] was assessed in animals by barometric plethysmography. **Results:** After the 5-week treatment period, normoxic and hypoxic ventilation was elevated in CIH-treated rats compared to control. The hypoxic ventilatory response was similar in the two groups. Thus, VE was 42.1±4.6 and 52.0±4.1 in air and hypoxia respectively in control animals and 49.3±4.3* and 61.3±6.4* in CIH-treated rats; mean±SEM, ml/min/100g, *P<0.05 ANOVA. Baseline normoxic ventilation was unaffected by CIA treatment (37.1±1.5 vs. 40.2±1.5) although the pattern of respiration was different following CIA. However, there was a significant blunting of the hypoxic ventilatory response in CIA-treated rats compared to controls (VE in hypoxia was 76.6±4.1 vs. 46.1±1.6*; control vs. CIA, *P<0.05 ANOVA). **Conclusions:** These results indicate that chronic intermittent hypoxia and asphyxia elicit plasticity in the respiratory control system. These changes have potentially adaptive and maladaptive consequences for respiratory homeostasis.

PW06-48

Anti-inflammatory treatment improved the lung functions in a rabbit model of meconium aspiration syndrome

Mokra D.[1], Mokry J.[2], Drgova A.[3], Calkovska A.[1], Petraskova M.[1], Bulikova J.[1], Redfors B.[1]
[1]Dept. Physiol.; [2]Dept. Pharmacol.; [3]Dept. Med. Biochem., Jessenius Med. Faculty, Comenius Univ., Martin, Slovakia; mokra@jfmed.uniba.sk

Aim: Anti-inflammatory treatment may diminish lung oedema, inflammation, airway hyperreactivity, and pulmonary vasoconstriction associated with meconium aspiration, and thereby improve lung functions. **Methods:** Air-ventilated adult rabbits intratracheally received 4ml/kg of saline (Sal group, n=5) or meconium suspension (25mg/ml). From this moment, all animals were oxygen-ventilated. When respiratory failure developed, meconium-instilled rabbits received dexamethasone (0.5mg/kg, Mec+Dex group, n=8), aminophylline (2.0mg/kg, Mec+Amin group, n=7) i.v., or budesonide (0.25mg/kg, Mec+Bud group, n=8) intratracheally by inpulsion effect of high-frequency jet ventilation at two doses 0.5 and 2.5h after meconium instillation, or were left without treatment (Mec group, n=8). Animals were oxygen-ventilated for additional 5h after the first treatment dose. Respiratory parameters, blood gases and white blood cells count (WBC) were evaluated regularly. Post mortem, *in vitro* airway reactivity was measured in tracheal and right lung strips, lung oedema was determined by wet/dry weight ratio, and markers of lipid and protein oxidation were estimated in lung homogenate. Left lungs were saline-lavaged and differential WBC was calculated in BAL sediment. **Results:** Treatment with Dex, Amin and Bud significantly improved gas exchange, decreased pulmonary shunting, central venous pressure, ventilatory pressures, reduced meconium-induced lung oedema, airway hyperreactivity, neutrophil count in BAL associated with higher WBC and neutrophils in blood, and diminished oxidative modifications of proteins and lipids in lung homogenate compared to non-treated Mec group (all $P<0.05$, 0.01 or 0.001). **Conclusion:** Anti-inflammatory treatment effectively improved the lung functions in meconium-instilled rabbits. *Supported by Grant of VEGA 1/2306/05.*

PW06-49

Facilitation of the cough response by afferent inputs from the nasal mucosa tested in animal experiments and clinical surveys

Plevkova J., Brozmanova M., Pecova R., Varechova S., Tatar M.
Department of Pathophysiology, Jessenius Faculty of Medicine, Comenius University, Slovak Republic

Aim: Nasal diseases are common causes of chronic cough. Accordingly to the idea of cough plasticity we designed the studies to address the effect of stimulation of nasal afferents on experimentally induced cough in laboratory animals and human volunteers.
Methods: SurveyA) Intranasal challenges with capsaicin (50μM) and histamine (16mM) were performed in anaesthetized cats (n=13) and guinea-pigs (n=15) and in 15 conscious guinea-pigs. Coughing was provoked after these challenges by mechanical stimulation of the larynx and tracheobronchial region in anaesthetized animals and by inhalation of 0.3M of citric acid aerosol in awake animals placed in bodyplethysmograph. Parameters of cough reflex were evaluated from either interpleural or esophageal pressures in anesthetized animals and from pneumotachographic traces in conscious guinea-pigs during cough expulsions. SurveyB) Healthy volunteers (32) and patients with allergic rhinitis (20), mean age 27.1 yr, 26M/26F, nonsmokers, with normal FVC/FEV1, without acute respiratory infection in past four weeks, were randomly challenged with intranasal capsaicin (750μM, 25μl) and histamine (4mg/ml 25 μl) versus saline. Nasal symptom score, cough sensitivity to inhaled capsaicin and number of coughs during provocation were determined. **Results:** Intranasal stimulation with capsaicin and histamine increases the cough response in anaesthetized and conscious animals. Nasal stimulation enhances the cough response in healthy humans and this facilitation of the cough response is exaggerated extremely in allergic subjects. **Conclusion:** We speculate that facilitation of the cough response could be the consequence of increased afferent inputs from the nose into the brainstem thus stimulating synaptic activity of cough pattern generator.

PW06-50

Cough provocation testing in children with gastro-oesophageal reflux disease and children with Diabetes mellitus

Varechova S., Plevkova J., Durdik P., Mikler J., Banovcin P., Hanacek J.
Institute of Pathological physiology, Clinic of children and adolescents, Jessenius Medical School, Martin, Slovakia

Aims: The aim of the study was to find out whether CRS is changed in children with gastro-oesophageal reflux disease (GORD) and children with Type 1 Diabetes mellitus (DM). Information from literature claim that heightened exposure of distal oesophagus to acid in adult patients causes an appreciable increase in cough reflex sensitivity. Opposite, CRS could be weakened according to the presence of autonomic neuropathy (DAN) in children with DM. **Methods:** The CRS test was performed in 35 children suffering from Diabetes mellitus type 1 and in 20 children with symptoms suggestive of GORD. The results were compared with the group of age-matched 27 healthy children. Cough was induced by inhalation of capsaicin aerosol in doubling concentrations (0.61-1250 μmol/l) for 400 ms each. CRS was defined as the lowest capsaicin concentration that evoked 2 or more coughs (C2 parameter). **Results:** A significant decrease (P=0.005) of CRS was found in diabetic children with DAN [n=12; C2: 221.0 μmol/l (95% CI: 75.7- 644.8 μmol/l)] compared with diabetic children without DAN [n=23; C2: 42.7 μmol/l (95% CI: 23.1-79.0 μmol/l)]. CRS in the group of children with suspected GORD [C2: 17.0 μmol/l (6.4-45.6 μmol/l)] and with confirmed GORD [C2: 13.4 μmol/l (3.6-50.9 μmol/l)] were significantly elevated (P<0.05) compared with healthy children [C2: 72.1 μmol/l (25.5-203.9 μmol/l)]. **Conclusion:** We have found that CRS is decreased in diabetic children with DAN when compared with diabetic children without DAN. The CRS changes in children suffering from GORD are similar to those described in adult patients with GORD.

PW07-51

Physiological responses to hand and foot dynamic exercise in healthy young men

Al-Kubati M.[1,2], Fiser B.[1], Geas A.H.[3], Al-Mahmodi N.A.[4]
[1]Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic; [2]1st Internal Cardiovascular Clinic in St. Anne's University Hospital, Brno, Czech Republic; [3]Dept. of Paediatric Radiology; [4]Dept. of Functional Diagnostics and Rehabilitation
mkubati7@yahoo.com

Aim: to investigate responses to hand (H-Ex) and foot (F-Ex) exercise regarding blood pressure (BP). **Methods:** in 15 healthy men (30±5 years) we used non-invasive methods for measuring systolic, diastolic, pulse and mean BP (SBP, DBP, PP and MBP), R-R interval and baroreflex sensitivity (BRS). Each measuring lasted 5 minutes and was repeated three-times all in standing position: once without exercise, once using dynamic handgrips (40 handgrips in 2 min.) and once during foot exercise by raising the heels to stand on the tip-toes on a 5 cm. high stair (40 times in 2 min.). **Results:** during H-Ex: R-R interval decreased from 660 ± 29.8 to 652.5 ± 39.2 ms, p=0.7 non-significant (NS), SBP, DBP, PP and MBP (in mmHg) increased from 109.9 ± 4.6 to 121.9 ± 10.4, p=0.004; from 73.1 ± 2.7 to 81 ± 6.3, p=0.02; from 36.8 to 40.9 (NS); and from 85.4 to 94.6, p= 0.007; respectively, BRS decreased from 5.73 to 4.0 ms/mmHg (NS). Comparing with F-Ex: R-R decreased to 631.9 ± 50.7 ms, (NS); SBP, DBP, PP and MBP (in mmHg) increased to 136.4 ± 14.1, p= 0.000001; to 86.1 ± 6.9, p= 0.0002; to 52.3, p= 0.0007; and to 103.6, p=0.000008, respectively; BRS decreased to 2.7 ms/mmHg, p= 0.0001. Comparing H-Ex with F-Ex: we found no significant differences in R-R, DBP and BRS but greater SBP, PP and MBP in F-Ex: p= 0.0005, p= 0.008 and p= 0.005 were found respectively. **Conclusion:** These results indicate that changes in R-R intervals, BRS and blood pressure are higher in F-Ex. We conclude that this exercise could be more helpful for subjects with orthostatic hypotension.

PW07-52

Neurophysiological view of Back-Shu and Huatuo-Jiaji acupuncture points of urinary bladder

Cabioglu M.T.[1], Surucu S.[2], Ergene N.[3]
[1]Acupuncture Treatment Unit, Department of Physiology, Faculty of Medicine, Baskent University, Ankara, Turkey; [2]Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey; [3]Department of Physiology, Meram Faculty of Medicine, Selcuk University, Konya, Turkey
neyhan@hotmail.com

Acupuncture, one of the traditional Chinese Medicine Method, at certain points on the body, can have analgesic and therapeutic effects in various diseases. Application on the Back-Shu and Huatuo-Jiaji points are especially effective on the visceral organs. The specific points on the back are called the Back-Shu points where these are points pertaining to the Urinary Bladder Channel located at the back 1.5 cun (special measurement of acupuncture) lateral to the Du channel. Huatuo-Jiaji is a group of 34 points on both sides of the spinal column, 0.5 cun lateral to the lower border of each spinous process from the first thoracic to the fifth lumbar vertebra. Application of acupuncture on the Back-Shu and Huatuo-Jiaji points of the urinary bladder affects the motility of the urinary bladder. This effect can be explained with cutaneo-visceral reflexes. It is considered that the segmental dispersal of the sympathetic and parasympathetic nervous systems of urinary bladder is related with the localization of the Back-Shu and Huatuo-Jiaji points of the urinary bladder. Particularly, application on the Back-Shu for the treatment of urinary disturbances, bladder disorders and nocturnal enuresis is effective. Back-Shu point corresponds dermatomically to the outlets of the parasympathetic chain of the urinary bladder plexus. Any changes on the motility of the urinary bladder caused by acupuncture application on the Back-Shu and Huatuo-Jiaji points of the urinary bladder can be explained with the modulation of the sympathetic and parasympathetic nervous systems.

PW07-53

Circadian changes of skin potential level related to human operator performance

Gorgo Y., Gorgo D., Bogdanov V.
Laboratory of Physiological Cybernetics and Psychophysiology, Taras Shevchenko National University of Kyiv, Ukraine
gorgo@biocc.univ.kiev.ua, yugorgo@ukr.net

Aims: The prediction of the performance of human operator of machine systems can be done by the monitoring of individual differences in the changes of physiological parameters. **Methods:** In the population of 40 young male subjects the measurements of skin potential level (SPL) in palmar area as an index of autonomic sympathetic arousal and short term memory test (recalling of listened in a series of 12 random 2 digit numbers) were conducted 5 times per day (on 9, 12, 15, 18, 21 h.) during 5 days. **Results:** It was shown that circadian changes of SPL can be considered as a trait – like property, because the pattern of the changes was stable in every subject during all 5 days of observation. In the groups of subjects with maximal SPL in the morning or in the evening the ratings of short term memory were lower (68.7 ± 4.3 and 61.2 ± 4.5) than in the group with random fluctuations, absent circadian structure of SPL (81.1 ± 5.5, $p< 0.05$ by t – test). The best rating of short term memory was observed in a subject in the time of individually highest SPL. **Conclusion:** Both current skin potential level and its circadian changes are good predictors of human operator performance.

PW07-54

Effect of leg massage on heart rate variability (HRV) following a bout of submaximal aerobic exercise

Jones G.E., Cotterrell D., McDowall I.
School of Applied and Health Sciences, Department of Biological Sciences, University of Chester, Chester, UK; g.jones@chester.ac.uk

Aims: To investigate the effects of leg massage on cardiac autonomic activity assessed by heart rate variability following a bout of Cycling Exercise at 80%HR_{max} (CE). **Methods:** HRV was measured for 5mins in a supine position pre- 20mins CE in six healthy males (21.5±3.4yrs) using the Polar Precision Performance software (Polar Electro, Finland). On completion of CE, subjects received either a manual leg massage (MM), vibratory leg massage (VM), or rested (R) in a supine position for 20mins. HRV was then measured again for 5mins. **Results:** Compared to post exercise Rest, heart rate for MM and VM were lower (Figure 1). This was accompanied by a significant decrease in an indicator of sympathetic activity (LFnorm) and increase in parasympathetic activity (HFnorm). LF:HF Ratio, which is an indication of sympatho-vagal balance, also decreased indicating a parasympathetic effect exerted by both manual and vibratory leg massage. In contrast, during R, heart rate and LFnorm were higher and HFnorm lower and not significantly different from Pre-Exercise. **Conclusion:** Leg massage is effective at decreasing cardiac sympathetic and increasing parasympathetic activity, thus inducing a relaxation effect following a bout of cycling exercise.

Figure 1. Time and Frequency domain HRV

	Pre-exercise	Post exercise Rest (R)	Manual leg massage (MM)	Vibratory leg massage (VM)
HR	62.7±8.3	66.6±6.9	56.2±6.2*	57.8±6.7*
LF norm (S)	69.6±14.7	73.3±9.7	61.2±9.0*	63.5±10.2*
HF norm (PS)	30.4±14.7	26.7±9.7	38.8±9.0*	36.5±10.2*
LF:HF Ratio	2.5±1.4	3.0±1.4	1.7±0.7*	1.9±0.8*

* Significant difference compared to Post exercise-Rest ($p<0.05$). S = Sympathetic; PS = Parasympathetic (mean±SD)

PW07-55

Does pre-exercise static stretching reduce post-exercise muscle soreness?

Cotterrell M.D., Curtis D., Fallows S., Kellett D.
Centre for Exercise and Nutrition Science, University of Chester, UK; National Training Centre, Dublin, Ireland
m.cotterrell@chester.ac.uk

Aims: Pre exercise static stretching is said to reduce post exercise muscle soreness. The purpose of this study was to test this hypothesis. **Methods:** Twelve female and eight male students aged 30.7±1.4 years (mean±SD) from the National Training Centre, Dublin, took part in the investigation. Pre exercise, each subject had one of their legs randomly assigned to a stretched leg group (SL group; n=20) which performed 4x20second static stretches, while their other leg acted as control in a non stretched leg group (NSL group; n=20). Following a 15-minute warm up (treadmill and 5 sub maximal eccentric muscle contractions), subjects performed 5 sets of 15 maximal voluntary eccentric muscle contractions, with a one-minute rest between sets, on a seated leg curl machine. Rated soreness was evaluated pre exercise and 24, 48 and 72 hours post exercise on a visual analogue scale (VAS), which ranged from 0 (no pain) to 10 (worst pain ever). **Results:** There was significant muscle soreness ($p<0.05$) compared with the baseline for all groups (peak 48hours) however the reduction in soreness seen in the test group was not significant at the three time points: VAS at 24 hours SL group = 4.70±1.17, NSL group = 4.95±1.19 (reduction=5.05%), 48 hours SL group =5.85±1.42, NSL group = 6.10±1.33 (reduction=4.1%) and 72 hours SL group =3.35±1.23, NSL group = 3.45±1.39 (reduction=2.9%). **Conclusion:** Pre exercise static stretching does not provide significant protection from post exercise muscle soreness if a warm up period is used.

PW07-56

Age-related changes in human balance control during stance

Abrahamova D., Hlavacka F.
Institute of Normal & Pathological Physiology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovak ia
diana.abrahamova@savba.sk

Aims: Human balance control change with age, resulting in a slight postural instability. The aim was to investigate age-related indications of small balance impairment. **Methods:** We examined 81 healthy subjects during the quiet stance on a firm surface and/or on a compliant support surface, with eyes either open or closed. Body sway was measured by force platform as centre of foot pressure (CoP) positions during 50 sec interval. The seven CoP parameters were evaluated and analyzed in three age groups: juniors (20-40 years), middle-aged (40-60 years) and seniors (60- 82 years). **Results:** The regression analysis showed evident increase of body sway over 60 years of age. These seven CoP parameters were significantly different when comparing juniors and seniors in all static conditions. New physiological ranges of root mean square (RMS) parameter in each condition for each age group of healthy subjects were determined. The most sensitive view on postural steadiness was provided by CoP amplitude and velocity in AP direction and by RMS. Analysis of postural responses to somatosensory input showed that initial amplitude, velocity and return velocity of CoP postural response to Achilles tendons vibration were significantly related to the age. **Conclusion:** Our results documented that CoP data from force platform in quiet stance may indicate small balance impairment due to age. The determined physiological ranges of CoP parameters will be useful to distinguish between small postural instability due to aging and pathological processes in human balance control.

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PW07-57

Lack of negative feedback of cortisol on adrenal steroidogenesis at adrenocortical level in humans

Kerlik J., Penesova A., Vlcek M., Radikova Z.
Laboratory of Human Endocrinology, Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia; jana.kerlik@savba.sk

Aim: The study was aimed at affirming the direct negative feedback of cortisol on adrenal steroidogenesis at adrenocortical level in humans. **Methods:** Ten young healthy women in the phollicular phase underwent four tests. In the repeated minimal ACTH-ACTH test, dose of 1μg of ACTH was administered intravenously twice: at 9am and 10am, respectively. The control test consisted of single ACTH administration at 10am. In the HC-ACTH test, 20mg of hydrocortisone (HC) was given per os and 1μg of ACTH was injected intravenously 90min after the HC administration. The control test with sole HC administration was performed. **Results:** The first ACTH administration in the ACTH-ACTH test caused a significant increase of plasma cortisol concentration. The cortisol response after the subsequent ACTH bolus was present (Δmax: 180.8 ± 27.5 nmol/l, $p<0.001$), but significantly lower ($p<0.011$) than after the first ACTH administration (Δmax: 280.7 ± 12.8 nmol/l, $p<0.001$). Responses of the other adrenal steroids 17α-OH-progesterone, Δ4-androstenedione and dehydroepiandrosterone after both ACTH administrations were comparable. ACTH administration during HC-induced hypercortisolemia maintained a plateau in cortisol concentration and prevented a decrease in cortisol level at this timepoint, which was seen after the HC administration alone. As known, the levels of the cortisol precursors decreased after the HC administration, followed by a significant rise after the ACTH bolus. **Conclusions:** Pharmacologically induced hypercortisolemia does not suppress the cortisol secretion from adrenals and does not influence the adrenal steroidogenesis. The present study suggests that there is not a direct inhibitory effect of cortisol on steroidogenesis on adrenocortical level in humans. *Supported by VEGA 2/6157/27.*

PW07-58

Glucocorticoids and selected adipose tissue parameters in relationship to insulin sensitivity

Vlcek M., Penesova A., Radikova Z., Imrich R., Krskova K., Zorad S., Skopkova M., Gasperikova D.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; Miroslav.Vlcek@savba.sk

Aim: Cortisol is one of the factors regulating glucose metabolism; which is obvious in diseases with its increased secretion. However, some studies showed associations of physiological levels of cortisol and free cortisol with insulin sensitivity. The aim of our study was to evaluate this association in respect of adipose tissue parameters. **Methods:** 60 healthy volunteers were examined (36M/24F, age 29.2±6.4 y., BMI 26.5±5.6 kg/m^2). Insulin sensitivity was measured by hyperinsulinaemic euglycaemic clamp (M/I); blood pressure (BP), total cholesterol (TCh), HDL cholesterol, triglycerides (TG), cortisol, transcortin (CBG) and free cortisol baseline levels were measured. Accumulation of fat in abdominal region was expressed as waist circumference. Adipocytes' cell size (ACS) was measured in subcutaneous adipose tissue obtained from biopsy in umbilical region. **Results:** Correlation analysis showed known relationship of BMI and waist circumference to insulin sensitivity, BP, TCh, HDL, TG and also relation of ACS to insulin sensitivity. However, we did not find any relationship of cortisol or free cortisol levels to measured parameters. Levels of CBG correlated with HDL. Results in males and females were comparable. Using linear regression analysis, independent predictors for insulin sensitivity (M/I) were BMI(-), ACS(-), sex (M>F); for TCh: BMI(+), age(+), CBG(+); for TG: waist circumference (+); for HDL: CBG(+) and for BP: waist circumference(+). **Conclusions:** In our study, we confirmed relationship of adiposity parameters (BMI, waist circumference, ACS) to insulin sensitivity. The relationship of cortisol or free cortisol to insulin sensitivity, lipid spectrum or BP was not significant.
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PW07-59

Recurrence quantification analysis of heart rate variability in young diabetic patients

Trunkvalterova Z., Javorka M., Tonhajzerova I., Javorkova J., Javorka K.
Institute of Physiology, Comenius University, Jessenius Faculty of Medicine and Paediatric Clinic, Martin Teaching Hospital, Martin, Slovakia
Trunkvalterova@lefa.sk

Aims: Analysis of heart rate variability (HRV) can provide information predominantly about parasympathetic control of the heart. Nonlinear phenomena are involved in HRV signal – therefore, methods of nonlinear dynamics are increasingly applied to this signal to improve the description of different pathological states influencing autonomic nervous system. Classical nonlinear measures (Lyapunov exponent, etc.) are of limited use in real biosignals analysis due to limited availability of long stationary data in living subjects. Therefore, new methods that are able to detect nonlinear characteristics are developed – one of them is recurrence plot with recurrence quantification analysis (RQA). The aim of this study was to find whether HRV parameters derived from RQA are different in young patients with DM compared to control group. **Methods:** Patients with type 1 DM (17, 10 females, 7 males) aged 12.9-31.5 years were investigated. The control group consisted of 17 healthy matched probands. The length of R-R intervals was measured using telemetric system. **Results:** From RQA measures based on diagonal lines in recurrence plots, we have found higher percentage of recurrence (%Rec) and of determinism (%Det) and increased maximal length of diagonal line in DM group. Trapping Time was higher in DM group compared to controls. **Conclusion:** These results suggest reduced complexity and increased predictability of heart rate dynamics even in young patients with DM. RQA parameters can be used together with other HRV parameters for better description of heart rate dysregulation.

PTh08-60

β-amyloid peptide induces changes in calcium signaling in hippocampal cells

Korol T., Korol S.
Department of General Physiology of Nervous System, Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine, Kiev, Ukraine; mtanyushka@ukr.net

Aims: It is known that a fragment of amyloid protein, $A\beta_{1-42}$, is lethal to hippocampal cells, producing recent memory deficits characteristic of Alzheimer's disease (AD). Dysfunction in calcium homeostasis is one of the events in the pathogenesis of AD. The aim of the study was to discover changes in calcium signaling in hippocampal cells with experimental induced AD. **Methods:** The changes in neuronal Ca^{2+} homeostasis were studied on rat hippocampal cell culture in control condition and under one-day β-amyloid-induced modification. The cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using fura-2 based microfluorometry. **Results:** The recovery of depolarization-induced $[Ca^{2+}]_i$ increase was delayed on 200 s in amyloid-treated neurons compared with recovery at control conditions. Basal $[Ca^{2+}]_i$ in cells exposed to β-amyloid was higher on 67±9 nM (mean SD, $P<0.05$) than that in control cells. The amplitude of depolarization-induced $[Ca^{2+}]_i$ increase in amyloid-treated neurons was lower on 189±26 nM ($P<0.05$) than depolarization-induced $[Ca^{2+}]_i$ increase in control cells. The morphology of neurons has been changed essentially by influence of β-amyloid. **Conclusion:** These results demonstrated strong β-amyloid affect on hippocampal cell culture. Smaller response of the β-amyloid-treated cells to depolarization compared with that in control condition could be explained by action of $A\beta_{1-42}$ on voltage-gated calcium channels. Longer after-depolarization recovery period and higher resting Ca^{2+} level peculiar to β-amyloid-modificated cells might be related with damaging of Ca^{2+} extrusion mechanisms. We conclude that β-amyloid causes a strong toxic effect on hippocampal cell culture by changing Ca^{2+} signaling in it.

PTh08-61

DHP analog cerebrocrast inhibits T-type $Ca_V3.1$ calcium channel

Drigelova M.[1], Dubur G.[2], Lacinova L.[1]
[1]Institute of Molecular Physiology and Genetics, Slovak Academy of Science, Bratislava, Slovakia; [2]Latvian Institute of Organic Synthesis, Riga, Latvia
maria.drigelova@savba.sk

Aim: Cerebrocrast is a novel analog of a 1, 4-dihydropyridine (DHP). The aim of our study was to evaluate the effect of cerebrocrast on the $Ca_V3.1$ calcium channel. **Methods:** We have used whole cell patch-clamp method. $Ca_V3.1$ calcium channel was permanently transfected into HEK 293 cells. Stock solution of cerebrocrast was prepared in DMSO in 10 mM concentration and was diluted into experimental concentration (10 nM, 100 nM, 1μM, 10 μM, 50 μM) prior to the experiment. The bath solution contained (mM): NaCl 135, HEPES 10, $CaCl_2$ 2, $MgCl_2$ 1, CsCl 5. The pipette consisted of (mM): CsCl 130, Na-ATP 5, TEA-Cl 10, HEPES 10, EGTA 10, $MgCl_2$ 5. **Results:** Effect of cerebrocrast was evaluated at holding potentials of –100 mV and –70 mV. Cerebrocrast inhibited current through the CaV3.1 calcium channel already in nanomolar concentrations. At both holding potentials 100 nM of cerebrocrast blocked 8% of the current amplitude. 50 μM of the drug inhibited 42 % of the current amplitude measured at a holding potential of -100 mV. At a holding potential of –70 mV drug inhibited 49 % of the current. Voltage-dependent enhancement of drug effectivity was not significant. 50 μM of cerebrocrast had no significant effect on the shape of the I-V curve and the position of their peaks. **Conclusion:** In contrast to known DHPs such as nifedipine or isradipine, cerebrocrast was able to inhibit also the T-type $Ca_V3.1$ calcium channel.

PTh08-62

Effect of DHP analog cerebrocrast on L-type calcium current through $Ca_V1.2$ channel

Tarabova B.[1], Dubur G.[2], Lacinova L.[1]
1Institute of Molecular Physiology and Genetics, Slovak Academy of Science, Bratislava, Slovakia, 2Latvian Institute of Organic Synthesis, Riga, Latvia
bohumila.tarabova@savba.sk

Aims: Cerebrocrast is a novel analog of dihydropyridines (DHP) synthesized in the Latvian Institute of Organic Synthesis. It was shown that cerebrocrast did not antagonize calcium influx in neuronal tissue and inhibited KCl – induced arterial contraction. It was hypothesized that cerebrocrast is a vascular – specific calcium antagonist. In our study we aimed to investigate direct effects of cerebrocrast on $Ca_V1.2$ L-type calcium channels in expression system. **Methods:** In experiments we used HEK 293 cells transiently transfected with cDNAs encoding main subunit for smooth muscle isoform of $Ca_V1.2$ calcium channel together with β_{2a} and $\alpha_2\delta$ auxiliary subunits. 10 mM of Ca^{2+} was used as a charge carrier. **Results:** We measured effects of cerebrocrast on current through $Ca_V1.2$ calcium channel at holding potential (HP) of -80 mV and depolarized HP of -50 mV. Cerebrocrast inhibited calcium current in submicromolar concentrations in a concentration dependent manner at both holding potentials. Fitting data by Hill equation yielded IC_{50} values of 585 ± 0.1 nM and 178 ± 0.1 nM and at HP -80 mV and -50 mV. Hill coefficient values were 0.63 ± 0.06 and 0.6 ± 0.2 at HP -80 mV and -50 mV. Cerebrocrast caused no significant changes of current voltage relationship of $Ca_V1.2$ calcium current at both tested holding potentials. **Conclusion:** In conclusion, similar to traditional DHPs like nimodipine and nifedipine, cerebrocrast inhibited current through the $Ca_V1.2$ calcium channel in a voltage-dependent manner. However, its efficiency was more than one order of magnitude lower.

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PTh08-63

Role of uppermost arginines in S4 segments of the $Ca_V3.1$ channel in channel deactivation

Pavlovicova M., Lacinova L.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia
michaela.pavlovicova@savba.sk

Aims: Positively charged S4 segments in domains I to IV form a putative voltage sensor of voltage-dependent calcium channels. We have investigated their participation in deactivation of the $Ca_V3.1$ calcium channel. **Methods:** Uppermost basic amino acids of the S4 segments from each domain were replaced by neutral cysteines using a PCR-based method. Four single mutants (R180C, R834C, R1379C, and R1717C) and four double mutants combining mutations in two neighbouring domains were constructed and transfected into HEK 293 cells. Whole-cell patch-clamp was used. **Results:** Replacement of an arginine in domain II accelerated, while other single mutations slowed down channel deactivation. Acceleration of deactivation by the mutation in domain II was further enhanced when mutation in the adjacent domain I or III was introduced. Voltage dependencies of deactivation time constants (τ_{deact}) were shifted along the voltage axis. Mutations, which increased τ_{deact}s shifted their voltage dependence to more negative potentials (IIIS4, IVS4 and III+IVS4). Decrease of τ_{deact} was accompanied by the shift towards more depolarised membrane potentials (I+IIS4). Voltage sensitivity of channel deactivation reflected in slope factor of voltage dependence of τ_{deact} was not altered by introduced mutations except for the single mutation in the domain IV. **Conclusion:** The kinetics of channel deactivation reflects the stability of the open channel state. Removal of a basic amino acid in domains IV and III and, to a lesser extent, in the domain I stabilised an open channel state while neutralisation of the basic amino acid in domain II destabilised it.

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PTh08-64

Involvement of intracellular chloride and promiscuous channels in apoptosis and cardioprotection

Ondrias K.[1], Malekova L.[1], Kominkova V.[1], Kopacek J.[2], Krizanova O.[1], Novakova M.[3]
[1]Institute of Molecular Physiology and Genetics; [2]Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia; [3]Faculty of Medicine, Masaryk University, Brno, Czech Republic
karol.ondrias@savba.sk

Aims: Purpose of the study was to test the hypothesis that intracellular chloride and mitochondrial promiscuous channels are involved in the apoptosis and cardioprotection. **Methods:** We measured effects of the chloride blockers DIDS, NPPB and Phloretin on H_2O_2-induced cardiomyocyte apoptosis and single channel properties of chloride and high conductance promiscuous channels derived from rat heart lysosomal and mitochondrial membranes incorporated in bilayer lipid membrane (BLM). Promiscuity of the single mitochondrial channels was measured as a voltage change of a voltage-dependent switch from K^+ to Cl^- selectivity and an opposite switch. **Results:** The chloride channel blockers (100 μmol/l) inhibited the H_2O_2 induced cardiomyocytes apoptosis. They inhibited the chloride channels at concentrations of 10-50 μmol/l by decreasing the channel open probability and the open dwell time. They affected promiscuity of the mitochondrial high conductance channels, influencing the voltage-switch and the K^+/Cl^- channel activity. **Conclusion:** The obtained results did not contradict the tested hypothesis. We may assume that the intracellular and the promiscuous channels may be involved in apoptosis and cardioprotection, however a definite proof is still missing. The results may contribute to understand a possible involvement of intracellular chloride and promiscuous channels in apoptosis and cardioprotection.

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PTh08-65

Expression of calcium channel Cav1.3 in rat spinal cord and brain stem

Sukiasyan N., Hultborn H., Zhang M.
Department of Neuroscience and Pharmacology, the Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark
natalya@mfi.ku.dk

Aims: It is well known that persistent inward currents (PICs) could trigger all-or none plateau potentials and self-sustained firing in motoneurones. It was demonstrated this PIC was mediated by a particular subgroup of L-type Ca^{2+} channels, the Cav1.3 channel. The goal of this study is to investigate the distribution of Cav1.3-immunoreractive (Cav1.3-IR) neurons in different spinal segments as well as in the brain stem in the rat. **Methods:** This issue is studied by immunohistochemistry. The immunostaining was performed using a polyclonal antibody to a segment of the α-1D subunit of rat brain voltage-gated calcium channel. Eight adult male Wistar rats were used. Four of the animals were used for retrograde labelling of tail motoneurons in the spinal cord by injecting fluorogold into tail muscles. Selected segments from the whole spinal cord and brain stem were investigated. **Results:** Cav1.3-IR neurons were found in the all the segments of the spinal cord, particularly in the ventral horn including the motor nuclei. In the brain stem Cav1.3- IR neurons were observed in many nuclei including all cranial motor nuclei, many sensory nuclei as well as some reticular nuclei and raphe nuclei. In retrograde labelled spinal motoneurons the Cav1.3-IR labelling is seen at the soma and proximal dendrites; few, if any, distal dendrites were labelled. **Conclusion:** These results in rat's spinal cord are very similar to the cat but differ from studies in mouse and turtle motoneurons where the immunoreactivity against these channels was described to localise to the (distal) dendrites.

PTh08-66

Permeability properties of rat cardiac ryanodine receptor

Tomaskova Z., Gaburjakova M.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic
zuzana.vareckova@savba.sk

Aims: Ryanodine receptor (RyR) is the major intracellular Ca^{2+} release channel required for excitation-contraction coupling in cardiac muscle. The crystallographic structure of this channel is currently unavailable. Thus, the architecture of the conductive pore involved in ion handling can be probed only indirectly by examining the permeability properties of the channel. All available lines of evidence are compatible with the proposal that the conduction pathway of the RyR channel is occupied by only one ion at a time. The purpose of our study was to re-examine this conclusion under asymmetrical ionic conditions that have not yet been tested. **Methods:** RyR channels isolated from the rat heart were reconstituted into planar lipid membrane. The zero-current potential was determined from the current-voltage relationship obtained under various asymmetrical ionic conditions. **Results:** The zero-current potential showed clear concentration dependence when Li^+ or Na^+ was present at the cytosolic and Ca^{2+} at the luminal side of the channel. In one set of experiments, concentration ratios $[Li^+]/[Ca^{2+}]$ and $[Na^+]/[Ca^{2+}]$ were held constant. Importantly, the concentration dependence of zero-current potential was abolished when the concentration ratio was lowered from 12 to 1. Furthermore, the zero-current potential did not show extremes with varying Ba^{2+}/Ca^{2+} molar ratio at the luminal face of the channel. **Conclusion:** In the light of the barrier model of ion permeation through channels, the concentration dependence of the zero-current potential is one of the characteristics predicted for multi-ion channels. Thus, our results weaken the hypothesis of the single-ion nature of the RyR channel conductive pathway.

PTh08-67

Pushing the door of the inactivation gate – a new mechanism of drug interaction with the voltage-gated sodium channel

Zarrabi T., Cervenka R., Koenig X., Zebedin E., Hilber K., Todt H.
Center of Biomolecular Medicine and Pharmacology, Medical University of Vienna, Vienna, Austria
hannes.todt@meduniwien.ac.at

Aims: Slow inactivated states in voltage-gated ion channels can be modulated by binding of molecules both to the outside and to the inside of the pore. Here, we explore the modulation of a very long-lived inactivated state, ultra-slow inactivation (I_{US}) by ligand-binding to the outer vestibule in voltage-gated Na^+ channels. **Methods:** rNav1.4 channels were heterologously expressed in *Xenopus laevis* oocytes and examined by means of the two electrode voltage clamp technique. **Results:** Binding of Cd^{2+} (30 μM) to a cysteine engineered to the selectivity filter (K1237C) substantially accelerated recovery from I_{US} (time constant of recovery = 145 ± 10 s at control; 2.5 ± 3 s during superfusion with Cd^{2+}; $P < 0.001$). Cd^{2+} only accelerated recovery from I_{US} at -120 mV but did not affect development of I_{US} at -20 mV. On the other hand, I_{US} was also modified by binding of the local anaesthetic lidocaine to the internal vestibule. These effects could be simulated by a kinetic model in which Cd^{2+} binds with high affinity to a slow inactivated state (I_S) which is transiently occupied during recovery from I_{US}. In support of this model, 50 μM Cd^{2+} produced a ~8 mV hyperpolarizing shift of the steady-state inactivation curve of I_S. **Conclusion:** We propose a molecular model in which binding of Cd^{2+} to C1237 promotes the closure of the selectivity filter region (=I_S gate), thereby hastening recovery from I_{US}. Thus, Cd^{2+} ions may act like a foot-*on*-the-door, kicking the I_S gate to close.

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PTh09-68

Effect of short intense and prolonged moderate stimulation on gene expression of Ca^{2+} -regulatory muscle membrane proteins

Manttari S., Pilegaard H., Madsen K.
Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, and Copenhagen Muscle Research Centre and Centre of Inflammation and Metabolism, University of Copenhagen, Denmark
smanttari@health.sdu.dk

Aims: Sarcoplasmic and t-tubule membrane proteins of skeletal muscle regulate intracellular Ca^{2+} concentration and play central role in muscle contraction and relaxation. The purpose of this study was to evaluate the effects of short intense (SHO) and prolonged moderate (PRO) stimulations on mRNA expression of receptor proteins involved in Ca^{2+} regulation. **Methods:** mRNA levels of dihydropyridine (Cacna1) and ryanodine (Ryl) receptor, Ca^{2+}-ATPase (SERCa1), and calsequestrin (CASQ1,2) in extensor digitorum longus (EDL) and soleus (SOL) muscles of rat were assessed by RT-PCR after *in vitro* stimulation (SHO: 60Hz, 5min; PRO: 20Hz, 40min) followed by a 300min recovery period. **Results:** In general, stimulation decreased mRNA expression of all proteins studied. Most prominent down-regulation ($p<0.01$) in relation to resting control was observed for Cacna1 mRNA content (19% after SHO in EDL; 21 and 24% after SHO and PRO in SOL). A significant decrease ($p<0.05$) was seen also for Ryl mRNA content (45% after PRO in SOL), SERCa1 (27% after SHO in SOL), CASQ1 (15% after SHO in EDL), and CASQ2 (65% after SHO in EDL, 55% after PRO in SOL). **Conclusion:** These results demonstrate contraction-induced mRNA responses of the main components of Ca^{2+} regulating system in rat skeletal muscle. The decreased mRNA levels of fast isoforms Cacna1, Ryl, SERCa1 and CASQ1 in slow-twitch SOL, and the slow isoform CASQ2 in fast-twitch EDL may contribute to phenotypic adaptations in response to muscle contractions. Furthermore, the results suggest that down-regulation of Ca^{2+} regulating proteins may be a mechanism explaining at least partly the long-lasting fatigue involved in the muscle dysfunction and pain.

PTh09-69

Hydrolysis of extracellular ATP elicits ERK1/2 activation via A2B receptors in differentiated human skeletal muscle cells

May C.[1], Weigl L.[2], Muller C.[3], Karel A.[1], Hohenegger M.[1]
[1]Institute of Pharmacology; [2]Clinic for General Anesthesia and Intensive Care Medicine, Medical University of Vienna, Austria; [3]Pharmaceutical Institute, University Bonn, Germany
martin.hohenegger@meduniwien.ac.at

Aims: In human skeletal muscle, ATP is released at the neuromuscular junction. We could recently show that ATP activates ERK1/2 via P2Y1 receptor under inhibitory conditions of extracellular nucleotide hydrolysis. Here we allowed hydrolysis of ATP to test the hypothesis that adenosine receptor might be activated. **Methods:** In the absence of an ATP regenerating system ATP was applied to differentiated human skeletal muscle cells and the formation of cAMP and the phosphorylation of ERK1/2 was investigated. **Results:** ATP is extensively hydrolysed when applied to differentiated human skeletal muscle cells in the absence of an ATP regenerating system. Basal and forskolin stimulated formation of cAMP was significantly enhanced in the presence of 100 µM ATP. This ATP induced increment in adenylyl cyclase activity was completely abrogated by the universal adenosine receptor antagonist xanthine amine congener (XAC). A cross-activation of the cAMP formation by ATP via P2Y receptors can be excluded because suramin failed to inhibit. Adenosine receptor mediated cAMP production was further corroborated by stimulation with the adenosine analogue 5'-N-ethyl-carboxamidoadenosine (NECA) which again was inhibited by XAC. Using the specific A2B receptor antagonist PSB-1115 and the potent inhibitor ZM241385 allowed for the pharmacological identification of an A2B receptor. This is further confirmed by the observation that already nanomolar concentrations of NECA are sufficient to activate ERK1/2. **Conclusion:** These results demonstrate for the first time that nanomolar concentrations of NECA are sufficient to trigger ERK1/2 phosphorylation via an A2B receptor while adenylyl cyclase was not activated under these conditions.

PTh09-70

The underlying mechanisms of delayed force recovery from fatigue are dependent on reactive oxygen species metabolism in skeletal muscle fibres

Place N., Yamada T., Bruton J., Westerblad H.
Department of Physiology and Pharmacology, Section of Muscle Physiology, Karolinska Institute, Stockholm, Sweden; nicolas.place@ki.se

Aims: The present study was conducted in order to test the hypothesis that differences in reactive oxygen species (ROS) metabolism are responsible for different causes of decreased force at low frequencies (i.e. decreased SR Ca^{2+} release vs. reduced myofibrillar Ca^{2+} sensitivity). **Methods:** Intact, single muscle fibres were dissected from flexor digitorum brevis muscles of rats and mice (wildtype and superoxide dismutase (SOD2) overexpressing). Force and myoplasmic free $[Ca^{2+}]$ ($[Ca^{2+}]_i$) were measured. Fibres were stimulated at frequencies varying from 15 to 100 Hz before and 30 min after fatigue induced by repeated tetani (70 Hz, 350 ms). **Results:** Force was markedly decreased (~60-70%, $P<0.05$) at low stimulation frequencies 30 min after fatiguing stimulation in all fibres. This reduction was associated with reduced tetanic $[Ca^{2+}]_i$ in wildtype mouse fibres (to 64 ± 11% of the original, $P<0.05$), which can not be reversed by application of the reducing agent dithiothreitol or the antioxidant N-acetylcysteine. In contrast, rat fibres and mouse SOD2 overexpressing fibres showed a significant ($P<0.05$) decreased myofibrillar Ca^{2+} sensitivity (assessed by measuring the $[Ca^{2+}]_i$ required to produce 50% of the maximal tetanic force), which can be partially reversed by application of the reducing agent dithiothreitol. **Conclusion:** In conclusion, the origin of the delayed force recovery seems to depend on the ROS metabolism. These findings may have clinical implications since ROS-mediated impairments in myofibrillar function can be counteracted by reductants and antioxidants, whereas changes in SR Ca^{2+} handling appear more robust.

PTh09-71

Purinergic nucleosides and nucleotides modulate migration in developing peripheral neurons

Erriquez J.[1], Bernascone S.[1], Toscani M.[1], Genazzani A.A.[1], Billington R.[1], Ferraro M.[2], Distasi C.[1]
[1]Dipartimento di Scienze Chimiche Alimentari Farmaceutiche e Farmacologiche, Universita del Piemonte Orientale "A. Avogadro", Novara, Italy; [2]Dipartimento di Fisica Sperimentale, Universita di Torino, Torino, Italy

Aims: Neuronal migration is a process in which neuron – glial cell interactions play a major role. In previous studies we described the ability of embryonic peripheral neurons to migrate in association with glial cells to form cellular aggregates. These results point to a key role of glia in the formation of neural cell networks. The purpose of this study was to characterize the effects of extracellular application of purinergic nucleosides and nucleotides on calcium signaling and their role in the regulation of the cell – complex migration. **Methods:** We used 1) Fura – 2 calcium to quantify calcium signals induced by extracellular application of 1 µM ATP and NAADP and 10 µM adenosine in dissociated E7 chick ciliary ganglion cells; 2) time – lapse experiments and quantitative analysis of cell trajectories to compare the velocity of migration in the above different conditions. **Results:** Adenosine, ATP and NAADP, when given extracellularly, directly activated a calcium response in both neurons and glia with different and independent mechanisms. Adenosine and NAADP strongly decreased the modulus of the velocity of the neuron – glial cell complex whereas no differences were observed in the presence of ATP. (CTR: v= 1.48 ± 0.06 µm/min, n= 24; ATP: v= 1.47 ± 0.06 µm/min, n= 8; NAADP: v= 1.02 ± 0.04 µm/min, n= 10; Ado v= 1.9 ± 0.04 µm/min, n= 20). **Conclusions:** These results provide evidence for a role of nucleoside and nucleotide signaling in the regulation of cellular migration in developing peripheral ganglion cells. Whether NAADP acts on intracellular Ca^{2+} – channels or on plasma membrane receptors remains to be established.

PTh09-72

Evidence of concurrent stimulation of PKA dependent and Epac1-Rap2-PLC-Ca^{2+} signaling in forskolin stimulated Cl secretion in T84 cells

Kazi M.H., van Rossum D.B., Zachos N.C., Tse C.M.
Division of Gastroenterology, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD-21205, USA.

Background & Aims: The exchange factor directly activated by cAMP (Epac) is a newly discovered direct target for cAMP and cAMP has been shown to stimulate intestinal Cl^- secretion. Thus, we studied the role of Epac in FSK stimulated Cl^- secretion in T84 cells. **Methods:** T84 cell monolayers grown on transwell insert were studied in Ussing chamber under voltage clamp condition for the measurement of agonist stimulated anion secretion. The Ca^{+2} sensitive fluorescence dye, Fura-2 was used for $[Ca^{2+}]_i$ measurement. Activation of Rap2 was determined by EZ-Dectect™ Rap activation kit (Pierce). **Results:** FSK stimulated Cl^- secretion was inhibited by PKC inhibitor, 5µM GO6976 and by the PKA inhibitor H-89 (1µM). Complete inhibition of Cl^- secretion was obtained by GO6976 plus H-89, suggesting that FSK stimulated Cl^- secretion was both PKA dependent and independent. FSK elevated $[Ca^{2+}]_i$ and FSK stimulated Cl^- secretion was inhibited by BAPTA/AM, an intracellular calcium chelator, and by the PLC inhibitor U73122, confirming the role of Ca^{2+} in FSK stimulated Cl^- secretion. By western blot and RT-PCR, Epac1 was found expressed in T84 cells. The Epac agonist, 8pCPT-2'-O-Me-cAMP stimulated Cl^- secretion, and this was inhibited by BAPTA/AM but not by H-89. It has been shown that Rap2 activates PLCε/Ca^{2+} signaling. FSK and 8pCPT-2'-O-Me-cAMP activated Rap2 and this suggests that Epac1 and Rap2 linked cAMP into PLC/Ca^{2+} signaling in this secretory process. **Conclusion:** We concluded that cAMP mediated epithelial Cl^- secretion was caused by the sum of PKA dependent and independent events, the latter was mediated through Epac1-Rap2-PLC-Ca^{2+} signaling.

PTh09-73

Glibenclamide reduces phosphatidylserine exposure on neutrophils induced by *ex-vivo* incubation of whole human heparinised blood

Schmid D., Spieckermann P.G., Moeslinger T.
Centre for Physiology and Pathophysiology, Medical University of Vienna, Austria
diethart.schmid@meduniwien.ac.at

Aims: Neutrophils were reported to take part in promoting the activation of the coagulation cascade by uptake of tissue-factor (TF) bearing microvesicles. Exposition of phosphatidylserine (PSE) is an essential cofactor of TF, which had been reported to be reduced in human monocytes by the KATP-channel blocker glibenclamide (GLIB). We tested the hypothesis, that GLIB might reduce PSE in neutrophils, too. **Methods:** Tightly closed vacuum tubes filled with whole heparinised venous blood of male healthy volunteers were incubated at 37°C in a shaking water bath for 0, 2, or 8 hours in absence and presence of 30μM GLIB. PSE was quantified using flow cytometry by staining with annexinV-FITC and counterstaining with CD14-PerCP in order to exclude monocytes. Neutrophils were gated on basis of forward and side scatter and mean annexin-V-FITC fluorescence was evaluated. **Results:** PSE of neutrophils significantly increased within 2 hours and to about 6 times after 8 hours of ex-vivo incubation. GLIB strongly reduced PSE both after 2 and 8 hours. **Conclusion:** PSE on neutrophils arose during ex-vivo incubation of whole blood in a time dependent way. This process, which accompanies neutrophils apoptosis, necrosis and activation of coagulation could be dramatically retarded by glibenclamide. This finding might suggest a role for KATP-channels in neutrophils in mediating "blood-borne" thrombosis as it is part of the mechanisms of stasis-induced coagulation.

PTh09-74

Regulation of hALC-1 promoter activity by vasopressin and sex hormones

Schulz L., Haase H., Morano I.
Dep. Molecular Muscle Physiology, Max-Delbruck-Center for Molecular Medicine, Berlin, Germany

The promoter of the human atrial light chain (*h*ALC-1) contains two transcriptional start sites, a proximal and a distal. To analyse their functional importance, we generated stably transfected H9c2 cardiomyoblasts either with the full length promoter or a deleted promoter. We demonstrated that the deleted promoter and the full length promoter have equal basal activities. However, both transcriptional start sites are required for vasopressin (5μM) stimulation ($p<0.001$). The treatment with estrogen (5μM) and testosterone (5μM) alone did not influence the *h*ALC-1 promoter activity. However estrogen (5μM) ($p<0.01$) and testosterone (5μM) ($p<0.01$) significantly upregulates the vasopressin stimulated *h*ALC-1 promoter activity. Thus the full length promoter is required for hypertrophic activation of the *h*ALC-1 gene.

PTh09-75

Neuroprotective influence of simvastatin to UPR reaction after global forebrain ischaemia/reperfusion

Urban P., Pavlikova M., Sivonova M., Dobrota D., Lehotsky J.
Department of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic; urban@jfmed.uniba.sk

Aims: The purpose of study is to evaluate the effect of simvastatin on ischemic brain injury. A variety of endoplasmic reticulum (ER) stresses trigger the unfolded protein response (UPR), a compensatory response whose most proximal sensors are the ER membrane bound proteins IRE1 and ATF6. **Methods:** We have simultaneously examined the activation of ATF6, IRE1, Grp78 and Xbp-1 at mRNA and protein levels after 15 minutes 4-VO ischemia and different times of reperfusion (1, 3 and 24h). We used Western blot with immunodetection to determine protein levels and RT-PCR to determine mRNA levels. **Results:** The results showed that simvastatin increased ATF6 mRNA levels and caused significant difference at I stage between treated and untreated animals. In simvastatin treated animals it follows normal ischemic behaviour of mRNA levels; however the response at R24 is not so sharp. Our results for Grp78 detect the highest mRNA level in 3h of reperfusion in cortex. In addition to this we observed increased mRNA level after simvastatin treatment. Results also showed that statins kept increasing level of mRNA between R3 and R24 in treated animals. Simvastatin lightly decreased mRNA level for Xbp1 during ischemia and 1h reperfusion. Protective effect is visible mainly in 24h of reperfusion. mRNA level of untreated animals showed no significant shift between 1 and 24h of reperfusion. **Conclusion:** These data indicates that statins, in addition to their cholesterol lowering effect, might exert a neuroprotective role in the attenuation of ER stress response after acute stroke/reperfusion.

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PTh10-76

Changes in melatonin concentration and melatonin receptor density in aorta of L-NAME hypertensive rats

Benova M., Stebelova K., Paulis L., Simko F., Zeman M.
Faculty of Natural Sciences, Department of Animal Physiology and Ethology, School of Medicine, Department of Pathophysiology, Comenius University in Bratislava, Slovakia; mbenova@fns.uniba.sk

Aims: Plasma melatonin concentrations are changed in hypertensive patients, but the role of melatonin in hypertension development is not understood. The purpose of our study was to test the hypothesis that melatonin and melatonin receptor levels are changed in rats during L-NAME (NG-nitro-L-arginine methyl ester) induced hypertension. L-NAME blocks nitric oxide synthase activity and contributes to hypertension development. **Methods:** Wistar rats (36) were treated with L-NAME (40 mg/kg) and another group (36) served as a control. Blood pressure was measured weekly. After 4 weeks, animals were sacrified over a 24h cycle in 4h intervals and plasma melatonin concentrations were measured using radioimmunoanalysis and the MT1 receptor density in aorta was quantified using western blot analysis. **Results:** The L-NAME treatment induced the expected increase in blood pressure (178±1 vs. control 118±1 mmHg). We found a clear cut daily rhythm of melatonin levels in pineal gland and plasma in both groups. Pineal melatonin concentrations in L-NAME treated rats were higher than in controls (3377±478 vs. 1752±329 pg/pineal gland) in the middle of the dark period. No differences between both groups were found in plasma melatonin levels. Melatonin receptors did not exhibit clear daily rhythm neither in control nor L-NAME treated rats. **Conclusion:** Results suggest that L-NAME treatment stimulates melatonin production through blocking of inhibitory effect of NO on adrenergic stimulation of melatonin biosynthesis. Increased utilization of melatonin in hypertensive rats may explain the absence of differences in plasma hormone concentration.

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PTh10-77

Connexin-43 in aorta of aged SHR after PUFA treatment

Dlugosova K.[1], Fialova M.[1], Sotnikova R.[2], Tribulova N.[1], Okruhlicova L.[1]
[1]Institute for Heart Research, [2]Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia; usrdkadl@savba.sk

Aims: Hypertension is a cardiovascular disease associated with an increase in cardiovascular complications characterized by endothelial dysfunction and structural remodeling of vessel wall. Intercellular gap junction connexin-43 (Cx43) channels ensure direct communication between adjacent cells of arteries. Polyunsaturated fatty acids (PUFA) supplementation enhances protection against cardiovascular events. Therefore the aim of this study was to examine the effect of PUFA on ultrastructure of endothelial intercellular junctions and expression of connexin43 (Cx43) gap junction protein in aorta of spontaneously hypertensive rats (SHR). **Methods:** 1-year-old male SHR and nonhypertensive Lewis (LEW) rats were divided into four groups: 1) SHR rats untreated, 2) SHR treated with PUFA (30mg/day) for 2 months, 3) LEW untreated and 4) LEW treated with PUFA. Thoracic aorta of SHR and LEW were processed for: transmission electron microscopy, immunofluorescence, Western blot analysis of Cx43. Physiological functions of aorta were also determined. **Results:** Electron microscopy revealed heterogeneous focal injury of structural integrity of aortic endothelial monolayer and intercellular junctions in aortas of untreated SHR while not in control LEW. It was demonstrated that there was reduced acetylcholine-induced relaxation of aorta in untreated SHR comparing with untreated LEW group. There were no differences in expression of Cx43 phosphorylated and dephosphorylated isoforms between untreated and treated SHR as well as untreated and treated LEW. **Conclusions:** The results indicate that PUFA supplementation did not significantly affect neither expression of Cx43 in aorta nor function of aorta in both SHR and LEW.

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PTh10-78

Homocysteine alters the profile of mechanisms involved in endothelium-dependent relaxation of rat small mesenteric arteries

Hurjui L.[1], Serban I.L.[1,2*], Serban D.N.[1,2], Branisteanu D.D.[3], Mungiu O.C.[2,4**]
[1]Dept. of Physiology; [2]Center for the Study and Therapy of Pain**; [3]Endocrinology Clinic; [4]Dept. of Pharmacology, Univ. of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania; dnserban@yahoo.com

Aims: Since endothelial dysfunction induced by increased blood plasma levels of homocysteine is not well understood, we started an investigation of homocysteine effects upon isolated resistance arteries. **Methods:** We used the wire myograph technique and small mesenteric arteries from male adult Wistar rats. Submaximal reference contractions were induced by 0.01 mM phenylephrine; results expressed as % active tension of the reference value (mean±SEM; n=6). **Results:** Homocysteine 0.05mM induces a slow and minor contraction of resting vessels (<10%), only in the presence of endothelium. This effect is completely blocked in arteries where tone is increased by inhibition of basal nitric oxide (NO) release (0.01 mM L-NAME), which confirms the ability of homocysteine to inhibit nitric oxide synthase. However, incubation for 2 hours with 0.05 mM homocysteine does not alter the contraction induced by phenylephrine (in presence/absence of endothelium) or endothelium-dependent relaxation (EDR) induced by 0.01mM carbachol. The EDHF response (EDR in presence of 0.01mM L-NAME and 0.01mM indomethacin) is augmented by 0.05mM homocysteine ($p<0.01$). This may reflect a physiological compensation of NO decrease by EDHF, or a novel ability of homocysteine to potentiate the EDHF component. This is difficult to reconcile with the inhibition by homocysteine of calcium-dependent potassium channels (BKCa, involved in EDHF effect) in smooth muscle from rat mesenteric arteries. **Conclusion:** Global EDR in resistance arteries is not impaired by short-time excessive homocysteine, since reduction in NO release is accompanied by enhancement of the EDHF component.

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PTh10-79

Evidences for the presence of c-kit positive interstitial cells in the wall of mouse mesenteric artery

Povstyan O.V.[1,2], Tsupikov O.M.[2], Gordienko D.V.[1,2], Prestwich S.A.[1], Bolton T.B.[1]
[1]St. George's University of London, United Kingdom; [2]Bogomoletz Institute of Physiology, Kyiv, Ukraine

Aim: The morphology of mouse mesenteric artery (MMA) and the characteristics of interstitial cells (ICs) discovered in the wall of this blood vessel were studied. **Methods:** Experiments were performed on segments of MMA after removal of fat and connective tissue and on freshly isolated single cells. MMA segments, after staining with DAPI and/or *anti-kit* antibodies, and single cells, after loading with the fluorescent Ca^{2+}-sensitive indicator fluo-4AM, were examined using a laser scanning confocal microscopy. Gene expression was detected by RT-PCR technique. **Results:** RT-PCR analysis of MMA tissue showed apart from the housekeeping gene β-actin, expression of mRNA for *c-kit*, the marker of ICs of Cajal. Z-scans of MMA segments fixed and stained with DAPI revealed at least three different cell types in the vessel wall. Besides an internal monolayer of endothelial cells, and circular smooth muscle cells in the media, some cells staining with *c-kit* antibodies were found in adventitia. This was confirmed by staining of living pressurized MMA segments. Enzymatic dispersion of MMA revealed a population (5-7 %) of ICs among numerous smooth muscle cells (SMCs). ICs had brighter fluorescence than SMCs, but no local or global calcium transients were seen, which are typical for SMCs at room temperature. Also ICs, in contrast to SMCs, did not contract in response to activation of receptors, depolarisation or mechanical disruption. However they responded with an increase in $[Ca^{2+}]_i$. **Conclusion:** Our results demonstrate the presense of novel ICs in the adventitia of MMA; their role is as yet unknown.

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PTh10-80

Mechanisms of vasoconstriction induced by angiotensin II and serotonin in human umbilical arteries

Slatineanu S.[1], Hogas M.M.[2], Serban I.L.[2,3], Varlam H.[4], Serban D.N.[2,3]
[1]Obstetrics-Gynecology Clinic IV; [2]Department of Physiology; [3]Center for the Study and Therapy of Pain*; [4]Department of Anatomy, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
dnserban@yahoo.com

Aims: We finalized our previous study regarding the involvement of L-type calcium channels (Ca_L) in the constriction of human umbilical vessels, with reference to the umbilical artery and the action of angiotensin II and serotonin, also investigating the participation of store-operated calcium channels (capacitative calcium influx). **Methods:** The 2 mm wide arterial rings were mechanically de-endothelised and equilibrated for 2 h under a resting tension of 2 g. The contraction force was normalised (%) to the contraction induced in the same ring by K^+ 80 mM (mean ± SEM; n=4). **Results:** We constructed dose-contraction curves for angiotensin II and serotonin in rings precontracted by 80 mM K^+ (complete activation of the contractile mechanism based on depolarization and CaL). Separately we obtained dose-relaxation curves for the Ca_L blocker D600, in rings precontracted by serotonin 0.01 mM and angiotensin II 0.001 mM. The residual contraction after D600 10^{-4} M was in both cases completely relaxed by blocking the calcium influx via store-operated channels ($NiCl_2$ 1 mM or 2-aminoetoxi-diphenylborane 0.1 mM). **Conclusion:** This is the first study devoted to the pathways that ensure calcium influx for constriction of human umbilical arteries. The physiological relevance is discussed in comparison with the few data available in animal umbilical arteries and with reference to some classical aspects observed in other arterial preparations.

**CNCSIS grant interdisciplinary platform /68*

PTh10-81

Vasomotor effects of Ach on hepatic venous vessels and mechanisms of its realization

Yanchuk P., Komarenko V., Pasichichenko O., Prikhodko T., Terekhov A., Tsybeko V.
National Taras Shevchenko University, Kyiv, Ukraine; Lusha2003@ukr.net

Aims: To investigate the influence of Ach on hepatic blood circulation and to find out mechanisms of its action on venous vessels of the rat liver. **Methods:** In acute experiments on anesthetized rats (by uretan – 1g/kg) the blood pressure in carotid artery (AP) and in portal vein (PV), local blood flow (LBF) in liver and changes in liver's blood volume (LBV) were registrated on intraportal administration of Ach. The thensometry was used for registration of constriactile activity of isolated and perfused by warmed Tyrode solution rat portal vein. **Results:** Ach (1 mkg/kg) caused decrease in AP on 35 %, LBV on 24 % and LBF on 22 % ($p<0.05$), and increase in Ppv on 38, that testifies to constricting intrahepatic venous vessels, hepatic veins and, probably, sinusoids. Reactions AP and LBV were removed to initial level by M-chlinolitic atropin (1mg/kg), and changes Ppv were blocked on 60-78 % by α-adrenoreceptors antagonist phentholamine (2 mg/kg). Atropin ($1\cdot10^{-8}$ mol/l) completely blocked Ach-induced constriction of isolated PV, nicotinic blocker tubocurarin ($1.5\cdot10^{-4}$ mol/l) – on 50 %, and phentolamine ($1\cdot10^{-4}$ mol/l) – on 40 % ($p<0.05$). At the same time Ach ($1\cdot10^{-5}$ mol/l) caused relaxation of hepatic vein (HV) on 1.4 ± 0.5 мН ($p<0.05$), wich were atropin- and tubocurarin-resistant, but sensitive to β_1-adrenolytics athenolol. **Conclusion:** Constrictor action Ach on PV can be realized through M-, N-cholinoreceptors and α-adrenoreceptors vessel wall. Adrenergic way, but without involving cholinoreceptors are realised vasoconstrictor influence of Ach, probably, on pre- and postsinusoidal small veins too, and vasodilatator effect of Ach on HV.

PTh10-82

The increased nitric oxide production contributes to beneficial effect of indapamide in low-dose combination therapy in spontaneous hypertension

Kojsova S.[1], Jendekova L.[1], Sladkova M.[1], Paulis L.[2], Pechanova O.[1]
[1]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences; [2]Medical Faculty, Comenius University, Bratislava, Slovak Republic; stanislava.kojsova@savba.sk

Aims: We compared the effects of different antihypertensive agents, thiazide-like diuretic (indapamide) and angiotensin-converting enzyme inhibitor (captopril) on the development of spontaneous hypertension. The combined effect of these agents was analyzed particularly. **Methods:** Six-week-old male SHR were treated with indapamide (1 mg/kg/day) or captopril (10 mg/kg/day) or with indapamide+captopril combination. After 6-week-treatment, nitric oxide synthase (NOS) activity, endothelial (eNOS) and neuronal (nNOS) protein expressions, and concentration of conjugated dienes (CD), a marker of oxidative damage, were determined in the left ventricle, aorta and kidney. **Results:** Indapamide (I), captopril (C) and I+C treatment significantly decreased blood pressure in young SHR. Indapamide, in contrast to captopril, increased NOS activity, as well as eNOS expression in the aorta and attenuated CD concentration in the kidney. I+C combined treatment increased NOS activity as well as eNOS expression and decreased CD concentration comparably to indapamide alone treated group. Moreover, indapamide, captopril, and I+C treatment increased nNOS expression in the left ventricle. Indapamide significantly increased acetylcholine-induced relaxations of the femoral artery. Captopril failed to affect these relaxations and simultaneous I+C treatment increased the relaxations similarly as indapamide alone. **Conclusion:** Indapamide treatment along with captopril had the additive effect on the prevention of blood pressure increase. On the other hand, this combination increased NOS activity, as well as eNOS expression in the aorta, similarly as indapamide alone. Our results suggested that indapamide is responsible for NOS activity and eNOS expression increase after the combined treatment. This effect of indapamide may contribute to its vasorelaxant and antihypertensive properties.

PTh10-83

Apocynin prevents reactive oxygen species production and improves nitric oxide availability in the experimental hypertension

Jendekova L., Kojsova S., Pechanova O.
Institute of Normal and Pathological Physiology SAS, Bratislava, Slovak Republic; lydia.jendekova@savba.sk

Aim: The purpose of this study was to investigate the preventive effect of NADPH oxidase inhibitor – apocynin (4-hydroxy-3-methoxyacetophenone) on the development of systolic blood pressure (SBP) in borderline hypertensive rats (BHR) and spontaneously hypertensive rats (SHR). **Methods:** Young 6-week-old male BHR (offspring of SHR dams and Wistar Kyoto sires) and SHR were treated with apocynin (30 mg/kg/day) for six weeks. SBP was measured by tail-cuff plethysmography. Nitric oxide synthase (NOS) activity was determined in the left ventricle and aorta. Protein expression of nuclear factor kappaB (NF-κB) and NAD(P)H oxidase subunits p67phox and p22phox as well as concentration of cGMP were determined in the left ventricle. **Results:** Apocynin significantly decreased SBP in all groups investigated. Administration of apocynin had no effect on NOS activity in either tissue studied. However, apocynin lowered protein expression of the NF-κB in all groups, decreased protein expression of NAD(P)H oxidase subunit p22phox in both hypertensive groups and attenuated protein expression of the NAD(P)H oxidase subunit p67phox in SHR. Moreover, apocynin was able to prevent lowering of concentration of cGMP in the left ventricle of both hypertensive groups. **Conclusion:** Our study demonstrated that apocynin treatment partially prevented SBP rise in borderline and spontaneously hypertensive rats without increasing activity of NOS in the left ventricle and aorta. However, apocynin was able to decrease production of reactive oxygen species in hypertensive rats and thus to prevent cGMP decrease.

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PTh10-84

Relation of blood pressure and L-NAME-sensitive component of vasorelaxation in rats

Puzserova A., Bernatova I.
Institute of Normal and Pathological Physiology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovak Republic; Angelika.Puzserova@savba.sk

Aims: The question whether endothelial dysfunction is a consequence or a cause of hypertension remains still opened. The aim of this study was to investigate nitric oxide (NO) production and L-NAME-sensitive component of endothelium-dependent vasorelaxation in adult normotensive Wistar-Kyoto (WKY), borderline hypertensive (BHR) and spontaneously hypertensive rats (SHR). **Methods:** Blood pressure (BP) was determined using tail-cuff plethysmography. Nitric oxide synthase (NOS) activity was determined by conversion of [^{3}H]-L-arginine in the aorta. L-NAME-sensitive component of vasorelaxation was investigated in the preconstricted femoral arteries using the wire myograph during isometric conditions as a difference between acetylcholine (ACh)-induced vasorelaxation before and after acute NOS inhibitor N^G-nitro-L-arginine methyl ester pre-treatment (L-NAME, 10^{-5} mol/l). **Results:** BP of WKY, BHR and SHR was 111±3, 140±4 and 184±6 mmHg, respectively. NOS activity was significantly higher in the aorta of BHR and SHR vs. WKY. ACh-induced vasorelaxation of SHR was significantly greater than that in WKY. There was a significant positive correlation between BP and L-NAME-sensitive component of vasorelaxation of the femoral artery ($r = 0.614$, $p<0.007$). **Conclusion:** Results suggest the absence of endothelial dysfunction in the femoral artery of adult borderline and spontaneously hypertensive rats and gradual elevation of L-NAME-sensitive component of vasorelaxation with increasing BP. This suggests that reduction of cardiovascular NO production and endothelial dysfunction do not participate in the initiation of hypertension in these experimental models.

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PTh10-85

Acute physiological effects of ecstasy (MDMA) in anaesthetised male rats

Srisawat R., Nontamart N.
Institute of Sciences, Suranaree University of Technology, Nakhon Ratchasima, Thailand
srisawat@sut.ac.th

Aims: Ecstasy (MDMA) has powerful acute physiological effects in humans: hyperthermia, hyponatraemia, hypertension and increase in heart rate. Little is known about what exactly MDMA does to those physiological responses. We, therefore, investigated acute effects of MDMA on arterial blood pressure, heart rate, respiratory rate and body temperature in anaesthetized rats. **Methods:** Invasive arterial blood pressure, heart rate, respiratory rate and body temperature were recorded in pentobarbital-anaesthetized male rats for 2 hours after single i.v. injected with vehicle (n=7), 5 mg/kg MDMA (n=8) and 10 mg/kg MDMA (n=8). **Results:** In comparison between groups, there was no significant difference in diastolic blood pressure and respiratory rate at any stage of measurement. Intragroup comparison (5 mg/kg MDMA and 10 mg/kg MDMA groups) showed that MDMA markedly decreased mean arterial blood pressure (MABP), systolic blood pressure and heart rate ($p<0.05$, compared with baseline). MDMA induced significant and non-dose-dependent decreases in MABP, systolic blood pressure and heart rate over 2 hours of investigation ($p<0.05$, compared with control group). There were significant decreases in body temperature over baseline in both MDMA groups ($p<0.05$). Interestingly, significant increases in body temperature were observed 20 minutes after administration of 5 mg/kg MDMA (34.98 ± 0.19 °C) and 10 mg/kg MDMA (34.88 ± 0.31 °C) compared with control group (34.33 ± 0.12 °C) ($P<0.05$, two way repeated measures ANOVA). **Conclusion:** This study provided the first evidence of transient hyperthermia response and prolonged cardiovascular effects (decreases in MABP, systolic blood pressure and heart rate) following acute MDMA in anaesthetised rats.

PTh10-86

Effect of urotensin II on adernergic activity in arteries from spontaneously hypertensive rats

Zemancikova A.[1,2], Torok J.[1], Zeman M.[2]
[1]Institute of Normal and Pathological Physiology and Centrum of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Aims: Urotensin II (U II) is known as the most potent mammalian vasoconstrictor identified and may play a role in aetiology of essential hypertension. We have investigated the possible role of this peptide in modulating vascular responses to adrenergic stimuli in normotensive and spontaneously hypertensive rats (SHR) and compared them with the action of angiotensin II (ANG II), which has similar physiological functions in blood vessels. **Methods:** Isolated arterial rings (rat main pulmonary and superior mesenteric artery) was set up for isometric tension recording. Adrenergic contractions were elicited by endogenous noradrenaline released from electrically stimulated (4 Hz) perivascular nerves and by exogenous noradrenaline applied to incubation medium. **Results:** The presence of U II in bathing solution caused inhibition of neurogenic contractions in similar extent as of those elicited by exogenous noradrenaline. There was no significant difference in this effect observed in normotensive and spontaneously hypertensive rats. In contrast, ANG II enhanced the contractile responses of arteries to transmural nerve stimulation. It has, however, no influence on contractions evoked by exogenous noradrenaline. Potentiation of neurogenic contractions by ANG II was less extensive in vessels from SHR compared with those from normotensive Wistar rats. **Conclusion:** These results suggested that modulation of neurogenic contractions in main pulmonary and mesenteric artery by U II and ANG II is mediated by different mechanisms.

PTh11-87

Nutritional modulation of ^{3}H-deoxy-glucose uptake by placental BeWo cells

Araujo J.R., Goncalves P., Azevedo I., Martel F.
Dept. Biochemistry, Faculty of Medicine, University of Porto, Portugal
fmartel@med.up.pt

Aim: The aim of this work was to test the acute and chronic effects of some dietary compounds upon the uptake of glucose by the human placenta. For this, we measured uptake of ^{3}H-2-deoxy-D-glucose (^{3}H-DG) by the human trophoblast (BeWo) cell line. **Methods:** The acute (26 min) and chronic (48 h) effect of several phenolic compounds and methylxanthines present in different drinks upon the uptake of ^{3}H-DG (1 μM) by BeWo cells was determined. Cellular uptake of ^{3}H-DG was measured by liquid scintillometry. The effect of these compounds upon cellular viability was assessed by the MTT assay. **Results:** Acutely, resveratrol (100 μM), quercetine (10-100 μM), epigallocatechin-3-gallate (100 μM), chrysin (10-100 μM), xanthohumol (100 μM) and acetaldehyde (30-100 mM) decreased ^{3}H-DG uptake, whereas rutin (10-100 μM), catechin (10-100 μM) and epicatechin (100 μM) increased it. Chronically, uptake of ^{3}H-DG was decreased by theophylline (0.1-1 μM) and increased by myricetin and rutin (0.1-1 μM). Acutely, none of the compounds tested, with the exception of crisin, caused a reduction on cellular viability. Chronically, cellular viability was increased in the presence of myricetin and rutin (0.1-1 μM), and decreased by theophylline (0.1-1 μM). **Conclusions:** ^{3}H-DG uptake is differentially modulated by different phenolic compounds and methylxanthines.

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PTh11-88

Effects of diabetes and insulin resistance in pregnant rats on *ex vivo* vascular reaction to magnesium

Arikawe A.P., Ettarh R.R.
Department of Physiology, College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria
arikawepaul2002@yahoo.co.uk

The purpose of this study was to investigate the effects of diabetes and insulin resistance during pregnancy on the *ex vivo* vascular reaction to magnesium. Female Sprague-Dawley rats were made diabetic by intravenous injection of alloxan, or insulin resistant by fructose feeding. The rats were allowed to mate and sacrificed on Day 19 of pregnancy. Aortic rings were isolated and mounted in organ baths for measurement of isometric tension. The rings were contracted with 10(-7) M phenylephrine and cumulative concentration-response curves for magnesium (1-12 mM) were determined in the presence and absence of 10(-4) M Nomega-nitro-L-arginine methyl ester (L-NAME) or 10 (-5) M indomethacin. The relaxation response to magnesium was significantly decreased in pregnant rats compared with non-pregnant rats. Pregnant rats with diabetes or insulin resistance had greater impairment in the relaxation responses to magnesium compared with normal pregnant rats. The effects of diabetes and insulin resistance on magnesium-induced relaxation in pregnant rats were not altered in the presence of L-NAME and indomethacin. The results suggest that diabetes and insulin resistance aggravate the alteration in magnesium-induced vascular relaxation observed in pregnancy, and this may be due in part to impairment to mechanisms other than the nitric oxide-cyclic guanosine monophosphate and cyclooxygenase pathways.

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PTh11-89

Lack of effect of high glucose upon the absorption of ^{3}H-folic acid by Caco-2 cells

Martel F., Goncalves P., Azevedo I.
Dept. Biochemistry, Faculty of Medicine, University of Porto, Portugal
pedrogon@med.up.pt

Aim: To investigate the effect of high glucose exposure on the absorption of folate (FA) by Caco-2 cells. **Results:** Apical high glucose did not affect the apical uptake of ^{3}H-FA. Both different concentrations of glucose (10-45 mM) and different exposure times (10 min – 24 h) were tested. Also, apical high glucose (30 mM) did not affect the intracellular steady-state levels of ^{3}H-FA, and simultaneous apical and basolateral high glucose (30 mM) did not change the apical-to-basolateral apparent permeability (P_{app}) to ^{3}H-FA. Both the apical uptake and the steady-state intracellular levels of ^{3}H-FA were strongly reduced by 5-methyltetrahydrofolate, methotrexate, SITS, DIDS and indomethacin, but were almost not affected by p – aminohippuric acid and fumitremorgin C. Moreover, DIDS and indomethacin significantly reduced (by 50-60%) the apical-to-basolateral P_{app} to ^{3}H-FA, but ^{3}H-FA present in the cells at the end of the experiment was higher in the case of indomethacin. Fumitremorgin C had no effect. The effect of the drugs was not changed or only hardly changed by high glucose. **Conclusions:** Absorption of ^{3}H-FA is not modulated by either apical or basolateral high glucose exposure in Caco-2 cells. Moreover, the apical uptake of ^{3}H-FA by Caco-2 cells seems to involve RFC (but not OAT), and MRP and/or OAT (but not BCRP) may mediate apical efflux of ^{3}H-FA.

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PTh11-90

Elevated leptin and AT1 receptor and reduced PPARγ and GLUT4 in age-induced obesity

Baculikova M., Tybitanclova K., Zorad S.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia
miroslava.baculikova@savba.sk

Aim: Angiotensin II (Ang II), an effector of renin-angiotensin system (RAS), is effective as a vasoconstrictor and also as a proliferative and hypertrophic agent. Others and we have shown that adipose tissue possesses a biologically active local RAS. The locally produced Ang II may be implicated in the regulation of adipocyte growth and differentiation. This study was aimed to assess the components of local RAS and some markers of insulin sensitivity in adipose tissue in rats at different age (9-, 12-, 20- and 26-weeks). **Methods:** Circulated levels of hormones in serum were determined by radioimmunoassay. Gene expression of adipokines and RAS components in adipose tissue were evaluated by RT-PCR. AT1 receptor protein was determined by immunoblot. **Results:** Hypertrophic adipose tissue contains large insulin resistant adipocytes. Our results show that fat tissue enlargement due to maturation of the rats is associated with an increase in AT1 receptor gene expression and protein, and with a decrease of angiotensin-converting enzyme and angiotensinogen gene expression. At the same time, leptin expression increased significantly in adult rats. On the contrary, PPARγ and GLUT4 were significantly reduced whilst adiponectin was not changed in the studied age intervals. **Conclusion:** Our results suggest a complex pattern of RAS involvement in adipose tissue enlargement with probably an inhibitory role of AT1 receptors on adipocyte differentiation leading to tissue hypertrophy and insulin resistance.

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PTh11-91

The effect of obesity on the clinical periodontal status and amount of gingival crevicular fluid

Duran I.[1], Okudan N.[2], Gokbel H.[2], Hakki S.S.[1]
[1]Department of Periodontology, Faculty of Dentistry; [2]Department of Physiology, Meram Faculty of Medicine, Selcuk University, Konya, Turkey
nokudan@hotmail.com

Aim: Obesity may associate with increased risk of periodontal disease. The aim of this study was to evaluate the effects of obesity on periodontal clinical parameters and volume of the gingival crevicular fluid (GCF). **Methods:** Forty-two obese and 12 nonobese women (control group) volunteered in the study. Obese women were included into obese group (30.0<BMI<40.0, n=19) or morbid obese group (BMI≥40, n=23). In all subjects Probing Depth (PD), Clinical Attachment Level (CAL), Gingival Index (GI) and Plaque Index (PI) were recorded and GCF sampling was performed. **Results:** Although mean PI was significantly greater in only morbid obese group when compared to the other groups, no significant difference was observed for other clinical parameters between groups. The amounts of GCF were also greater in only morbid obese group compared to the other groups, but this difference was not statistically significant. **Conclusion:** Data obtained from this study suggests that obesity is not directly a risk factor for periodontal diseases.

PTh11-92

Influence of carbohydrates on the methanogenesis in the cattle rumen

Luchka I., Herasymiv M., Solohub L., Salyha Y.
Institute of Animal Biology UAAS, Lviv, Ukraine
livvan@ukr.net

Aims: The study of methanogenesis in the ruminant rumen and investigations of approaches to its decrease is significant from two points of view. At the first – the methane formation is energy undesirable process; at the second – the emission of methane by cattle increases significantly the greenhouse effect. The aim of our study was to investigate the effect of some carbohydrates and volatile fatty acids on intensity of methanogenesis in the cattle rumen. **Methods:** Investigation was carried out in vitro by incubation of rumen liquid with different carbohydrates in anaerobic condition during 24 hours at 38 °C. After the incubation the amount of methane produced was determined on the gas chromatograph SRI 8610 B. **Results:** The carbohydrates have different effect on the methane productions by microorganisms in the rumen of steers. Among the substrates added to the incubation medium the decreased effect on methanogenesis reveal starch (-30 %; $P < 0.05$) > inulin (-19 %; $P < 0.05$) > glucose (-19 %; $P < 0.05$). On the contrary cellulose (+17 %; $P < 0.05$), acetate and propionate stimulate the methane production. **Conclusion:** With the aim of decrease of methanogenesis in the rumen of cattle it is advisable to maintenance of animals' rations with higher levels of structural and lover levels of nonstructural carbohydrates.

PTh11-93

Alcohol intake alters endocrine activity of rat adipose tissue

Pravdova E., Macho L., Fickova M.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic; ueenelis@savba.sk

Aims: We have studied the effect of short and long-term alcohol intake on serum levels and gene expression in rat adipose tissue of three adipokines – leptin, adiponectin and resistin. **Methods:** Alcohol (A10, A28) and control (C) Wistar rats were fed standard laboratory diet *ad libitum*. Rats had *ad libitum* access to 6% ethanol solution in tap water as the only drinking fluid. Pair-fed (PF) animals had the same caloric intake in the form of pelleted diet as A rats had consumed during the preceding 24 h. Gene expression of adipokines in epididymal adipose tissue was determined by the rtPCR method and serum levels of adipokines by ELISA method. **Results:** Alcohol intake (A10, A28) significantly increased serum concentrations of all three adipokines compared to C. Alcohol consumption (A10, A28) resulted in the decrease of leptin and adiponectin mRNA levels in adipose tissue. Resistin gene expression in adipose tissue was not affected neither by short or long-time alcohol intake. Only increased adiponectin gene expression positively correlated with elevated adiponectin serum levels. **Conclusion:** We demonstrated that both short and long-term alcohol intake altered serum levels of leptin, adiponectin and resistin and also modified adipokines gene expression in epididymal adipose tissue. Increased adipokines serum concentrations could be involved in changed metabolic processes manifested by smaller adipocyte size, increased glycemia, attenuation of insulin effect on glucose transport in isolated adipocytes, decreased liver glycogen content, and modified serum and liver lipid profile.

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PTh11-94

Some biological fitness and biochemical parameters of *Pimpla turionellae* adults reared on hosts exposed to ethyl parathion

Buyukguzel E.[1], Acikgoz S.[2]
[1]Department of Biology, Faculty of Arts and Science; [2]Department of Biochemistry, Faculty of Medicine, University of Zonguldak Karaelmas University, Zonguldak, Turkey; endericen@hotmail.com

Aims: We investigated effects of organophosphorus insecticide, ethyl parathion on survival, development and some biochemical parameters of endoparasitoid *Pimpla turionellae* (L.) reared on host insect, greater wax moth, *Galleria mellonella* (L.) pupae. **Methods:** Newly hatched larvae of *G. mellonella* were orally exposed to 0.01, 0.1, 1.0 or 10 ppm of the insecticide by rearing on an artificial diet. The pupae emerged from these larvae were used as ethyl parathion-contaminated hosts for *P. turionellae*. As bioindicators of long-term physiological stress responses to such hosts, survival and development of the parasitoids were assessed. Furthermore alterations in activities of various enzymes involved in certain metabolic functions such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), acetylcholinesterase (AChE), acid phosphatase (ACP), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), glucose-6-phosphate dehydrogenase (G6PDH), and content of total protein (TP) and lactate (LAC) were determined. **Results:** Ethyl parathion did not significantly affect the survival while all tested concentrations caused significant increase in time required to reach fifth instar. Significant reduction in postlarval survival and increase in developmental time were obtained for high concentrations of the insecticide. Ethyl parathion significantly increased total protein content and activities of AST, ALT and GGT. The activities of ACP, AChE, and G6PDH were decreased by all concentrations of ethyl parathion. However, the insecticide at 0.1 ppm and above resulted in decreased lactate content and LDH activity. **Conclusion:** This study infers that ethyl parathion-induced metabolic dysfunction is a causal factor in deterioration of survival and development of *P. turionellae*. **Key words:** Ethyl parathion, *Pimpla turionellae*, survival, transaminases, glucose-6-phosphate dehydrogenase, total protein.

PTh11-95

Effects of sodium tetraborate on some biological fitness parameters of *Galleria mellonella* L. (Lepidoptera: Pyralidae)

Durmus Y., Buyukguzel K.
University of Karaelmas, Faculty of Science, Department of Biology, Zonguldak, Turkey; buyukguzelk@hotmail.com

Aims: Inorganic insecticides are commonly used in pest management because of their low toxicity to nontargets beneficial insects, mammals and environment. In this paper we test the effects of sodium tetraborate on life parameters of *Galleria mellonella* (L.) for assessing its sublethal toxicity on insects. **Methods:** The effects of sodium tetraborate on survival, development, adult longevity, and fecundity of greater wax moth *Galleria mellonella* L. were investigated by rearing the larvae on artificial diets containing 0.005, 0.1, 0.2 or 0.3 g per 100 g of diets. **Results:** The highest concentration of sodium tetraborate (0.3 g) significantly decreased the survival rate of 7th instars to 17.5 ± 2.9% and prolonged the time required to reach 7th instar by 5 days. The diet containing this concentration produced 12.5% of both pupae and adult yields and also prolonged development by 5 days. When compared to adult longevity (9.0 ± 0.7 days) from control diets, adults reared with 0.2 g of sodium tetraborate survived by about 4.2 days longer (13.2 ± 1.9 days). The highest concentration of sodium tetraborate resulted in decreased adult longevity in comparison to control. Diet containing 0.005 and 0.2 g sodium tetraborate concentrations caused a significant decrease in egg production and hatchability of *G. mellonella* females. Oviposition of survivors in highest sodium tetraborate treatment (0.3%) was completely inhibited. **Conclusion:** From a practical standpoint, sodium tetraborate incorporated into a bait appears to be an ideal replacement for insecticides with high toxicity to environment and nontargets. We infer that survival, development, longevity and fecundity are effective biological fitness parameters for assessing toxicity of this boron compound.

PTh12-96

Analysis of circadian system pathways in TGR (mREN – 2) 27 rats

Herichova I.[1], Monosikova J.[1], Mravec B.[2], Zeman M.[1]
[1]Department of Animal Physiology and Ethology, Comenius University, Bratislava, Slovak Republic; [2]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic
herichova@fns.uniba.sk

Aims: Circadian system consisting from central and peripheral oscillators employs several pathways to achieve optimal phase relationships between biological rhythms inside the body. TGR (mREN – 2)27 rats exerting normal rhythm of the heart rate and locomotor activity are characterized by gradual development of inverted blood pressure (BP) profile. Our study was aimed on possible involvement of melatonin and/or angiotensin II in this process. **Methods:** TGR and control rats were synchronized to the light:dark cycle 12:12. Angiotensin II receptors AT1 mRNA expression was measured in the SCN, nucleus of the solitary tract (NTS) and area postrema. Melatonin was administered in a rhythmic manner and effect of its amplified rhythm on clock gene expression in the suprachiasmatic nucleus (SCN) and paraventricular nucleus (PVN) was investigated. Brain nuclei were isolated by punching technique and gene expression was measured by real time PCR. **Results:** Melatonin administration caused increase in per2 expression in SCN at the end of the night in TGR as well as control rats. In PVN effect of melatonin on per2 expression was detectable only in control rats. Expression of AT1 mRNA was increased in the area postrema and NTS in TGR rats in comparison with control. **Conclusion:** Effect of melatonin on clock gene expression seems to be tissue specific. This phenomenon may reflect melatonin receptor distribution. Increased expression of AT1 receptors in the brainstem indicates involvement of angiotensin II in generation of inverted BP in TGR rats that have up regulated rennin – angiotensin system.

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PTh12-97

Lack of correlation between intracellular calcium and *in vitro* proliferation of splenocytes from siberian hamsters (*Phodopus sungorus*)

Pawlak J., Markowska M., Skwarlo-Sonta K.
Department of Animal Physiology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Aim: The aim of this study was to investigate the effect of photoperiod and melatonin (Mel) in vitro on Siberian hamster (*Phodopus sungorus*) splenocyte proliferation and intracellular calcium concentration ($[Ca^{2+}]_i$). **Methods:** Spleens were isolated at midday and midnight from Siberian hamster adult males kept in long (LD 16:8) or short (SD 8:16) photoperiods. Erythrocyte-free spleen leukocytes were incubated without or in presence of Mel and/or a mitogen phytohaemagglutinin (PHA). Freshly-isolated splenocytes were used for $[Ca^{2+}]_i$ spectrofluorometric measurement using fluorescent dye Fura-2AM. Additionally, cells were cultured in vitro (for 72 h) and proliferation was assessed by incorporation of [^{3}H]-thymidine. **Results:** In splenocytes obtained from hamsters housed in both photoperiods Mel increased $[Ca^{2+}]_i$ but only when used in pharmacological concentrations. Additionally, it enhanced a stimulatory effect of PHA on splenocyte $[Ca^{2+}]_i$. Although Mel decreased PHA-induced in vitro proliferation in cultured splenocytes isolated from hamsters kept in both photoperiods, this effect was time of day-dependent, i.e. melatonin-sensitive were either "diurnal" or "nocturnal" splenocytes from LD or SD hamsters, respectively. Moreover, Mel inhibited spontaneous proliferation of nocturnal SD-splenocytes. **Conclusions:** The first time measured effect of exogenous Mel on hamster spleenocyte $[Ca^{2+}]_i$ suggests lack of correlation with that on their proliferation in vitro, but seems to be photoperiod (thus endogenous Mel)-dependent phenomenon. It suggests a possible involvement of differences in Mel receptor expression, associated with time of day and photoperiod. Subsequent investigations to elucidate these observations are in progress.

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PTh12-98

Expression of clock gene hPer2 in patients with colorectal carcinoma

Vician M.[1], Herichova I.[2], Reis R.[1], Monosikova J.[2], Zeman M.[2]
[1]First Surgery Department, Medical Faculty and Hospital, Comenius University, Bratislava, Slovak Republic; [2]Department of Animal Physiology and Ethology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic

Aims: Circadian system is involved in control of cell proliferation and apoptosis. Mice with *Per1* and *Per2* deletion showed a marked increase in tumour development and reduced apoptosis. The aim of our study was to measure expression of human *Per2* in patients who underwent surgery for colorectal carcinoma. **Methods:** Our study included 24 patients of both sexes with progressing colon cancer. All patients were exposed to a standard hospital practice and exposed to light from 6:00 until 21:00 daily. The protocol was explained to each patient and informal consent was obtained. Tissue samples were taken at the time of surgery. They were collected from the tumour as well as from the proximal (at least 10 cm) and distal (at least 2 cm from the tumour) parts of the surgery. Surgery was performed during morning hours. Samples were put into liquid N_2 and kept at -80°C until RNA extraction. Expression of *Per2* mRNA was performed by real time PCR. Ribosomal S17 gene was used as a reference gene. **Results:** Expression of *Per2* was substantially lower in tumours classified as G1 in comparison with G2. Moreover, there was a significant negative correlation between *Per2* expression and tumour staging Expression of *Per2* did not correlate with localization of the tumour in the proximal, transversal or distal colon. **Conclusion:** Data demonstrated significant *hPer2* deregulation in tumour tissue in comparison with ectomised tissue without malignancy in patients with colorectal carcinoma and suggest one way how the circadian system can influence tumourigenesis.

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PTh12-99

Turkey retina and pineal gland differentially respond to prolonged exposure to constant darkness and light: studies on serotonin N-acetyltransferase activity

Zawilska J., Lorenc A., Berezinska M.
Departments of Pharmacodynamics and Pharmacology, Medical University of Lodz, Centre for Medical Biology, Polish Academy of Sciences, Lodz, Poland; jzawilska@pharm.am.lodz.pl

Aims: Vertebrate pineal gland and retina synthesize melatonin in light-dependent rhythmic fashion controlled by endogenous circadian clock. This work was aimed at studying effects of prolonged exposure of turkeys to constant darkness (DD) and continuous light (LL) on serotonin N-acetyltransferase (AANAT; a key regulatory enzyme in melatonin biosynthesis) activity in the pineal gland and retina. **Methods:** Newly hatched turkeys after adaptation to 12 h light: 12 h dark (LD) cycle were kept under DD for 14 days or LL for 7 days. The animals were killed at 4-h intervals, isolated pineal glands and retinas were used for measurements of AANAT activity. **Results:** AANAT activity in the turkey pineal gland oscillated in circadian rhythm for 7 days of DD (tau = 23.8 ± 0.02 h) or LL (tau = 24.3 ± 0.05 h). Under DD AANAT activity in the retina also exhibited circadian rhythm (tau = 23.7 ± 0.05 h), but its amplitude progressively dampened, and no rhythmicity was observed after five days of DD. Low amplitude rhythmic changes in retinal AANAT activity disappeared on the third day of LL. **Conclusions:** AANAT activity in the turkey pineal gland and retina is regulated by endogenous circadian pacemaker and light. The melatonin-generating system in the turkey retina is more sensitive to changes in the environmental light than is the system of pineal. In contrast to mammals, in pineal gland of galliforms the circadian melatonin rhythm persists under conditions of LL.

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PTh12-100

Expression of arginine vasopressin and vasoactive intestinal peptide in hypothalamic nuclei of TGR[mREN2]27 hypertensive and control rats

Kovacikova Z.[1], Kiss A.[2], Jezova D.[2], Zeman M.[1]
[1]Dept. Animal Physiol. Ethol., Fac. Nat. Sci., Comenius University; [2]Inst. Exp. Endocr., Slovak Acad. Sci., Bratislava, Slovakia, kovacikovaz@fns.uniba.sk

Aims: To elucidate expression of arginine vasopressin (*Avp*) and vasoactive intestinal peptide (*Vip*) gene in the suprachiasmatic nucleus (SCN) and in the paraventricular nucleus (PVN) of the hypothalamus in TGR[mREN2]27 hypertensive (TGR) and control Sprague-Dawley (SD) normotensive rats. TGR rats have an additional mouse renin gene that results in higher activity of the renin-angiotensin system (RAS), subsequent systemic hypertension and an inverted daily blood pressure profile. **Methods:** Rats were kept under 12h light: 12h dark regimen. Animals were decapitated in 4h intervals during 24-hour period. Brains were extirpated and frozen on dry ice. Expression of *Avp* and *Vip* was assessed by *in situ* hybridization on 14 μm thick coronal sections of the hypothalamus with ^{35}S radiolabelled oligoprobes. Results were expressed as relative optical density and data obtained in single time points were statistically analyzed by Student t-test between TGR and SD groups. **Results:** Expression of *Avp* gene in the SCN was rhythmic both in SD and TGR rats with peak during the light phase. Up regulated *Avp* expression in TGR as compared to control animals was found at the beginning and at the end of the light phase. No significant differences were found in *Vip* expression in the SCN between TGR and SD rats. **Conclusion:** Up regulated RAS in TGR animals resulted in increased *Avp* expression at the beginning and at the end of the light phase in the SCN compared to control animals while expression of *Vip* was not altered. *Supported by grants APVV 20-022-704 and UK/209/200.*

PTh12-101

Development of light sensitivity of clock genes *Period1*, *Period2* and immediate early gene *c-Fos* within suprachiasmatic nucleus of the rat

Mateju K., Sladek M., El-Hennamy R., Sosniyenko S., Bendova Z., Illnerova H., Sumova A.
Department of Neurohumoral Regulations, Institute of Physiology of Czech Academy of Science, v. v. i., Praha, Czech Republic
laurinova@centrum.cz

Aims: Light entrains circadian rhythms to the 24-h period of solar day through shifting the phase of the endogenous circadian clock in suprachiasmatic nuclei of hypothalamus (SCN). Light sensitivity of the circadian core clock is controlled by the core clock mechanism itself. Aim of our study was to determine when during postnatal development expression of clock genes *Period1* (*Per1*) and *Period2* (*Per2*), i.e. parts of the molecular mechanism underlying circadian rhythmicity, and of *c-Fos*, an immediate early gene, became light sensitive and when clock begins to control the photosensitivity. **Methods:** Adult rats and pups at postnatal day 1 (P1), P3 or P10 maintained at LD 12:12 were released into darkness at circadian time (CT) 0. Animals were exposed to a 30min light pulse during the fist (CT15) or second (CT21) part of subjective night or during the subjective day (CT7) and sampled 30min, 1h and 2h after the start of each pulse together with untreated controls. Levels of *Per1*, *Per2* and *c-Fos* mRNA in the SCN were assessed by in-situ hybridization. **Results:** At P1, photoinduction of *Per1* and *c-Fos* mRNA occurred during the day as well as during the night. At P3, the photoinduction was present during the night and not during the day, hence, clock started to control it. Development of *Per2* mRNA photoinduction seems to be different from that of *Per1* mRNA. **Conclusions:** Development of the gating mechanism of light sensitivity of circadian clock is gradual and non-parallel.

PTh12-102

Different clock gene expression in the area postrema in hypertensive TGR (mREN-27) rats

Monosikova J.[1], Herichova I.[1], Mravec B.[2], Zeman M.[1]
[1]Department of Animal Physiology and Ethology, Comenius University, Bratislava, Slovak Republic; [2]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic
monosikova@fns.uniba.sk

Aims: Hypertensive TGR (mREN-27) rats are characterized by hypertension and inverted blood pressure (BP) profile. The purpose of the study was to test the hypothesis that inverted BP profile correlates with clock genes expression in the brain areas responsible for BP regulation (paraventricular nucleus, area postrema). **Methods:** Hypertensive TGR and normotensive Sprague-Dawley rats were synchronized to the lighting schedule 12L:12D. Brain nuclei were dissected by punching technique. Daily profile of clock genes expression was measured by real time PCR in the hypothalamic nuclei, brainstem and the circumventricular organ area postrema (AP). Plasma renin activity (PRA) was determined by radioimmunoassay. **Results:** PRA was significantly higher in TGR in comparison with control animals. Expression of *per2* was rhythmic in the most of analyzed nuclei and reached the maximum at beginning of the night. Expression of *bmal1* was also expressed in rhythmic manner in the most nuclei and peaked at the beginning of the day. Most performed differences in clock gene expression we detected in the AP of TGR rats. Expression of *per2* was phase delayed and expression *bmal1* was phase advanced in the TGR rats in comparison with controls. Genes involved in protein and glucose metabolism were expressed in rhythmic manner in AP of TGR rats with peak levels at night. **Conclusion:** Results confirmed that TGR rats exerted a higher PRA. Daily profile of clock and metabolic gene expression were different in AP in TGR rats in comparison with control animals.

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PTh12-103

Infradian dynamics of salivary sex steroids

Celec P., Ostatnikova D.
Institute of Pathophysiology, Department of Molecular Biology, Institute of Physiology, Comenius University, Bratislava, Slovakia
petercelec@gmail.com

Aim: Salivary levels of steroid hormones have been shown to correlate to with the free "bioactive" fraction in plasma and are, thus, widely used in endocrine research. One potential source of bias is intraindividual variability. The aim of our study was to analyze infradian dynamics of salivary sex steroids in both sexes. **Methods:** In two separate cohorts young healthy volunteers (17F & 45M; 18-25 years) were asked to collect saliva samples daily or every second day during a period of 30 or 75 days, respectively. Testosterone, estradiol and progesterone were measured in saliva samples by radioimmunoassay. Time series analysis was performed using repeated measures ANOVA and analysis of rhythmic variance (ANORVA). **Results:** All analyzed hormones showed high intraindividual variability in both genders reaching coefficients of variation of more than 25%. In women the effects of menstrual cycle phases on salivary hormonal levels were confirmed. ANORVA showed no further cyclical components in any sex steroid. In men strong infradian rhythmic variations were found for testosterone (periods of 20 and 30 days) and estradiol (period of 12 days). **Conclusion:** The relatively high intraindividual variability indicates the importance of infradian dynamics of salivary sex hormones. Our study shows that the widely used single time point samplings make the interpretation of salivary sex hormone levels difficult and its information value vague. The findings of infradian rhythms of sex hormones in men should be considered in endocrine research. Further studies are needed to uncover the causes and consequences of infradian hormonal rhythms in men.

PTh13-104

***In vitro* characterisation of a chimeric CD7 mini-antibody**

Akramiene D.[1], Peipp M.[2], Staudinger M.[2], Kevelaitis E.[1], Fey G.[3], Gramatzki M.[2], Repp R.[2]
[1]Department of Physiology, Kaunas University of Medicine, Kaunas, Lithuania; [2]Division of Stem Cell Transplantation and Immunotherapy, 2nd Medical Department, University of Kiel, Kiel, Germany; [3]Chair of Genetics, University of Erlangen-Nuremberg, Erlangen, Germany

Aims: Despite aggressive chemotherapy the majority of T-cell malignancies have an unfavourable prognosis. Therefore, more potent targeted therapies with greater specificity and more favourable toxicity profiles are needed. One of the prerequisites for successful immunotherapy is the selection of an appropriate target antigen, which ideally should be T-cell specific and expressed on most T-cell lymphomas and leukaemias but absent on at least a portion of normal T lymphocytes. The T-cell differentiation antigen CD7 meets these requirements. A high affinity CD7 hybridoma antibody – generated by our group – produced significant anti-tumour effects in xenotransplanted athymic nude and SCID mice. The Fc portion was essential for the anti-tumour effect in vivo. Here the construction and expression of a chimeric CD7 mini-antibody – to enhance recruitment of human effector cells – is described. **Methods:** The CD7 mini-antibody was expressed in 293T cells and purified to homogeneity by two-step purification. To monitor binding to antigen positive and negative cells, FACS analyses were performed. Lytic activity was tested in 51Cr-release assays. **Results:** The recombinant protein was successfully expressed and specifically bound to CD7-positive CEM cells. In ADCC experiments the mini-antibody mediated efficient lysis of CEM cells with MNC as effector cells. No killing was observed with CD7-negative ARH-77 cells. **Conclusions:** The CD7 mini-antibody retained its antigen specificity and significantly mediated ADCC – an important mechanism of action of therapeutic antibodies – with human MNC as effector cells. This novel reagent might have potential for the treatment of T-cell malignancies.

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PTh13-105

Changes in brainstem catecholaminergic neurons activity in tumour bearing rats

Mravec B.[1,3], Pirnik Z.[1], Bundzikova J.[1], Bizik J.[2], Hulin I.[3], Kiss A.[1]
[1]Laboratory of Functional Neuromorphology, Institute of Experimental Endocrinology; [2]Laboratory of Experimental Oncology, Cancer Research Institute, Slovak Academy of Sciences; [3]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
ueenmrav@savba.sk

Aims: It is well established that immune system plays important role in tumourigenesis. Based on functional interconnections between immune and nervous system, it is assumed that tumour progression might activate specific brain areas. **Methods:** Using dual Fos/tyrosine hydroxylase immunohistochemistry we investigated whether advanced stadium of cancer induced by a single injection of 0.5 millions of BP6-TU2 fibrosarcoma cells to male Wistar rats may affect the activity of the brainstem noradrenergic (NA) neurons. Our intent was also to consider whether the process of tumourigenesis may sensitize the brainstem NA neurons in rats exposed to an immobilization (IMO) stress. **Results:** We found that in tumour bearing rats sacrificed 28 days after the initiation of the tumourigenesis only A2 and in less extent A1 NA cells showed presence of Fos protein in their perikarya. However, effect of 60 min IMO on Fos expression in the brainstem NA cell groups of tumour bearing rats did not differ from controls. **Conclusion:** Our data indicate that advanced tumorigenesis has only moderate but targeted impact on the activity of brainstem NA cell groups. In addition, tumour bearing animals did not show signs of altered sensitivity to strong physical stimulus. Our findings support the hypothesis that the brain is informed about the progression of the peripheral cancer.

PTh13-106

Characterisation of melatonin receptor (MT1) expression in human primary breast cancer specimens

Rogelsperger O.[1], Svoboda M.[1], Huber S.[1], Krenbek D.[1], Konigsberg R.[3], Klimpfinger M.[4], Jager W.[2], Thalhammer T.[1]
[1]Center Physiol. Pathophysiol., Med. Univ. of Vienna; [2]Dept. Clin. Pharmacy and Diagnostics, Univ. of Vienna; [3]3rd Med. Dept., Center Oncology Haematol.,; [4]Ins. Bacteriol. Pathol., Kaiser-Franz-Josef-Spital, Vienna, Austria; olga.roegelsperger@meduniwien.ac.at

Aim: To elucidate the role of the G-protein coupled melatonin receptor 1 (MT1) in breast tumours, paraffin-embedded tissue sections from tumorous (Tu) and adjacent non-tumorous (NTu) tissue samples from 42 breast cancer patients of different tumour stage and grade were investigated for cellular levels and subcellular localization of the receptor. **Methods:** A mAB directed against the 2nd cytoplasmic domain of MT1 was applied in the immunohistochemistry and tissue sections were examined in a Zeiss Axioplan2 microscope. Based on the staining intensity and the percentage of positively stained epithelial cells, an immunoreactive score (IRS) ranging from 0-12 points was used to classify MT1 levels. **Results:** MT1 staining was observed in 40/42 Tu and 38/42 NTu specimens. In 21/42 cases, the IRS for MT1 staining in the Tu exceeded that of the adjacent NTu. In 12/42 specimens, MT1 levels in the Tu were lower than in NTu sections. No difference was seen in 9/42 cases. Remarkably, the pattern of MT1 staining of cellular structures varied between Tu and NTu specimens. Membrane staining was predominant in 26/42 NTu, but only in 3/42 Tu samples, whereas cytoplasmic staining was apparent in 32/42 Tu but only in 10/42 NTu specimens. Additional staining of nuclear compartments was seen in 15/42 Tu and 10/42 NTu samples. **Conclusion:** We show that MT1 is present in the majority of Tu and NTu specimens from breast cancer patients with primary untreated tumours. Whether an altered subcellular localization might influence the function of MT1 in breast cancer has to be elucidated.

PTh13-107

Extracellular calcium inhibits secretory response of insulinoma cell line INS-1E to cell swelling

Orecna M., Bacova Z., Hafko R., Strbak V.
Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; martina.orecna@savba.sk

Aim: To further characterise osmotically-induced insulin secretion. **Methods:** We compared response of freshly isolated rat pancreatic islets and INS-1 and INS-1E tumour cell lines to 15 mmol/l glucose, 30% hypotonic medium and 20% hypertonic medium using static incubations and perifusion by Ca^{2+} containing or depleted medium. **Results:** In Ca^{2+} containing medium glucose induced insulin release in all three cell types. Hypotonicity induced insulin secretion from islets and INS-1 cells but not from INS-1E cells, in which secretion was inhibited despite a significant increase in cell volume. $GdCl_3$ (100 µmol/l) did not affect insulin response from INS-1E cells to hypotonic challenge. Hypertonic medium inhibited glucose-induced insulin secretion from islets but not from tumour cells. Noradrenalin (1 µmol/l) inhibited glucose-induced but not swelling-induced insulin secretion from INS-1 cells. Surprisingly, perifusion with Ca^{2+} depleted medium showed distinct secretory response of INS-1E cells to hypotonicity that was even higher than that of INS-1 cells. **Conclusion:** Functioning glucose-induced insulin secretion is not sufficient prerequisite for hypotonicity-induced response in INS-1E cells suggesting that swelling-induced exocytosis is not essential step in the mechanism mediating glucose-induced insulin secretion. Both cell lines are resistant to inhibitory effect of hyperosmolarity on glucose-induced insulin secretion. Response of INS-1E cells to hypotonicity is inhibited by the presence of Ca^{2+} in medium.
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PTh13-108

Effects of PCBs 52 and 77 on Th1/Th2 balance in human lymphocyte cell cultures

Yilmaz B.[1], Sandal S.[2], Ozdemir G.[2], Godekmerdan A.[3]
[1]Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey; Departments of [2]Physiology and [3]Immunology, Firat University, Faculty of Medicine, Elazig, Turkey; byilmaz@yeditepe.edu.tr

Aims: Polychlorinated biphenyls (PCBs) are persistent industrial chemicals that were widely used in the world. Toxic effects of different PCB congeners are structure-dependent. We have tested a non-coplanar congener, 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), and a coplanar congener, 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), for their effects on the T helper 1 (Th1) and T helper 2 (Th2) lymphocyte balance. **Methods:** Venous blood samples were obtained from eight healthy male volunteers (age between 18 and 20). Lymphocytes were separated by density gradient centrifugation method. They were pooled and then cultured in 24-well plates at a seeding density of 3×10^4/ml. The experiments were performed on 10 µg/ml concanavalin A (Con A)-stimulated and non-stimulated lymphocytes for determination of cytokine production profiles. Interferon-γ (IFN-γ, produced by Th1 cells) and interleukin (IL)-13 (produced by Th2 cells) concentrations were measured by ELISA in the supernatants at 24 and 48 hr after treatment with PCBs. **Results:** The highest concentration of PCB 52 caused significant increases in IFN-γ levels at 48 hr in both Con A-stimulated and non-stimulated media ($p<0.05$). At 10 µM PCB 77 significantly raised IFN-γ levels at 48 hr in Con A-stimulated medium ($p<0.05$), but not non-stimulated medium. IL-13 levels were not significantly altered by PCBs (52 and 77) in both media (non- and Con A-stimulated). **Conclusion:** Our results indicate that the cytokine production profile was significantly shifted to Th1 by coplanar and non-coplanar PCB congeners in human lymphocyte cultures, but effect of non-coplanar PCB congener was more potent than the coplanar PCB congener.
Acknowledgement: This study was supported by Firat University Research Foundation (FUBAP-1351).

PTh13-109

Erythrocyte deformability changes in oncologic and diabetic patients

Beder I.[1], Mataseje A.[1], Carsky J.[2], Zahorec R.[3], Kittova M.[1], Pinakova Z.[3]
[1]Inst. Physiol.; [2]Inst. Med. Chem., Biochem. and Clinical Biochemistry, Medical School, Comenius Univ., Bratislava, Slovakia; [3]Cancer Institute St. Elisabeth, Bratislava, Slovakia; igor.beder@fmed.uniba.sk

Aims: The work was focused on the study of functional properties of erythrocytes in microcirculation in selected oncologic and metabolic diseases. **Methods:** Erythrocyte deformability (ED), as a basic criterion of erythrocyte transport properties, haematologic and selected biochemical variables as well as acid-base balance variables were determined. ED was measured by filtration method and studied in two groups of oncologic patients: 15 subjects with carcinoma (CA) of the colon and 17 subjects with CA of the breast. The third examined group consisted of 16 patients with type 1 diabetes mellitus. The control group involved 15 healthy subjects. **Results:** In oncologic patients ED values were significantly impaired in comparison with the control group. Surgical stress in patients with CA of the colon lowered ED from 65.6±3.2 to 62.0±4.0 ($P<0.05$) and following adjustment of ED values was observed in postoperative days. Significant decrease in erythrocyte count, haemoglobin concentration and increased reticulocyte count was recorded simultaneously. Radiotherapy in patients with CA of the breast resulted in ED decrease by 6.8% after the first week and continued by 9.4% and 8.6% after the third and fourth week of radiotherapy ($P<0.05$) and significant decrease of blood elements counts. Membrane active substances (aminoguanidine, pyridoxiliden-aminoguanidine, pyridoxal) demonstrated protective effect on erythrocyte elasticity. **Conclusion:** Surgical stress lowered ED values in oncologic and in operated non-oncologic patients as well. Cytotoxic effect of radiotherapy on ED was found. Significant deformability improvement was obtained by pyridoxal in diabetic patients ($P<0.05$).

PTh13-110

Investigation of the role of steroid hormone 1α,25(OH)$_2$D3 on the calcium regulation in human peripheral blood mononuclear cells

Lajdova I.[1], Chorvat D.[2] Jr., Spustova V.[1], Chorvatova A.[3]
[1]Dept. Clin. & Exp. Pharmacotherapy, Slovak Medical Univ., Bratislava, Slovakia; [2]Internat. Laser Centre, Bratislava, Slovakia; [3]Research Centre, CHU Sainte Justine, Dept. of Pediatrics, Univ. of Montreal, Canada; ingrid.lajdova@szu.sk

Aims: 1α,25(OH)$_2$D3 has been shown to exert its effects by both genomic and nongenomic mechanisms. We have examined rapid nongenomic effects of the hormone on calcium mobilization and entry through calcium release activated (CRAC) and L-type calcium channels, as well as possible implication of the purinergic P2X$_7$ receptors in this action. **Methods:** Human peripheral blood mononuclear cells were obtained from blood samples of healthy volunteers. Intracellular calcium was measured using Fluo 3-AM fluorimetry. P2X$_7$ pore function was assessed by ethidium bromide confocal imaging. **Results:** 1α,25(OH)$_2$D3 induced time-dependent rise in $[Ca^{2+}]_i$, which stabilized after 10 min. Sensitivity of the initial 1α,25(OH)$_2$D3-stimulated calcium rise to thapsigargin pointed to its origins in the calcium release from intracellular stores. 2APB, the inhibitor of capacitative entry, but not nifedipine, the L-type calcium channel inhibitor, prevented the hormone-stimulated $[Ca^{2+}]_i$ increase. 1α,25(OH)$_2$D3 inhibited calcium entry stimulated by BzATP, a specific agonist of P2X$_7$ receptor, while KN-62, a P2X$_7$ receptor antagonist, had no effect on cells pretreated with 1α,25(OH)$_2$D3. Furthermore, 1α,25(OH)$_2$D3 significantly reduced the BzATP stimulated ethidium bromide fluorescence, confirming inhibitory effect of the hormone on calcium influx through P2X$_7$ channel. **Conclusion:** Our results demonstrate that 1) 1α,25(OH)$_2$D3 promotes a two-step calcium response through calcium release from internal stores, followed by store refilling via CRAC, but not L-type calcium channels, while 2) the steroid hormone also induces a nongenomic inhibitory effect on Ca^{2+} entry and the permeability through pore-forming P2X$_7$ receptor. *Supported by Grants No APVT-21-033002 & No APVT-21-019702.*

PTh14-111 EYPS Keynote Lecture

Probing the mechanical properties of cardiac titin by molecular combing and atomic force microscopy

Graham H.K.[1], Sherratt M.J.[2], Trafford A.W.[1]
[1]Unit of Cardiac Physiology; [2]Tissue Injury and Repair Group, The University of Manchester, Manchester, United Kingdom

Over 49% of elderly patients with heart failure exhibit left ventricular (LV) diastolic dysfunction, characterised by preserved LV ejection fraction, impaired relaxation and increased myocardial stiffness. Two major determinants of myocardial stiffness are i) the turnover and organisation of the extracellular matrix (ECM) and ii) the mechanical properties of the giant sarcomeric protein titin. We have previously reported that during heart failure, reduction in collagen content is correlated with a simultaneous increase in the activity of the collagen-degrading matrix metalloproteinase MMP-9. **Aim:** In order to determine if titin contributes to altered compliance in diseased hearts, we have applied molecular combing techniques to characterise the nano-mechanical properties of isolated native cardiac titin molecules[1]. **Methods:** Following extraction from ferret LV[2], titin molecules were exposed to tensile forces ~110-160 pN. **Results:** Visualisation of uncombed titin molecules by atomic force microscopy revealed a highly coiled, spring-like conformation. In contrast, combed molecules were both straightened and aligned. Fitting Gaussian curves to measured contour length distributions from combed and uncombed molecules revealed a tensile force induced 40% increase in titin monomer length from 1.2 to 1.7 μm. Mean axial heights were also reduced following combing (0.62 ± 0.21nm uncombed vs. 0.58 ± 0.2 nm, n= 40-42 molecules, $p< 0.01$) which may indicate the unfolding of domains. **Conclusion:** The molecular combing technique is capable of aligning and stretching isolated titin molecules and has the potential to provide valuable information on the role of titin mechanical properties in disease processes.

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(2) Soteriou A. et al. J Cell Sci 1993; 104:119 - 23.

PTh14-112

Micro positron emission tomography as an imaging tool for monitoring of tumours and defined brain structures activity

Jakubikova L., Petrak J., Ondkova S., Macejova D., Mravec B., Brtko J., Kvetnansky R., Kovac P., Strbak V.
Institute of Experimental Endocrinology, Slovak Academy of Sciences (IEE SAS); Joined research MicroPET Laboratory of IEE SAS and BIONT a.s., Bratislava, Slovak Republic; ueenjaku@savba.sk

Aims: The objective of this study was to analyse *in vivo* monitoring of disease progression or a state of metabolic/therapeutic processes in the small laboratory animals by micro positron emission tomography (μPET). Uptake of [^{18}F]-Fluoro-2-deoxyglucose (FDG) reflecting metabolic activity of the tissue can be used for monitoring of response to chemotherapy in some forms of cancers. Moreover FDG is also a marker of neuronal activity. **Methods:** Longitudinal development of response to a specific new chemotherapy of tumours was measured by uptake of FDG in tumours as a "pre-therapeutic" staging and three months after therapeutic intervention as "therapeutic monitoring". We also investigated whether FDG uptake will reveal activation of discrete brain structures in rats exposed to stressful stimuli. **Results:** Images of individual tumours allowed quantification of tumour's volume, metabolic activity and their possible dependence and changes of their relation in time. μPET imaging revealed to be useful to analyze activity of discrete brain structures like thalamus, hypothalamus, frontal cortex, striatum and quantify their metabolism. **Conclusion:** μPET for small laboratory animals is suitable non-invasive imaging method for repeated monitoring of tumour diseases and effect of possible therapy. The images of FDG accumulation also revealed feasible application of μPET in study of neuroendocrine regulation. *This work was supported by project of the ESF No 13120200067 and APVV Grant No 0148-06.*

PTh14-113

Changes in the dependence of the somatosensory evoked potential on the parameters of stimulation in rats acutely treated with environmental neurotoxicants

Papp A., Banko S., Takacs S.
Department of Public Health, University of Szeged Faculty of Medicine, Szeged, Hungary
ppp@puhe.szote.u-szeged.hu

Aims: Numerous environmental xenobiotics are neurotoxic. The best way to detect and follow-up nervous system damage would be the use of functional biomarkers but these still have to be worked out. **Methods:** Adult male Wistar rats were anesthetized with urethane, the left hemisphere was exposed and a silver recording electrode was placed on the projection area of the whiskers. The whisker pad was stimulated with electric square pulses, in trains of 50, and the cortical response was recorded. The intensity of the stimulus was varied between 25% and 100% (just supramaximal), and its frequency, between 1 and 10 Hz. Three control records were taken, in 30 min interval, then one of the neurotoxicants (3-nitrporopionic acid, a mitochondrial toxin of microfungal origin: 20 mg/kg b.w.; dimethoate, an organophosphorus insceticide: 44 mg/kg b.w.; or manganese, a heavy metal: 50 mg/kg b.w. in chloride form) was injected ip. and further four records were taken. **Results:** The effect of the three toxicants was dissimilar. Administration of dimethoate caused no significant change in the evoked response parameters or their dependence on stimulation. Mn caused increased amplitude and latency for strong stimuli, but intensified frequency-dependent fatigue. 3-nitropropionic acid caused amplitude increase without altered latency, and had less effect on frequency dependence. **Conclusion:** The experiments carried out relied on techniques applied routinely also in human neurology. The alterations observed, if they prove to be sufficiently sensitive and/or specific in further studies, have the potency to be developed to functional biomarkers.

PTh14-114

Q-method for high-resolution measurement of cell impedance parameters using square wave stimulation.

Novak P., Zahradnik I.
Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia
ivan.zahradnik@savba.sk

Aims: High-resolution measurements of cell impedance provide valuable information on various cellular processes such as exocytosis, ion channel gating, or fertilization. The best recent techniques have limited applicability due to their inherent constrains and high complexity. **Methods:** We report here the Q-method, a simple method of high-resolution impedance measurement based on measurements of charge by integrating the cell current in response to square wave stimulation. The charge is decomposed into specific components related to segments of the voltage stimulus and analyzed using simple relations for fast and direct estimation of the cell impedance parameters. **Results:** The major advantages of the Q-method are its inherently low sensitivity to low-pass filtering, rejection of periodic interference signals, the capacitance resolution at theoretical limits, and simultaneous high-resolution low-crosstalk monitoring of the membrane resistance, the series resistance and the parasite capacitance, in addition to the membrane capacitance. High-resolution recordings of the cardiac myocyte capacitance and resistance under unstimulated control conditions are presented and their fluctuation is analyzed using the detrended analysis. **Conclusion:** We show that Rm and Cm may behave independently during experiment and therefore may reflect independent cellulular processes that indicate collective changes in the state of the cell that have to be understood.

PTh14-115

Chromophore exchange from A2 to A1 in salamander rod opsin: spectral blue-shift correlates with decrease in thermal "dark" noise

Donner K., Ala-Laurila P., Crouch R.K., Cornwall M.C.
Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland; Department of Physiology and Biophysics, Boston University School of Medicine, Boston MA, USA; Department of Ophthalmology, Medical University of South Carolina, Charleston SC, USA
kristian.donner@helsinki.fi

Aims: To investigate changes in the "dark" current noise of a rod photoreceptor cell as the absorption spectrum of the visual pigment is shifted to shorter wavelengths by switching chromophore from 11-*cis*-3,4-dehydroretinal (A2) to 11-cis-retinal (A1) (pigment λ_{max} = 528 and 502 nm, respectively). **Methods:** Dark noise, light-induced noise and responses to brief flashes of light were recorded with the suction electrode technique in the membrane current of isolated red rods from the retina of larval tiger salamander: (i) in the "native" state, with mainly A2 pigment and (ii) in the "final" state, after bleaching most of the native pigment and regenerating with A1. The proportions of A1 and A2 pigment (A1/A2 ratios) in the native and final states were determined by measurements of spectral sensitivity and absorbance. The rates of spontaneous activations of visual pigment were estimated from power spectra of dark current noise. **Results:** The estimated rate of spontaneous pigment activations per rod changed from 0.238 ± 0.026 $rod^{-1}s^{-1}$ to 0.030 ± 0.006 $rod^{-1}s^{-1}$ (mean ± SEM), as the A1/A2 ratio changed from 0.2/0.8 (native state) to 0.9/0.1 (final state). Extrapolation to pure A1 and pure A2 pigment indicates that the A1 pigment is at least 30 times more stable than its A2 pair. **Conclusion:** The results support the hypothesis (Barlow H.B.: Purkinje shift and retinal noise, Nature 179:255-256, 1957) that spectral tuning of visual pigments towards shorter wavelengths is coupled with a decreased rate of spontaneous (thermal) activations.

PTh14-116

Occurence of arsenic and lead compounds in umbilical blood

Morvova M., Kunecova D., Foltin V., Foltinova J.
Comenius University Bratislava, Slovakia: FMFIUK – Depts.: Astronomy, Earth Physics & Meteorology; Experimental Physics; LFUK – Inst. Histology and Embryology
morvova@fmph.uniba.sk; vfoltin@fmph.uniba.sk

Introduction: Heavy metals dangerous for organism commonly occur in the environment (industrial exhalations, dyes, ceramics products) what leads to increasing number of health damages caused by them. We focused on the occurrence of heavy metals in umbilical blood (UB). **Method:** UB of patients was processed by KBr pellet technique. Pellets (200mg of spectrally pure KBr and 1-10mg UB) were transparent in infrared region what enabled us to take infrared absorption spectra in FIR, MIR, NIR regions with FTIR spectrometer Perkin Elmer Spectrum BX from 6000 to 200 cm^{-1}. Metal compounds can be detected from IR spectrum most of all in the FIR region (library of spectra [1]). **Results:** We proved occurrence of arsenic in form of As_2O_3 (doublet between 330 and 350 cm^{-1}) and lead in form of Pb_3O_4 (singlet band 670 cm^{-1}). **Conclusion:** We assume occurrence of As and Pb in UB to be a result of inhaling air polluted with fall having its origin in production of technical glass-plumbiferous glass purified by arsenic compounds. Sublimation of the added raw material above the hot surface of smelting glass gives rise to aerosol that becomes a part of exhalations.
Funding: VEGA 1/3068/06 and APVV 0267-06

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PF15-117

Pyridoindole antioxidant stobadine influences ATP-utilisation by renal Na^+,K^+-ATPase in rats with streptozotocin-induced diabetes

Javorkova V.[1], Strelkova S.[1], Vlkovicova J.[1], Mezesova L.[1], Stefek M.[2], Kyselova Z.[2], Gajdosikova A.[2], Vrbjar N.[1]
[1]Institute for Heart Research, Department of Biochemistry, Slovak Academy of Sciences; [2]Institute of Experimental Pharmacology, Slovak Academy of Sciences, Slovak Republic

Aim: The aim of this study was to assess the effect of the pyridoindole antioxidant stobadine on diabetes-induced changes of Na,K-ATPase, especially those concerning the utilisation of its substrate ATP. **Methods:** Diabetes type-1 was induced in 8-week-old rats, by a single i.v. injection of streptozotocin (STZ) in a dose 55 $mg.kg^{-1}$. ATP-kinetics of Na,K-ATPase was estimated by measuring the hydrolysis of ATP. **Results:** Sixteen weeks of streptozotocin-induced diabetes was followed by decrease of the enzyme activity. This effect was emphasised in the presence of higher concentrations of ATP and in the presence of 8 $mmol \cdot l^{-1}$ ATP represented 20%. It might be a consequence of altered functional properties of Na,K-ATPase as suggested by 20% decrease of the V_{max} (maximum velocity) value along with decrease of the K_m value by 20% (K_m-concentration of ATP necessary for half-maximal activation of the enzyme). Administration of 0.05% (w/w) stobadine in the diet to diabetic rats improved the function of renal Na K-ATPase with respect to utilisation of ATP as suggested by significant increase of the enzyme activity in the whole concentration range of ATP investigated as a consequence of V_{max} elevation to the level comparable to absolute controls. **Conclusion:** Stobadine may play a positive role in restoring the functional properties of renal Na,K-ATPase, especially concerning the utilisation of energy derived from hydrolysis of ATP, improving thus the maintenance of ionic homeostasis during diabetes.
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PF15-118

Cardiac Na^+,K^+-ATPase in three various animal models of hypertension

Vlkovicova J.[1], Javorkova V.[1], Mezesova L.[1], Pechanova O.[2], Vrbjar N.[1]
[1]Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovakia; [2]Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovakia
usrdjavl@savba.sk

Aim: The aim of present study was the investigation of regulatory role of nitric oxide (NO) on functional properties of the cardiac Na^+,K^+-ATPase during hypertension. **Methods:** The first group was represented by spontaneously hypertensive rats (SHR) with increased synthesis of NO (Sh1). The second group of SHR revealed decreased synthesis of NO (Sh2) and in the third group (LN), the hypertension was induced by administration of L-NAME (40mg/kg/day). The kinetics of Na^+,K^+-ATPase were estimated by measuring the splitting of ATP by 50 µg of sarcolemmal proteins in dependence of the substrate and the cofactor concentrations. **Results:** Studying the utilization of energy substrate ATP we observed higher Na^+,K^+-ATPase activity for Sh1, and depressed activity in Sh2 and LN. Evaluation of kinetic parameters revealed increased V_{max} in Sh1 and decreased values in Sh2 and LN. The affinity of the ATP binding site was improved in Sh1 as indicated by the lowered K_m. In Sh2 and LN groups the K_m value remained unchanged. During the activation with Na^+ the V_{max} and K_{Na} values increased in the Sh1. The Sh2 revealed decreased V_{max} and increased K_{Na}. In LN the enzyme activity was depressed mainly at lower concentrations of NaCl, showing unchanged V_{max} with increased K_{Na}. **Conclusion:** Our data indicate a positive role of increased NO-synthesis in improvement of utilization of ATP as well as enhanced Na^+-binding by the cardiac Na^+,K^+-ATPase.
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PF15-119

Cerebral ischaemia/reperfusion injury in rats: impact to expression of novel secretory pathways Ca^{2+} pump (SPCA)

Pavlikova M., Sivonova M., Tatarkova Z., Murin R., Urban P., Lehotsky J.
Dept. of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia; Martina.Pavlikova@jfmed.uniba.sk

Forebrain ischaemia/reperfusion injury (IRI) initiates cascade of events which eventually lead to cellular death. Dysregulation of calcium homeostasis is believed to be one of the causative phenomena linked with delayed neuronal death after IRI. Proper function of secretory pathways is important for neural cells. They secrete many neurotransmitters and secretory proteins necessary for growth and reorganization of neuronal circuits. A newly recognized calcium pump of Golgi apparatus, Ca^{2+}-ATPase (SPCA1) has important secretory function in rat forebrain. We investigated the presence and distribution of the SPCA1 pump protein in homogenates prepared from both the rat brain and the cell cultures of neurons and glial cells. Experiments show that SPCA1 pump protein in neural cells is localized to structures distinct from endoplasmic reticulum. In addition, mRNA expression pattern of SPCA1 gene was analyzed by RT-PCR after global forebrain IRI in rat. Forebrain ischaemia was initiated by four-vessel occlusion for 15min and reperfusion for 1h, 3h and 24h (IRI). RT-PCR analysis clearly detected expression of SPCA1 gene in injured area after IRI. In addition, mRNA expression pattern follows time dependent manner in reperfusion period. We observed, in reperfusion time, expression of SPCA1 gene follows a bell shaped pattern. In both areas of brain (cortex and hippocampus) the expression of SPCA1 was significantly increased with maximum at 3h after insult. Since the pump plays major role in the refilling of Ca^{2+} stores, we discuss here its possible contribution to altered intracellular Ca^{2+} signaling in neural cells in ischaemia/reperfusion event in context of involvement of secretory pathways in stress sensing and transduction of apoptotic signal. *Supported by VEGA 3380/06, MVTS 39.*

PF15-120

The influence of chlorpyriphos on Na^+,K^+-ATPase activity

Krstic D.[1], Colovic M.[3], Vujacic A.[2], Djuric D.[3], Vasic V.[2]
[1]Inst. Chem.; University School of Medicine, Belgrade, Serbia; [2]Vinca Inst. of Nuclear Sciences, Dept. of Physical Chemistry, Belgrade, Serbia; [3]Inst. of Medical Physiology; Univ. School of Medicine, Belgrade, Serbia

Aims: The toxic effects of organophosphorus compounds are primarily based on inhibition of acetylcholinesterase enzyme which participates in the transfer of impulse in central synapses of the cholinergic nervous system through a chemical mediator, acetylcholine. At the same time, organophosphates and their metabolites are extruded from the organism by the kidney through the secretory pathway of the organic anions. This secretion is active and depends on the activity of tubular ATPases, mainly Na^+/K^+-ATPase. The aim of this work was to investigate the influence of organophosphate chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor-2-pyridinyl-thiophoshate) on ATP hydrolysis catalyzed by Na^+/K^+-ATPase. **Methods:** The Na^+/K^+-ATPase activity was assayed in the absence (control) and presence of inhibitor (within the range $1x10^{-8}$ - $1x10^{-3}$mol/l) in standard medium containing 50mM Tris-HCl (pH 7.4), 100mM NaCl, 20mM KCl, 5mM $MgCl_2$, 2mM ATP and 25µg enzyme in a final volume of 200µl. Incubation mixtures were preincubated for 10min at 37°C in the presence of organophosphate chlorpyrifos or distilled water (control). The inorganic orthophosphate (P_i) liberated from the hydrolysis of ATP, was measured using modified spectrophotometric procedure. **Results:** The increasing concentrations of chlorpyrifos induced inhibition of enzymatic activity in a concentration-dependent manner. Dependence of the Na^+/K^+-ATPase activity, expressed as the percent of control value, on the inhibitor concentration fitted a sigmoidal function. The half-maximum inhibitory concentration (IC_{50} values): (1.3±0.1) x 10^{-4}mol/l, was obtained from the inhibition curve. **Conclusions:** Chlorpyrifos, beside specific inhibition of acetylcholinesterase, alters activity of Na^+/K^+-ATPase, plasma membrane enzyme essential for functioning and homeostasis of almost all animal cells.

PF15-121

Membrane currents underlying characteristics firing patterns of developing spinal neurons in *Xenopus*

Winlove C.I.P., Roberts A.
University of Bristol, Bristol, UK
c.i.p.winlove@bristol.ac.uk

Aim: In hatchling *Xenopus laevis* tadpoles 7 classes of neuron have been identified anatomically and functionally in the developing spinal cord. These neurons control swimming behaviour and demonstrate 4 firing phenotypes to current injection: single action potential (S); repetitive firing without adaptation (R); repetitive firing with rapid adaptation (RA); and delayed-onset firing (DO). We explore membrane currents underlying the firing of S and R neurons. **Method:** *In vivo* whole-cell patch recordings were made from neuron somata exposed in the spinal cord of animals ~40hours post-fertilisation. Neurons were identified by anatomy, and responses to current. **Results:** Current-clamp recordings investigated firing patterns to current injection. S neurons fire a single long-duration action potential, the duration decreasing with reduced external calcium. Firing was unaltered by Muscarine (M-current antagonist) or 4-AP (A-current antagonist). In contrast, TEA (delayed rectifier antagonist) converts S neurons to multiple-firing R-like phenotypes. Voltage-clamp recordings revealed outward currents in S neurons activate more slowly than those in R neurons; both cell-types show limited current inactivation. The outward current reversed close to the potassium equilibrium potential, and was eliminated by TEA. These characteristics are typical of delayed rectifiers. In both neuron classes an inactivating outward current (A-current) was seen in a minority of cells, and calcium currents were small. **Conclusion:** We suggest that in S neurons the slow outward current balances late stage inward currents, preventing further depolarisation. This slow activation may underlie the long duration action potential seen in S neurons. Kinetic differences may be sufficient to explain divergent firing phenotypes.

PF15-122

Subunit stochiometry of heterologously expressed G-protein activated inwardly rectifying potassium channels analysed by FIR

Grasser E., Steinecker B., Ahammer H., Schreibmayer W.
Molecular Physiology Laboratory, Institute for Biophysics, Medical University of Graz, Graz, Austria
wolfgang.schreibmayer@meduni-graz.at

Aims: Heterologous expression in oocytes of *Xenopus laevis* allows the controlled simultaneous expression of up to >10 different proteins. Little data exist, so far, on the exact quantitation of the protein stochiometry of expressed excitability proteins. We studied the exact protein stochiometry between the GIRK1 and GIRK4 subunits of I_{K+ACh} at different ratios of injected RNA. **Methods:** N-terminal fusion proteins between the GIRK1 and GIRK4 subunits and variants of the green fluorescence protein (eGFP, eCFP, eYFP) were engineered and expressed in *Xenopus laevis* oocytes at different RNA ratio's. Using confocal miscroscopy, the subunit stochiometry was analysed by the Fluorescence Intensity Ratio (FIR) method. **Results:** It was found, that the subunit stochiometry GIRK1:GIRK4 was 0.46 ± 0.02 (N= 9 different batches of oocytes) at low magnification, using the 20x objective, when equal amounts of RNA were injected (10 ng per oocyte for each RNA species). When the ratio of injected GIRK1 RNA to GIRK4 RNA is raised to 5:1 and 25:1 the protein subunit stochiometry GIRK1:GIRK4 changes to 1.46 ± 0.13 (N= 9) and 2.82 ± 0.38 (N= 9), respectively. When oocytes were viewed at high magnification and a fluorescence marker for the endoplasmic reticulum (ER) was used, it could be shown that the subunit stochiometry is identical in the plasmamembrane and in the ER. **Conclusion:** These results demonstrate the coexistence of GIRK1/ GIRK4 heterooligomeric as well as of GIRK4 homooligomeric complexes in the plasmamembrane upon injection of equal RNA amounts encoding both subunits. The GIRK1:GIRK4 stochiometry can be shifted towards a 3:1 ratio, by injecting GIRK1 RNA in excess.

PF15-123

Kv7 potassium channels of the inhibitory interneurons of hippocampal culture

Grygorov O., Moskalyuk A., Dosenko V., Fedulova S., Veselovsky N., Brown D.
Bogomoletz Institute of Physiology of NASU, Kyiv, Ukraine; University College London, London, UK; grigorov@biph.kiev.ua

Aims: The Kv7 (KCNQ) potassium channels are involved in a generation of non-inactivating potassium current (I_M) and play a significant role in the regulation of excitability of different types of neurons, e.g. hippocampal pyramidal cells. The purpose of this study was to investigate the presence of Kv7 channels in GABAergic hippocampal interneurons and their ability to regulate functioning of these cells. **Methods:** Experiments were carried out on GABAergic neurons of 18-27-day-old primary culture of hippocampi of newborn rats. Single-cell RT-PCR technique was used for identification of expression of KCNQ2-KCNQ5 genes, the whole cell patch-clamp method was performed for the recording of membrane currents or potentials. **Results:** High level of expression of the genes coding Kv7.2 and Kv7.3 subunits was revealed in the any neuron studied. Small level of Kv7.5 subunits expression was detected in 38% of the cells. It was shown the non-inactivating current take major part (83 ± 1.7%) in the forming of total depolarization-evoked potassium current of the cells studied. Non – inactivating current revealed activation threshold about -60 mV and displayed biphasic kinetics of deactivation with fast (22 ± 3 ms) and slow (306 ± 40 ms) time constants. Retigabine (15 mcM) shifted its I-V plot to more negative values, increased current's amplitude at -20 mV by 66 ± 14% ($P < 0.001$) and prevented the ability of neurons to generate spike series. **Conclusion:** These results demonstrated that Kv7 channels are highly involved in the providing of repolarisation and in the regulation of activity of inhibitory hippocampal interneurons.

PF15-124

$G\alpha_i\beta\gamma$ heterotrimer regulates the function of an effector of $G\beta\gamma$, the neuronal G protein gated K^+ channel (GIRK)

Peleg S.[1*], Keren-Raifman T.[1*], Rubinstein M.[1*], Berlin S.[1], Varon D.[1], Dessauer C.[2], Ivanina T.[1], Dascal N.[1]
[1]Dept. Physiol. Pharmacol., Sackler School of Med., Tel Aviv Univ., Israel; [2]Dept. Integrative Biol. Pharmacol., Univ. Texas-Houston Med. School, Houston, USA,*Equal contributors; moranrub@post.tau.ac.il

G protein activated K^+ channels (GIRK, Kir3) mediate postsynaptic inhibitory effects of neurotransmitters by direct binding of $G\beta\gamma$ following activation of $G_{i/o}$ proteins via numerous G protein coupled receptors. Using *Xenopus* oocytes, we demonstrate that $G\alpha_i^{GDP}\beta\gamma$ actively regulates GIRK gating. Two $G\alpha_{i3}$ mutants were utilized: "constitutively active" $G\alpha_{i3}Q204L$ ("QL"; poor GTPase) and "constitutively inactive" $G\alpha_{i3}G203A$ ("GA") which forms a stable complex with $G\beta\gamma$. GIRK was activated by coexpression of $G\beta\gamma$ (whole-cell experiments) or by application of purified $G\beta\gamma$ to excised membrane patches. $G\alpha_{i3}GA$, with clear difference from other $G\beta\gamma$ scavengers, mimicked the wild-type $G\alpha_{i3}$ in reducing the basal current and priming GIRK for activation by $G\beta\gamma$. $G\alpha_{i3}QL$ elicited no effect. In *in vitro* protein interaction studies, purified $G\beta\gamma$ enhanced the binding of the whole cytosolic domain of GIRK1 to $G\alpha_{i3}^{GDP}$ or $G\alpha_{i3}^{GTP}$. This enhancement was not observed with GIRK2. In addition, using two-electrode voltage clamp, we tested $G\alpha$ and $G\beta\gamma$ regulation of homotetramers of GIRK1 and GIRK2. While GIRK2 behaved like a "classical" $G\beta\gamma$ effector, showing very low basal activity and strong $G\beta\gamma$-dependent activation, GIRK1 could not be activated by coexpressed $G\beta\gamma$ whilst retaining activation by agonist; $G\alpha_{i3}GA$ restored the ability of $G\beta\gamma$ to activate GIRK1, indicating that $G\alpha_i$ (probably as $G\alpha_i\beta\gamma$ heterotrimer) allosterically regulates the $G\beta\gamma$ gating of GIRK. These results suggest a specific role for GIRK1 as the scaffold for $G\alpha\beta\gamma$ within GIRK-G protein signaling complex, and imply that the $G\alpha_{i/o}\beta\gamma$ heterotrimers are not only precursors of free $G\beta\gamma$, but also active regulators of GIRK gating.

PF15-125

A tale of switched functions: from cyclooxygenase inhibition to M-channel modulation in new diphenylamine derivatives

Peretz A.[1], Degani-Katzav N.[1], Talmon M.[2], Danieli E.[3], Gopin A.[3], Malka E.[1], Nachman R.[1], Raz A.[2], Shabat D.[3], Attali B.[1]
[1]Dept. Physiol. & Pharmacol., Sackler Faculty of Med.; [2]Dept. of Biochem., Georges Wise Faculty of Life Sciences; [3]School of Chemistry, Faculty of Exact Sciences of Tel-Aviv Univ., Tel Aviv, Israel; aperetz@post.tau.ac.il

Aims: Cyclooxygenase (COX) enzymes are molecular targets of nonsteroidal anti-inflammatory drugs (NSAIDs), the most used medication worldwide. However, the COX enzymes are not the sole molecular targets of NSAIDs. Recently, we showed that two NSAIDs, diclofenac and meclofenamate, also act as openers of Kv7.2/3 K^+ channels underlying the neuronal M-current. Here we designed new derivatives of diphenylamine carboxylate to dissociate the M-channel opener property from COX inhibition. **Methods:** The carboxylate moiety was derivatised into amides or esters and linked to various alkyl and ether chains. The voltage- and current-clamp configurations of the whole-cell patch-clamp technique were used to study the drugs in neurons and transfected CHO cells. **Results:** Powerful M-channel openers were generated, provided that the diphenylamine moiety and a terminal hydroxyl group are preserved. In CHO cells, the openers activated recombinant Kv7.2/3 K^+ channels, causing a hyperpolarizing shift of current activation. In sensory dorsal root ganglion and hippocampal neurons, the openers hyperpolarized the membrane potential and robustly depressed evoked spike discharges. They also decreased hippocampal glutamate and GABA release by reducing the frequency of spontaneous excitatory and inhibitory post-synaptic currents. *In vivo*, the openers exhibited anti-convulsant activity, as measured in mice by the maximal electroshock seizure model. Conversion of the carboxylate function into amide abolished COX inhibition but preserved M-channel modulation. Remarkably, the very same template let us generating potent M-channel blockers. **Conclusions:** Our results reveal a new and crucial determinant of NSAID-mediated COX inhibition. They also provide a structural framework for designing novel M-channel modulators, including openers and blockers.

PF15-126

Voltage-gated potassium current in uterine artery myocytes is decreased in late pregnancy

Telezhkin V., Bonev A., Osol G., Gokina N.I.
Depts. of Obstetrics and Gynaecology, and Pharmacology, Univ. of Vermont, College of Medicine, Burlington, VT, USA; vst20@bath.ac.uk

Voltage-gated potassium channels (K_v) are involved in the control of myogenic tone of resistance uterine arteries therefore they could determine the blood flow to the placenta and fetus. **Aims:** We explored biophysical characteristics of transmembrane outward ion currents in smooth muscle cells (SMCs) of uterine arteries from late pregnant rats (LP) compared to those form nonpregnant (NP) controls. **Methods:** SMCs from radial uterine arteries of NP or LP were isolated using papain and 1,4-dithioerythritol. Currents were recorded using conventional whole-cell voltage-clamp technique by Axopatch 200B amplifier, Digidata 1322A and pCLAMP-9 software (Axon Instruments, CA). **Results:** SMCs from LP had a significantly larger cell capacitance compared to SMCs from NP. Outward current density was reduced in LP myocytes compared to NP. 4-AP (5mM) dramatically decreased current amplitude. The density of the 4-AP-sensitive K_v currents was significantly reduced in SMCs from LP animals relative to NP controls. Steady-state activation and inactivation studies displayed that the entire window of the current in SMCs of LP moved to rather negative voltages, was relatively wider, with steeper kinetics than in SMCs of NP. **Conclusion:** These data demonstrate that the density of outward currents through 4-AP-sensitive K_v channels was significantly diminished in SMCs of LP vessels compared to those of NP. It correlates with the increased capacitance of SMCs, which most likely reflects a significant enlargement of SMCs during gestation. Our data suggest that pregnancy may down-regulate function of K_v channels in rat uterine arteries. *Supported by NIH R01 HL-067250.*

PF16-127

The effects of bactofection mediated gene therapy using hypoxia inducible factor 1 alpha gene on markers of oxidative stress and angiogenesis in a model of intestinal ischaemia in rats

Gardlik R., Palffy R., Behuliak M., Hodosy J., Celec P.
BiomeD Research and Publishing Group, Institute of Pathophysiology and Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
romangardlik@gmail.com

Aims: The aim of this study was to prove whether hypoxia inducible factor 1 alpha (HIF) gene therapy decreases oxidative stress in a model of intestinal ischemia in rats. **Methods:** Male Wistar rats with a surgically induced ischemia of colon (caecum) or sham operated rats were treated by daily per os application of LB medium or E. coli with plasmids encoding listeriolysin and invasin (needed for the bactofection) and HIF. After one week, rats were sacrified, plasma and tissue samples were taken for analysis. Markers of oxidative stress – malondialdehyde, advanced oxidation protein products (AOPP) were measured. The expression of SOD1, SOD2 and VEGF was analyzed using real time PCR. **Results:** No significant differences were found in the analysis of oxidative stress markers. A tendency to alleviate the tissue damage measured by AOPP was seen in HIF treated animals. Interestingly, all treatments reduced VEGF expression in comparison to ischemic group without treatment ($p<0.02$). No significant differences were found in SOD1 and SOD2 expression, although the results for SOD2 indicated that HIF gene therapy might increase the antioxidant status. The differences were, however, marginally not significant. **Conclusion:** Our results show some potential for bactofection mediated HIF gene therapy, but further studies are needed to improve the bacterial vector and, thus, the efficacy of gene transfer.

PF16-128

Influence of the social stress and lead intoxication on the processes of the lipoperoxidation

Khavrona O., Fartushok N., Fedevych Y., Kobylinska L., Melekh B., Sklyarov A.
Department of Biochemistry, Danylo Halytskyi Lviv National Medical University, Ukraine

Aim: Acute lead intoxication induces changes at all levels of organisms metabolism, its functional and structural organisation. On cell level damage factors include damage of membranous apparatus and fermental systems. The organism is influenced with a significant amount of adverse ecological factors among which can be social stress (SS). Effects of the combined influence of social stress and plumbum-ions practically are still not studied. **Methods:** SS predetermined by isolation of Wistar male rats (m≈200g) during 2 days, with only water in daily ration. The following 10 days of stress rats were injected with a solution of plumbum nitrate (PN). Animals of the I group received 1/20 fatal dozes (FD) of PN, the II group – 1/200 FD. All parameters were estimated in liver homogenate. A level of MDA estimated by method of Timirbylatov and Seleznev (1981), catalase (CAT) activity – by Korolyk's method (1988); concentration of NO_2^- – by Green L.C. (1982), and diene conjugates (DC) – by Plocer's method (1982). **Results:** MDA levels (mmol/l) were the highest in the I group (579.09±5.08) compared to II group (451.208±3.59) and control group (443.69±4.09). CAT activity (mmol $[H_2O_2]$/min/mg): I group (10.29±0.049), II group (10.11±0.03), control (2.83±0.06). NO_2^- levels (mmol/l): I group (12.39±0.27), II group (11.10±0.21), control (9.76±0.12). DC (c.u.): I group (0.76±0.005), II group (0.706±0.003), control (0.087±0.008). IP: I group (0.023±0.005), II group (0.032±0.002), control (0.073±0.007). **Conclusion:** our data reveal activation of lipid peroxidation, which displays metabolic shifts under conditions of oxidation stress induced by heavy metals intoxication. As a result there is a compensatory activation of antioxidant enzymes (CAT) and NO hyperproduction. Decrease of IP at research group of animals confirms disbalance between processes of synthesis of free radicals and their inactivation. The received results depend on a dose of PN.

PF16-129

Signaling towards expression of hypoxic genes in the diabetic rat heart: role of the mitochonria

Ziegelhoeffer A.[1], Ferko M.[1], Holotnakova T.[2], Mujkosova J.[1], Pechanova O.[3], Waczulikova I.[4], Pastorekova S.[2], Pastorek J.[2]
[1]Institute for Heart Research, Centre of Excellence for Cardiovascular Research; [2]Institute of Virology; [3]Institute of Normal and Pathological Physiology, all Slovak Academy of Sciences; [4]Faculty of Mathematics Physics and Informatics, Comenius University; Bratislava, Slovakia

Background: Hearts (H) with streptozotocin (STZ) diabetes (DIA) are pseudo-hypoxic due to decreased capability of mitochondria (MIT) to utilise oxygen. However, besides pathological alterations they also exhibit compensatory and adaptation changes. **Aims:** i) Elucidation of mechanisms and outcome of STZ-DIA-induced functional alterations in H-MIT; ii) Investigation of signals by which DIA-H-MIT may crosstalk with other subcellular organells and modulate the expression of hypoxic genes. **Experimental:** DIA induced in male Wistar rats (220+20 g b. wt.) by 65 $mg.kg^{-1}$ STZ i.p., was terminated on 8th day after STZ application. MIT were isolated by differential centrifugation + protease. Estimations: MIT function, MIT-NO-synthase, conjugated dienes and membrane fluidity. MIT-signaling towards hypoxic genes (carbonic anhydrase, CA IX expression by RT PCR) was studied with diazoxide (DZO), tempol (TL), N-acetylcystein (NAC) etc., as modulators. **Results and Discussion:** DIA represented by increase in blood glucose, glycohemoglobin, triacylglycerols, cholesterol and decrease in insulin: +235.8, +89.5, +270.4, +53.6 and -53.9 % resp. MIT O_2 consumption, phosphorylation rate and NO synthase activity were also decreased ($p<0.05$), but energy transport from MIT to the cytoplasma was facilitated. DZO and TL stimulated CA IX expression, the former via inhibition of succinate oxidation followed by its efflux from the MIT, the latter may be by acting as prooxidant. NAC as antioxidant and BA as inhibitor of ROS release from MIT prevented CA IX expression. **Conclusions:** Release of succinate and radicals from functionally remodeled DIA-H-MIT represent signals for expression of hypoxic genes in the myocardium. Increased NO-signaling was not confirmed.

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PF16-130

Effect of the folic acid and of the cyanocobalamin treatment on the peripheral circulation in patients with type 2 diabetes

Muresan M., Micle L., Micle O., Demian L., Moldovan C., Vatca L., Muresan I., Dorofteiu M.
Faculty of Medicine and Pharmaceutics, University of Oradea, Oradea, Romania; marianamur2002@yahoo.com

Introduction: Previous reports show that high level of homocysteine in the blood is related to a higher risk of coronary heart disease, stroke, atherosclerosis and peripheral vascular disease. Homocysteine is an intermediate in synthesis of methionine and it is metabolised by enzymes that are dependent on vitamin B12 and folic acid. **Aim:** Our study had like target to establishment of a correlation between the reactive oxygen species and the intervention of homocysteine in the development of the cardiovascular disorders. **Material and Method:** The researches were performed on 21 patients with type 2 diabetes before and after a daily oral treatment during 3 weeks with 1 mg folic acid/day and 2 mcg cyancobalamin/day. Before and after the therapy the levels of malondialdehyde (MDA) (triobarbituric acid method) carbonylated proteins (guanidine hydrochloride method), and ceruloplasmin (Ravin method) were assessed. For the assessment of blood speed in the peripheral vessels the echo Doppler method was used. The level of blood glucose, cholesterol, creatinine and triglyceride was also determined. **Results:** The MDA is significantly raised and the treatment with folic acid and vitamin B12 is able to reduce its level, but not to the normal value. The carbonylated proteins have also a high concentration in comparison to the control group. **Conclusions:** The treatment was not able to improve the peripheral circulation but it was efficient in the removal of the oxidative stress.

PF16-131

Vascular reactivity in oxidative stress

Tkachenko M.N., Kotsjuruba A.V., Sagach V.F.
A.A. Bogomolets Institute of Physiology NAS Ukraine, Kiev, Ukraine
circul@biph.kiev.ua

Aim: Nitric oxide (NO)-dependent mechanisms of vascular regulation and metabolism of the reactive oxygen species (ROS) and stable NO metabolites were studied in the aorta of BALB/c mice in conditions of oxidative stress induced by long-term exposure to low doses of external γ-irradiation. **Methods:** Endothelium-dependent and endothelium-independent vascular reactions of relaxation, content of ROS and stable NO metabolites were studied on aorta preparations of two groups of BALB/c mice: I – control (6-8 mo.); II – irradiated (equivalent dose of 96.9 mcSv/h) 8-mo. mice (cumulative dose of 0.43 Sv). **Results:** Low doses of radiation were found to inhibit endothelium-dependent reactions of aortal smooth muscles to acetylcholine and to partially impair endothelium-independent relaxation to sodium nitroprusside. This is accompanied by a formation of high levels of superoxide anion, hydroxyl radical, and significant changes in the pools of stable NO metabolites (nitrite- and nitrate-anions). In increased simultaneous generation of $\cdot O^{2-}$ and NO they may bind and thus form a toxic substance peroxynitrite. This can be confirmed by the low doses of nitrite, which are formed spontaneously in the presence of molecular oxygen against the background of increased or control levels of nitrate, which is formed mainly at the degradation of peroxynitrite, i.e. at high levels of superoxide anion. **Conclusion:** Long-term exposure to low doses of γ-irradiation results in the development of oxidative stress with an increase of ROS content and disturbance of vascular reactivity.

PF16-132

The roles of TRP channel and NCX in mediating hypoxia-induced $[Ca^{2+}]_i$ elevation in PC12 cells

To W., Meng F., Wang S., Kumar P., Gu Y.
Department of Physiology, University of Birmingham, B15 2TT, UK
Contact y.gu@bham.ac.uk

Mammalian cells require a constant O_2 supply to maintain adequate energy production and sophisticated mechanisms have evolved to allow cells to sense hypoxia. In this study, we investigated the role of transient receptor potential (TRP) channels and the Na-Ca exchanger (NCX) in mediating hypoxia-induced $[Ca^{2+}]_i$ elevation in a rat pheochromocytoma (PC12) cell line, a model of O_2-sensing. In these cells, PCR and western blot confirmed the presence of TRPC1, 3 and 6 as well as NCX. Hypoxia consistently induced a reversible, Ca^{2+} elevation, which was sensitive to nifedipine (20μM). The block of TRPC by 2-APB (100 μM) and SKF 96365 (40 μM) significantly reduced hypoxia-mediated $[Ca^{2+}]_i$ elevation by 52 ± 7.5% and 56 ± 9.1%, respectively. The activation of TRPC in mediating the Ca^{2+} response to hypoxia was, in part, via the G-protein and PLC pathway as it was blocked by 27-30% by suramin (50μM) and U73122 (10μM). In addition to TRPC, NCX also contributed to the hypoxia response and blockage of NCX by KBR9473 (10μM) significantly attenuated the hypoxia induced $[Ca^{2+}]_i$ elevation by 41 ± 7.6%. Our results show that hypoxia induced Ca^{2+} can occur via TRPC and NCX channels in PC12 cells. We suggest that TRPC channels may play a major role in mediating the hypoxia response in these cells, perhaps by contributing to cell depolarization.

PF16-133

Variability of thiobarbituric acid reacting substances in saliva

Behuliak M., Palffy R., Celec P.
Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
michalbehuliak@gmail.com

Aim: Salivary thiobarbituric acid reacting substances (TBARS) have been proved as a potential marker of intraoral oxidative stress and parodontal status. This study was aimed at the analysis of intra- and interindividual variability of TBARS in saliva. **Methods:** Twenty two young healthy volunteers (12F & 10M) collected saliva samples daily in the morning during a period of 30 consecutive days. Salivary TBARS were measured spectrophotometrically. Time series analysis was done using standard statistical methods. **Results:** Repeated measures ANOVA showed significant between day variations of salivary MDA ($p<0.001$). The dynamics did not differ between genders, however, the data was not synchronized, and thus, gender differences in endogenous dynamics cannot be ruled out. Intraindividual variability was very high in both genders with coefficients of variation of more than 70%. Interindividual variability was higher in men than in women (63% vs. 20%; $p<0.001$). **Discussion:** The relatively high intraindividual variability indicates that repeated samplings and subsequent measurements are needed for individual diagnostics. Gender differences and interindividual variability will be taken into account in running clinical studies on patients with periodontal and dental diseases. Factors influencing the variability of salivary TBARS including infradian biorhythms should be uncovered by further studies.

PF17-134

Omega-3 fatty acids protect male and female aged hypertensive rat heart against ventricular fibrillation

Fialova M.[1], Dlugosova K.[1], Knezl V.[2], Okruhlicova L.[1], Drimal J.[2], Tribulova N.[1]
[1]Institute for Heart Research, SAS; [2]Institute of Experimental Pharmacology, SAS, Bratislava, Slovak Republic
usrdmafi@savba.sk

Aims: We have shown previously that hypertension-related structural and gap junction connexin-43 (GJCx43) remodelling is involved in the development of ventricular fibrillation (VF). Clinical and experimental studies suggest antiarrhythmic effects of omega-3 fatty acids (PUFA), however, mechanisms are not fully elucidated. Aim of the study was to examine whether PUFA affect susceptibility of aged spontaneously hypertensive rats (SHR) to VF and expression of GJCx43, which ensures cell-to-cell synchronisation. **Methods:** Male and female 14 month-old PUFA fed (20 mg/day/2 months) SHR and age-matched controls were used. Electrically inducible VF was performed on isolated heart preparation. Myocardial GJCx43 was detected using immunofluorescence and western blotting (WB) and intercellular synchronisation using ultrastructure examination. **Results:** PUFA resulted in significant blood pressure reduction in both male and female SHR. All untreated SHR hearts were prone to develop sustained VF. In contrast, VF was suppressed by 57% and 67% in PUFA – treated male and female SHR despite myocardial remodelling, i.e. fibrosis and hypertrophy were not eliminated. Immunolabelling of GJCx43 showed that neither hypertension-related remodelling of GJCx43 nor its levels was affected by PUFA. However, WB revealed elevated GJCx43 phospho-isoforms. Moreover, cardiomyocyte ultrastructure alterations and impairment of cell-to-cell synchronisation was attenuated in PUFA-treated SHR hearts. **Conclusion:** PUFA exert clear anti-fibrillating effects in aged male and female SHR, whereby modulation of GJCx43 channel function may be one of the mechanisms involved in cardioprotection.
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PF17-135

Distinct connexin-43 expression contributes to gender differences in occurrence of lethal arrhythmias

Knezl V., Drimal J., Fialova M., Dlugosova K., Okruhlicova L., Tribulova N.
Institute of Experimental Pharmacology, Institute for Heart research, SAS, Bratislava, Slovak Republic; exfaknem@savba.sk

Aims: Various clinical and experimental data have identified sex differences in cardiac pathophysiology and incidence of cardiac arrhythmias. The mechanisms responsible for the disparities are still being elucidated. Since gap junction conexin-43 (Cx43) channels determine myocardial conduction velocity and synchronisation we hypothesized that expression and/or distribution of Cx43 may differ between males and females. **Methods:** Experiments were conducted on aged (>1 year-old) male and female Wistar and spontaneously hypertensive (SHR) rats. Ventricular tissue taken from excised hearts was immediately frozen in liquid nitrogen and processed either for immunofluorescence labelling or western blotting of Cx43 using mouse MAb. Susceptibility to lethal ventricular fibrillation (VF) was examined using isolated heart preparation and electrical burst stimulation or hypokalemic perfusion. **Results:** Both male and female SHR rats were more vulnerable to VF compared to Wistar counterparts. In correlation, Cx43 examination revealed that its ventricular tissue expression was significantly lower in hypertensive (SHR) than normotensive (Wistar) rats. In addition, SHR hearts exhibited abnormal myocardial distribution of Cx43. Comparing to male either SHR or Wistar rat hearts female ones were significantly less susceptible to VF. It correlated with significantly higher Cx43 expression in females comparing to males. **Conclusion:** Taken together these findings indicate that the level of myocardial Cx43 expression is linked with lethal arrhythmia susceptibility and it may help to explain gender-related differences.
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PF17-136

Structural correlate of increased cardiac susceptibility to malignant arrhythmias

Slezak J.[1], Fialova M.[1], Dlugosova K.[1], Okruhlicova L.[1], Knezl V.[2], Kristek F.[3], Tribulova N.[1]
[1]Institute for Heart Research; [2]Institute of Exper. Pharmacology; [3]Institute of Normal and Pathol Physiology, SAS, Bratislava, Slovak Republic
jan.slezak@up.upsav.sk

Aims: Rapid spreading of the electrical impulse throughout the heart, ensured by electrical coupling at the gap junctions (GJs), is essential for synchronised contraction. Alterations in myocardial architecture and topology of GJs may result in conduction abnormalities facilitating occurrence of lethal arrhythmias. We examined ultrastructure and arrhythmia susceptibility of the hearts from various models of diseased rats. **Methods:** Ventricular tissues of male, 12-16 week-old rats suffering from hypertension (spontaneous and L-NAME-induced), diabetes and hereditary hypertriglyceridaemia as well as age-matched controls were routinely processed for electron microscopic examination. Lethal arrhythmia susceptibility, i.e. incidence of electrically- or hypokalemia-induced ventricular fibrillation (VF) was monitored in isolated heart preparation. **Results:** Marked subcellular alterations indicating hypertrophy of cardiomyocytes were found in both models of hypertensive while not in diabetic or hypertriglyceridaemic rat hearts. Extracellular matrix alteration, i.e. replacement fibrosis was pronounced in hearts of L-NAME and HTG and to lesser extent in diabetic rats. Unlike to control rat hearts with majority of GJs confined to intercalated disc (end-to-end type), diseased rat hearts were characterized by enhanced number of lateral (side-to-side-type) GJs. Lateralization was most pronounced in hypertensive and hypertriglyceridaemic, while much less in diabetic rat hearts. In correlation with structural and GJs changes, highest susceptibility to VF exhibited hypertensive and hypertriglyceridaemic rat hearts, while lower diabetic and lowest control rat hearts. **Conclusion:** The findings support hypothesis that structural and gap junctions remodelling increase susceptibility of the heart to lethal arrhythmias.
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PF17-137

Up-regulation of connexin-43 is associated with decrease while down-regulation with increased susceptibility of rat heart to life-threatening arrhythmias

Tribulova N.[1], Fialova M.[1], Knezl V.[2], Dlugosova K.[1], Hailin H.[3], Imanaga I.[3], Okruhlicova L.[1]
[1]Inst. Heart Res.; [2]Inst. Exp. Pharmacol., SAS, Bratislava, Slovakia; [3]Dept. Physiol. Fukuoka Univ., Fukuoka, Japan; narcisa.tribulova@savba.sk

Aims: Functional electrical and metabolic communication at the gap junction connexin channels ensures myocardial synchronisation, while connexin abnormalities are thought to be arrhythmogenic. We examined, therefore, topology, expression and phosphorylation of connexin-43 (Cx43) as well as susceptibility of the heart to ventricular fibrillation (VF) in various models of cardiomyopathy. **Methods:** Experiments were conducted on male adult spontaneously hypertensive rat, STZ-diabetic rat and hyperthyroid rat hearts. Distribution of Cx43, its expression and phosphorylation were analysed using immunodetection and Western blotting with mouse MAb. Susceptibility to VF was examined in isolated heart preparation using electrical stimulation or hypokalemic perfusion. **Results:** Immunodetection revealed besides end-to-end (intercalated disc-related) enhanced expression of side-to-side Cx43 positive gap junctions in hypertensive and hyperthyroid rat hearts. In addition, total Cx43 and its phosphorylated isoforms were significantly decreased in hyperthyroid and hypertensive, while increased in diabetic rat hearts. In correlation diabetic rats were less while hypertensive and hyperthyroid rats much more prone to develop VF. Interestingly, susceptibility to VF was increased in diabetic rats treated with thyroid hormone that was linked with suppression of Cx43 expression and phosphorylation. **Conclusions:** These findings indicate that intercellular channel protein Cx43 is involved in the modulation of susceptibility of the heart to malignant arrhythmias. Abnormal distribution and down-regulation of Cx43 increase a risk for VF.

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PF17-138

Detection of endothelin-A receptors in cultured myocytes using patients' sera autoantibodies

Okruhlicova L.[1], Schulze W.[2], Bartel S.[2], Wallukat G.[2]
[1]Institute for Heart Research, SAS, Bratislava, Slovak Republic; [2]Max-Delbruck-Center, Berlin, Germany

Aim: Endothelin-1 (ET) is involved in many processes of heart function. Its effects are mediated via specific G-protein-coupled receptor subtypes ETA and ETB. Autoantibodies (AAB) against ETA-R were found in sera of patients with cardiovascular diseases. They exert a negative chronotropic effect in cultured rat cardiomyocytes antagonized by the ETA-R antagonist BQ 610. We examined cellular localization of ETA-R in cultured human smooth muscle cells and cell cultures of 3-day-old heart myocytes isolated from neonatal Wistar rats. **Methods:** Cell cultures were incubated with AAB against ETA-R (1:100) for 1h. AAB were affinity purified from sera of patients. Incubation of cells with commercial ETA-R-AB, ET-1 peptide and free of primary AB served as controls. Thereafter, cells were fixed and stained with corresponding secondary AB for immunofluorescence. **Results:** Presence of ETA-R was demonstrated on surface of cells of smooth muscle and heart. The intensity of immunofluorescence was different in variable cell types. In lesser extent ETA-R were also detected in the cell nuclei. In controls, no specific reaction was seen. **Conclusion:** Results indicate that AAB against ETA-R found in sera of patients with heart diseases might bind to membrane ETA-R and contribute to progression of heart dysfunction.

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PF17-139

Stress induced changes in the gene expression of adrenoceptors in rat heart

Tillinger A.[1], Myslivecek J.[2], Petrak J.[1], Krizanova O.[3], Kvetnansky R.[1]
[1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia; [2]First Faculty of Medicine, Charles University, Prague, Czech Republic; [3]Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovakia.
ueentill@savba.sk

Aims: Stress-induced elevation of plasma noradrenaline and adrenaline levels is well established response of the organism, which should stimulate adrenoceptors (AR) and lead to changes in mRNA and protein levels in the heart. The aim of this study was to measure mRNA levels of AR in heart atria and ventricles during exposure to immobilization (IMO) or cold stress. **Methods:** Changes in gene expression of AR were studied by RT PCR in animals exposed to acute and repeated/chronic IMO and cold. **Results:** No significant changes in mRNA levels of β_1-AR in the hearts of rats exposed to IMO or cold were found. On the other hand a significant decrease in β_2-AR mRNA levels in atria and ventricles of immobilized animals were shown. In hearts of cold stressed animals no changes of the β_2-AR at mRNA levels were detected. Unexpectedly, we found a significant increase of β_3-AR mRNA levels in atria and ventricles of IMO rats. Increase in β_3-AR mRNA levels was also observed in ventricle of cold stressed animals. No significant changes of α_{1B}-AR mRNA levels were observed in hearts of animals exposed to IMO. In cold stressed animals we found a significant decrease of α_{1B}-AR mRNA levels in atria and ventricles. **Conclusion:** Our results point to different regulation of individual types of AR by various stressors. Also, since β_2- , β_3-AR and α1B-AR are expressed in the opposite manner, their role in the process of heart adaptation to stress is likely possible. Nevertheless, further experiments must verify this proposal.

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PF18-140

Endothelium moderates anaphylactic reaction of small isolated arteries in guinea pig

Laukeviciene A.[1], Korotkich I.[1], Civinskiene G.[1], Kevelaitis E.[1], Stankevicius E.[1,2], Simonsen U.[2]
[1]Department of Physiology, Kaunas University of Medicine, Kaunas, Lithuania; [2]Department of Pharmacology, University of Aarhus, Aarhus, Denmark
ale.laukeviciene@googlemail.com

Aim: Cardiovascular events during anaphylaxis may result in life dangerous state – circulatory shock. The role of endothelium in different vascular beds during this pathological state remains to be elucidated. The purpose of this study was to assess the possible implication of vascular endothelium into vascular events during anaphylaxis. **Methods:** Guinea pigs were actively sensitized using horse serum. For investigation we used small mesenteric and coronary arteries (350-450 μm in diameter) from sensitized and sham sensitized guinea pigs. Anaphylaxis of blood vessels was performed in vitro adding 1% of horse serum to the organ bath. Vascular effects of anaphylaxis were quantified using small blood vessels wire myography method. **Results:** Antigen challenge resulted in anaphylactic contraction in both mesenteric (3.69±1.72 mN) and coronary (3.45±1.02 mN) arteries. This contractile response remained after the removal of endothelium, but it was decreased till 56.3±11.8% ($p<0.05$) in coronary vessels and till 90.8±8.6 % ($p>0.05$) in mesenteric vessels. The inhibition of nitric oxide synthase using L-NAME decreased the anaphylactic contraction in mesenteric arteries (till 64.2±20.8%; $p>0.05$), but increased in coronary arteries (till 187.8±32.3%; $p<0.05$). **Conclusion:** The endothelial factors play modulating role in anaphylaxis of small isolated coronary and mesenteric arteries in guinea pig, acting against anaphylactic contraction.

PF18-141

New data regarding the influence of contracting agent upon the profile of mechanisms that ensure endothelium-dependent relaxation in resistance arteries

Serban I.L.[1,2*], Serban D.N.[1,2], Hogas S.M.[3], Varlam H.[4], Mungiu O.C.[2,5]
[1]Department of Physiology; [2]Center for the Study and Therapy of Pain**; [3]Clinic of Nephrology; [4]Department of Anatomy; [5]Department of Pharmacology, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania; dnserban@yahoo.com

Aims: Endothelium-dependent relaxation (EDR) presents multiple differences according to species, age, sex, hormonal status, vascular territory and arterial caliber, the mechanical or chemical vasodilating agent, pre-existing vascular tone and its determining factors. Regarding the latter things are surprisingly little investigated explicitly. We noticed that in rat mesenteric resistance arteries (second order branches) EDHF-mediated relaxation is increased when they are precontracted by prostaglandin $F_{2\alpha}$ (PGF) compared to phenylephrine (PHE) and we investigated the participation of K^+ channels (K_{Ca} and K_{ATP}) in the observed effects. We extended this study upon angiotensin II and upon vascular fragments from first order branches. **Methods:** EDR was evaluated by isometric myography in rings prepared from branches of the mesenteric artery (first and second order). **Results:** In the case of angiotensin the phasic component of contraction is affected similarly with the PHE response, while the tonic one resembles the PGF response. The EDHF component is stronger in second order branches only when precontraction is induced by PHE and against the phasic response elicited by angiotensin II. **Conclusion:** Others have shown that the EDHF phenomenon increases in relative importance towards periphery, but it seems that this depends upon the contracting agent used, a novel observation that may change interpretation of the physiological relevance of existing data regarding EDHF and nitric oxide as mediators of EDR in resistance arteries.
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PF18-142

The role of physical exercise in improving the endothelial dysfunction induced by chronic exposure to cigarette smoke: a study on thoracic aorta at guinea pigs

Pantea V.[1], Noveanu L.[1], Ordodi V.[1], Oancea C.[1], Fira-Mladinescu O.[2], Mihalas G.[1]
[1]Department of Physiology UMPh „Victor Babes" Timisoara, Romania; [2]Department of Pathophysiology UMPh „Victor Babes" Timisoara, Romania; vlad_pantea@yahoo.com

Aim: Chronic exposure to cigarette smoke induces endothelial dysfunction by decreasing the endothelial-dependent vasodilator response to acetylcholine, without changing the endothelial-independent vasodilator response to sodium nitroprusside. The purpose of the study was to test if the physical exercise improves the endothelial dysfunction induced by chronic exposure to cigarette smoke. **Methods:** A number of 16 guinea pigs were exposed for 10 weeks to cigarette smoke (5 cigarettes/day, 3 cigarettes/ hour, 20 minutes interval, twice a day). From these 16 guinea pigs, 8 were trained on an ergonomic device (a moving carpet), increasing the time (60 – 90 min.) and the intensity of the physical effort (0° – 20° slope), with the speed of 0.25 m/s, for 10 weeks. The results were compared with the control group (n=8). In the isolated organ bath, the vascular preparations' reactivity was evaluated by determining the dose – effect curves to acetylcholine (10^{-9} – 10^{-4}M) and sodium nitroprusside (10^{-9} – 10^{-4}M), obtained after the precontraction with phenylephrine 10^{-5}M. **Results:** The dose-effect curves were compared based on all of the three parameters from Hill's sigmoidal equation: the maximal relaxation (%), EC50% (-log [M]) and the Hill coefficient. These curves showed a significant difference $p<0,001$ between the groups regarding the vasodilator response to acetylcholine. **Conclusion:** The physical exercise decreased the effects of chronic exposure to cigarette smoke on the endothelial-dependent vasodilatation and did not change the vascular smooth muscle responsivity.

PF18-143

Mechanisms of endothelium-dependent relaxation in the renal interlobar artery

Serban I.L.[1,2*], Maranduca M.A.[1], Serban D.N.[1,2], Slatineanu S.M.[1], Petrescu G.[1]
[1]Department of Physiology; [2]Center for the Study and Therapy of Pain**, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
dnserban@yahoo.com

Aims: Based on our experience regarding endothelium-dependent relaxation (EDR) in resistance mesenteric arteries, we started an investigation upon renal interlobar arteries of the rat. **Methods:** Isometric myography was used to study interlobar renal arteries (1 mm rings, ~200 μm diameter) from adult male Wistar rat. We used a bicarbonate buffer physiological saline solution, at 37°C, bubbled with 95%O_2 + 5% CO_2; pH 7.2 – 7.4. After 2 h equilibration under 1 g tension, in the ring precontracted by 0.01 mM phenylephrine we have separately examined: global EDR induced by 0.01 mM carbachol, the EDHF component of EDR (in presence of 0.01 mM L-NAME and 0.01 mM indomethacin, to inhibit cyclooxygenase and nitric-oxide-synthase), and the participation of calcium-dependent potassium channels (K_{Ca}) in the EDHF phenomenon, using tetraethylammonium 0.01 mM as inhibitor. The results are expressed as residual active tension, % from the precontraction level (mean ± ESM; n=4). **Results:** The EDHF response (42.5 ± 3.3 %) is blocked by the K_{Ca} inhibitor (97.5 ± 5.4 %). The results are compared to those obtained by other authors in pig interlobar renal arteries and in upstream renal vascular fragments from Wistar-Kyoto and Sprague-Dawley rats, mice and rabbits. **Conclusion:** This is the first study regarding the physiological relevance of EDHF in rat interlobar renal arteries and data show that it has a participation comparable with NO, involving K_{Ca}.
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PF18-144

The effect of cigarette-extract-smoke exposure on vasodilatation induced by 5'adenosine on guinea pigs thoracic aorta

Sturza A.[1], Noveanu L.[1], Fira-Mladinescu O.[2], Ordodi V.[1], Mihalas G.[1]
[1]Department of Physiology, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania; [2]Department of Physiopathology, University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania

Aim: Arterial endothelium is a mechanic and biologic "barrier" and also an "organ" which secrets active factors necessary for vasomotoricity and integrity of vascular wall. One of the most important of these factors is nitric oxide which is released from endothelial cell at the action of acethylcholine or adenosine on specific receptors. **Methods:** Our study performed, using an isolated organ bath, analysed the vascular reactivity of 20 guinea pigs thoracic aorta. We analyzed the dosage-effect curves to phenylephrine-PHE, adenosine-5'ADP and sodium nitroprusside-NPS, before and after an incubation with hydrosoluble cigarette extract smoke 5% during 1 hour. The statistics completed with Student t test and ANOVA test, show that exposure of guinea pigs to cigarette extract smoke determined a significant increase of the contractile response at PHE 10^{-5} mol/l ($p<0.05$) and a significant reduction of the endothelial-dependent relaxing response at 5'ADP 10^{-5} mol/l ($p<0.005$), but does not significantly modify the endothelial-independent relaxing response to NPS 10^{-5} mol/l ($p=0.05$). **Conclusions:** In conclusion, incubation with cigarette extract smoke of vascular rings has diminished the production of NO on action of ADP (endothelial-dependent relaxation), without the modification of the muscular response on action of NO from NPS (endothelial-independent relaxation).

PF18-145

Manganese supplementation affects endothelium-dependent relaxation in rats

Serban D.N.[1,2], Stoica B.[3], Hogas M.[1], Serban I.L.[1,2*], Budurca A.R.[4], Nechifor M.[5]
[1]Department of Physiology; [2]Center for the Study and Therapy of Pain**; [3]Department of Biochemistry; [4]Clinic of Reparatory and Plastic Surgery; [5]Department of Pharmacology, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
dnserban@yahoo.com

Aims: We have previously studied the effect of Mn ($MnCl_2$ 3 mg/kg, 7 days), in rats, healthy and with alloxan-induced diabetes and shown there is no correlation between Mn influences upon blood glucose and total antioxidant status. Then we found among others that endothelium-dependent relaxation (EDR) induced by carbachol (10^{-5} M) could be affected by Mn. **Methods:** We have repeated the experiments in healthy rats using a higher Mn dose ($MnCl_2$ 10 mg/kg, 7 days). The animals were sacrificed to study the contractile activity in ring fragments from the aorta and small mesenteric arteries, in isometric conditions. **Results:** We observed the reduction down to disappearance of EDR in both vessel types, with no changes in the contracting effects of K 40 mM and phenylephrine 10^{-5} M, or in the relaxing effects of nitroglycerine 10^{-6} M (upon both precontractions) and methoxy-verapamil (D600) 10^{-5} M (in K-contracted rings). In resistance arteries the EDHF component of EDR (in presence of 0.01 mM L-NAME and 0.01 mM indomethacin) was increased, partially compensating the reduction by Mn of nitric oxide dependent relaxation. **Conclusion:** We show for the first time the appearance of toxic Mn effects upon endothelium at doses way bellow those recognized as toxic at the hepatic and neural level.

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PF18-146

Effect of arterial wall shear stress on the dynamic elasticity of a conduit artery

Kelly R.F.[1], Snow H.M.[2]
[1]Hatter Institute for Cardiology Research, Chris Barnard Building, University of Cape Town, Cape Town, South Africa; [2]University College Cork, Cork, Ireland
Roikelly@capeheart.uct.ac.za

Aims: The interaction between smooth muscle and collagen largely determine the dynamic stress strain characteristics (E_{dyn}) of the artery wall. The purpose of this investigation was to determine the effects of increases in WSS (which causes smooth muscle relaxation) on E_{dyn}. **Methods:** Experiments were carried out in 4 female anaesthetized pigs (24-31kg) (induction pentobarbitone 30mg/kg i.v., maintenance 6mg/kg/h). The iliac artery and vein were connected by a shunt with a variable resistance which allowed blood flow and therefore WSS to be regulated. Stepwise increases in WSS were obtained without causing significant changes in MAP. E_{dyn} was calculated from diameter and pressure throughout a minimum of 5 cardiac cycles. **Results:** Increases in WSS caused a significant increases in diameter from 3.663±0.215mm to 4.488±0.163mm (mean±sem. $P<0.05$) and a corresponding fractional increase in diameter (fD) (range 1 – 1.5) with no significant change in MAP, 108±2mmHg to 106±1mmHg (mean±sem). The average value of E_{dyn} per cardiac cycle at baseline (fD= 1) was $2.17\pm0.10.10^3$kPa and increased to a maximum of $9.23\pm1.0.10^3$kPa when fD was 1.5. The relationship between E_{dyn} and fD is curvilinear (E_{dyn} = tan [3.43(fD-1.08)]+2.3) and remains relatively constant until fD exceeds 1.3. **Conclusion:** These findings are consistent with a model of an arterial wall in which collagen is recruited both by passive stretch, in response to an increase in wall tension, and by a contraction of smooth muscle. This confirms that in a conduit artery, during WSS induced dilatation, the interaction between smooth muscle and collagen operates so as to maintain E_{dyn} relatively constant.

PF18-147

K-influence on motor and electrical vesical oscillations: stretch-dependent channels

Martin D., Gornik E., Foltin V., Seidenbusch W., Michailov M.C., Neu E.
Inst. Umweltmedizin c/o ICSD e.V. Muenchen-Paris-Sofia/Kazanlak & Univ. Erl.-Nuernberg; Inst. Physik, Univ. Bratislava; Inst. Exp.Physik, Univ. Innsbruck-Vienna; eva_neu15@hotmail.com

Introduction: In the last two decades we reported on various kinds (uniform, regular or not, burst-like) of vesical (and pyeloureteral) motor and electrical patterns [Proc. IUPS 17 (Helsinki) 529/1989; 18 (Glasgow) 140.31&140.32/1993; 21 (San Diego) Faseb J., 19/4, A585/2005]. Presently will be informed on their K-dependence. **Method:** Motor (isometric), electrical (intracellular-recording) vesical-activity (guinea-pig). **Results:** Spontaneous phasic detrusor-contractions (SPC): Detrusor-amplitudes (4.1±2.4 mN=100%) increased after 3xKCl (normal 5.6 mM=1x, McEwen-solution) 354% (at 3 mN) resp. 467% (50 mN stretch), frequency was unchanged (4.0±0.7/min=100%). Tonic trigonum-contractions (STC): Amplitudes (17.2±11.6 mN) were unchanged after 3xKCl, but frequency (0.3±0.1/min=100%) increased to 198% (3mN)/764% (50 mN). K-effect on electrical action potentials of vesical myocytes: Not only spike (S) activity was transformed into a burst-plateau (BP) one by stretch (3 to 80 mN), but also 2-5x [KCl] induced BP with decreased duration and increased frequency (proportionally to [KCl]). The K-phenomena appeared also in whole bladder preparations in vitro and in toto (isovolumetric cystotonometry) (total n=60). **Conclusion:** Various kinds of electrical and motor detrusor patterns are related probably to stretch-dependent K-channels, also to gap-junctions (intercellular excitation-conduction) [Eur. J. Physiol. 443, S334/2002; 430, 163/1995; 420, R99/1992; 419, R98/1991; Br. J. Urol. 94/2, 258/9/2004; Urol. 68/5A, 78-9/2006]. Further investigations could open new possibilities for a vesical pharmacological and electrotherapy (incontinence, overactive-bladder, etc.).

PF18-148

Human uterine motor oscillations and tocolytic therapy

Neu E., Srivastava-Werner A., Welscher U., Gupta S.K., Martin D., Michailov M.C.
Inst. Umweltmedizin c/o ICSD e.V. Muenchen-Paris-Sofia/Kazanlak & Univ. Erl.-Nuernberg/Germany; All Indian Inst.Med.Sci., New Delhi/India
eva_neu15@hotmail.com

Introduction: Myometrial motor oscillations are fundamentals for parturition. Investigations demonstrate a probable relation between motor activity of myometrium, uterine artery, vesical trigone from surgical tissue (Abstract-Book-1, Int. Congr. Gyn. Kuala-Lumpur, 64/2006; Br. J. Urol. 94/2, 258-9/2004; Urol. 68/5A, 78-9/2006). Recent and earlier results support the possibility for a more effective treatment of pathological myometrial activity. **Method:** Motor (isotonic) activity of preparations (see introd.). **Results:** Human myometrium generates spontaneous slow tonic contractions (STC: 0.22±0.01/min) with amplitudes of 30-5% (mean=12%) of prep. length (n=101): STC can be transformed into spontaneous fast phasic contractions, similar to uterine tube (SPC: 0.65±0.35/min), with small amplitudes (about 1-6%) by combined effects of hormones and drugs [1 nM – 10 µM: met-/leu-enkephalins, melatonine, cAMP, PGE1; herbal drugs, antihistaminics, phosophodiesterase inhibitors, ß-sympathomimetics, Ca^{++}-antagonists]. Hormones (adrenaline, 5-HT) induced STC in uterine artery. Human (and guinea pig, n=40) trigone generated also STC (0.3±0.1/min); detrusor SPC (3.8±0.9/min) could be transformed into STC by TEA/$BaCl_2$. Anaesthetics (e.g. procaine): 0.1-10 µM inhibited STC/electro-induced contractions (10Hz, 0.3-40ms, 3s). **Conclusion:** SPC/STC are probably related to electrical stretch-dependent spike/burst-plateau activities, similar to vesical myocytes (Eur. J. Physiol. 443S, 334/2002; Faseb J. 19/4, A585/2005). Systematic investigations of myometrial electro- (K-/Ca-stretch channels) and pharmaco-physiology could open new approaches for more effective (incl. tokolytic) therapy of abortus, partus pramaturus et serotinus, dysmenorrhoe, etc.

PF18-149

Vascular effects and mechanisms of action for certain enkephalin-related peptides

Serban D.N.[1,2], Serban I.L.[1,2]*, Jaba I.M.[2,3], Tamba B.[2,3]**, Mungiu O.C.[2,3]***
[1]Department of Physiology; [2]Center for the Study and Therapy of Pain***; [3]Department of Pharmacology, University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania
dnserban@yahoo.com

Aims: Opioid peptides exert direct vascular actions (via opioid receptors located in the vascular wall), explained to a certain extent by the presynaptic inhibition of the neuro-effector junction and by the release of nitric oxide from the endothelium, but there are few studies regarding their action in resistance arteries. Based on our own studies upon analgesic properties, we have selected for testing the vascular effects the following peptides (10^{-9}-10^{-6} M): leucin-enkephalin, methionin-enkephalin, endomorphine 1, endomorphine 2, morphiceptin, Phe-Gly-Gly and Phe-Gly (the C-terminal tri-peptide and di-peptide from nociceptin). **Methods:** We used isometric myography to study the effect of these peptides in small mesenteric arteries (~2 mm wide and ~0.15 mm diameter rings) from adult male Wistar rats. In preparations precontracted by 0.01 mM phenylephrine we examined the relaxing effect of each peptide in three circumstances: de-endothelised rings, control intact rings and intact rings under inhibition of nitric-oxide-synthase by 0.01 mM L-NAME. The results are expressed as remnant active tension, % from the precontraction level (mean ± ESM; n=4). **Results:** We noticed, among others, the weak effect of methionin-enkephalin, the partial endothelium-dependence and NO-independence of the effect for the nociceptin fragments, the presence of the effect for small concentrations and the NO-dependence in the case of endomorphins. **Conclusion:** All these represent novel findings and the possible physiological relevance is discussed in the context of few available data on the vascular action of enkephalin-related peptides.

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PF18-150

Beneficial effect of sodium selenate on vascular dysfunction in diabetic rats

Zeydanli E.N., Tanriverdi E., Seymen A., Tuncay E., Gurdal H., Koksoy A., Turan B.
Dept. of Biophysics, Ankara University, Turkey
ezeydanli@yahoo.com

Aims: Since selenium compounds can restore some metabolic parameters and structural alterations of diabetic rat tissues, we tempted to investigate whether these beneficial effects extend to the diabetic rat isolated thoracic aorta dysfunctions. **Methods:** Diabetes was induced by streptozotocin (50 mg/kg body weight) and rats were treated with sodium selenate (15 μmol/kg body weight/day) for 4 weeks. **Results:** Sodium selenate treatment of diabetic rats induced a significant recovery (80% wrt diabetes) in the depressed phenylephrine (10^{-8}–10^{-5} M) stimulated isometric contractions (50% wrt control) in aorta while we obtained 100% recovery in the depressed relaxation responses (30% wrt control) with isoproterenol (10^{-9}–10^{-4} M) without any significant changes in Log50 values. Sodium selenate treatment of the diabetic rats also restored the altered activities of several antioxidants enzymes of which are involved in the glutathione metabolism of the heart as well as the levels of glutathione and oxidized protein sulfhydryls while no significant effect on high blood glucose level. Our data indicate that an oxidant shift of cellular thiolic pools can modulate contraction-relaxation activities of thoracic aorta in diabetic rats. **Conclusion:** It can be summarized that selenium employs important roles in altered contraction-relaxation activities of thoracic aorta via affecting the glutathione redox cycle to combat oxidative stress in diabetes and small doses of selenium compounds may be useful as an adjunctive therapy in human diabetes.

PF18-151

Preliminary data on the influence of resting tension upon endothelium-dependent relaxation

Serban I.L.[1,2]*, Oprisa C.[1,2], Tucaliuc S.E.[1], Branisteanu D.D.[3], Serban D.N.[1,2]
[1]Department of Physiology; [2]Center for the Study and Therapy of Pain**; [3]Clinic of Endocrinology, University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania
dnserban@yahoo.com

Aims: Endothelium-dependent relaxation (EDR) differs upon species, age, sex, hormonal status, vascular territory and caliber, vasodilating agent, pre-existing tone. There are few studies regarding the effect of tone, so we investigated the effect of resting tension upon the EDHF response. **Methods:** We used isometric myography and mesenteric resistance arteries (2 mm rings; ~0.15 mm diameter) from male Wistar rats (200-250 g). After 2 h equilibration under a tension of 1 g or 2 g, the ring was precontracted by 0.01 mM phenylephrine and we tested EDR induced by 0.01 mM carbachol or the EDHF component of EDR (in presence of 0.01 mM L-NAME and 0.01 mM indomethacin). Results are expressed as residual active tension (RAT), % of precontraction level (mean ± SEM; n=4). **Results:** The chosen preparation does not display a myogenic response to stretch and carbachol does not decrease baseline tension. Contraction is ~25 % stronger in rings pretensioned at 2 g, EDR is complete for both pretension levels, while the EDHF component is more ample ($p<0.01$; Student's t-test) at 2 g (45.4 ± 3.1 % RAT), vs. 1 g (62.7 ± 2.8 % RAT). **Conclusion:** The antagonism between myogenic response and the EDHF phenomenon is well known, but our novel observations indicate that the release/action of EDHF is potentiated by a simple increase of parietal tension, which may be of functional relevance in both acute and chronic increases of arterial pressure.

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PF19-152

The effects of gabapentin therapy on pruritus, life quality, depression and sleep in pruritic haemodialysis patients

Tol H.[1], Atalay H.[2], Guney I.[3], Gokbel H.[3], Altintepe L.[4], Buyukbas S.[5], Bodur S.[6], Selcuk N.Y.[2], Tonbul H.Z.[2], Yeksan M.[2], Engin B.[1], Turk S.[2]
Departments of [1]Dermatology; [2]Nephrology; [3]Physiology; [5]Biochemistry; [6]Statistics, Selcuk University, Meram School of Medicine, Konya, Turkey; [4]Department of Nephrology, Meram Research and Training Hospital, Konya, Turkey
hakkig@selcuk.edu.tr

Aim: It was aimed to determine possible changes in pruritus, life quality, depression and sleep quality in pruritic haemodialysis (HD) patients with gabapentin therapy. **Methods:** Fourteen adult HD patients who had histories of pruritus more than 8 weeks were assigned to receive gabapentin (300 mg) therapy for 8 weeks. The daily pruritus scores using a visual analogue scale were collected for each period of the study during 1 week preceding the trial, the active treatment phase, the placebo phase and the intervening 1-week washout period. Sleep quality was determined with a modified post-sleep inventory, quality of life with a short form of Medical Outcomes Study (SF-36), depression using the Beck Depression Inventory. **Results:** The mean pruritus score decreased significantly from 7.6 ± 1.2 to 1.3 ± 1.4, total of post-sleep inventory significantly from 5.8 ± 3.3 to 1.8 ± 1.8 with gabapentin therapy. Physical and mental component scales of SF-36 increased, cognitive and somatic depression index decreased with gabapentin. **Conclusion:** We concluded that beneficial effects of gabapentin therapy on pruritus, quality of life, depression and sleep quality are important clinically in pruritus haemodialysis patients. Gabapentin therapy should be taken into account as a serious choice of therapy in pruritic haemodialysis patients.

PF19-153

EKG and EEG changes during tonic seizures in metaphit-injected rats challenged by sound stimulation

Hrncic D., Rasic A., Susic V., Radosavljevic T., Loncar-Stevanovic H., Djuric D., Stanojlovic O.
Institute of Physiology, Institute of Pathophysiology, School of Medicine, Belgrade, Serbia, and Serbian Academy of Sciences and Arts, Belgrade, Serbia
solja@afrodita.rcub.bg.ac.yu

Aims: Seizures are associated with marked autonomic changes during ictal and interictal states, while recently performed studies have demonstrated changes in cardiovascular function during and immediately following the onset of a seizure in experimental animals and in humans with various forms of epilepsy, but yet these issues have not been definitively answered to date. The objective of the present study was to determine specific EKG and EEG correlates of tonic sound-induced seizures in metaphit (1-[1(3 isothiocyanatophenyl)-cyclohexyl]-piperidine) – treated rats. **Methods:** Male Wistar albino rats with three recording electrodes implanted over the skull were randomly divided into the following groups: 1. saline-treated control group (n=6) and 2. metaphit-injected experimental group (n=8). Following the treatment, rats were exposed to an intense sound stimulation (110±3 dB, 3-5 kHz, 60s) at hourly intervals during the experiment and EEG and EKG monitoring have been performed during the seizure events. Recorded tracings of tonic seizures were retrospectively analysed. **Results:** Bursts of multiple high-voltage spikes and spike-wave complexes were recorded in EEG during tonic seizures in metaphit-treated rats. Ictal events in metaphit-treated rats were accompanied by EKG changes. Sinus tachycardia and falling ST depression were noted in all recorded ictal EKG tracings. No changes in EEG and EKG were noted in control animals during entire experiment. **Conclusion:** These findings indicate that metaphit model of epilepsy could be a suitable model for studying cardiovascular manifestations of generalized seizures.

PF19-154

Impairment of learning in water maze caused by a short epileptic seizure – possible role of reactive oxygen species

Mares J.[1], Pometlova M.[1], Rokyta R.[1], Pokorny J.[2]
[1]Institute of Normal, Pathological and Clinical Physiology, 3rd Faculty of Medicine; [2]Institute of Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Aim: Experimental status epilepticus usually causes learning impairment. It is commonly connected with loss of hippocampal neurons. This damage is widely accepted as a reason of impaired learning. We tested whether disturbances of learning also occur without cell death and if it is possible to block their occurrence. **Methods:** We developed other model of learning impairment in water maze. This impairment was evoked by one epileptic seizure elicited by Flurothyl. **Results:** This seizure lasted maximally 3 minutes. We did not observe any morphological changes after the seizure. The learning could be preserved by hypoxic preconditioning (3 days prior the seizure) and the same effect occurred after the application of melatonin, reduced glutathione and tempol prior the seizure. We suppose that this effect is probably related to the scavenger activity of the substances. Pretreatment with melatonin 1h prior the hypobaric hypoxia decreased preconditioning effect. **Conclusion:** Our results strongly support the possibility of involvement of free radicals in some functional changes caused by epileptic seizure and its role in preconditioning in the central nervous system.

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PF19-155

Dose-dependent antiseizure effects of ethanol on experimental lindane-induced epilepsy in rats

Mladenovic D., Hrncic D., Radosavljevic T., Vucevic D., Duric D., Rasic A., Loncar-Stevanovic H., Stanojlovic O.
Institute of Pathophysiology, and Institute of Physiology, School of Medicine, Belgrade, Serbia
solja@afrodita.rcub.bg.ac.yu

Aims: Lindane, an organochlorine pesticide, was found to provoke generalized tonic-clonic seizures in rats. Acute administration of ethanol may suppress seizure activity. The aim of our study was to investigate possible correlation between dose of ethanol and its antiseizure activity in lindane-treated rats, measured by behavioural and electroencephalographic changes. **Methods:** Male Wistar rats (n=50) were divided into following groups: 1. control (0.9% NaCl); 2. dimethylsulfoxide- treated group; 3. lindane- treated (L) group (8 mg/kg); 4. groups that received ethanol in doses 2 g/kg, 1 g/kg and 0.5 g/kg 30 minutes prior to lindane; E2, E1 and E0.5 group, respectively. All substances were administered intraperitoneally. For EEG recordings, animals were implanted electrodes into frontal, parietal and occipital lobe. Epileptic behaviours were scored according to a scale: 0-no response, 1-lower jaw twitching, head nodding, 2-myoclonic body jerks, 3-generalized tonic-clonic convulsions, 4-status epilepticus. **Results:** The incidence and median grade of epileptic behaviour were significantly higher in E0.5 group compared to E1 and E2 groups ($p<0.05$) and were similar to the incidence and median grade in L group. The incidence of epileptic behaviour was higher in E1 than in E2 group, but this difference was not statistically significant. Latencies (time to the onset of the first seizure) were not significantly different among experimental groups. EEG findings correlate with behavioural changes. Ethanol, in all doses applied, reduced epileptiform activity to sporadic spikes, the phenomenon seen in animals with grade1 behavioural sign. **Conclusions:** Our results indicate that ethanol reduces the incidence and severity of seizures in lindane-treated rats in a dose-dependent manner.

PF19-156

The effects of peripheral and central administration of *Hypericum perforatum* L. on chronic and acute pain in male rats

Biranvand F.[1], Khaksarian M.[1], Sonboli A.[2]
[1]Herbal Medicines Research Center of Medical Sciences; Loreastan, Iran; [2]Institute of Medicinal Plants, Shaheed Beheshti University, Tehran, Iran

Aim: In this study, *Hypericum perforatum* L. (HP) aqueous extract was administered intraperitoneally (i.p.) for evaluation of its antinociceptive effect. **Methods:** For assessment of its site of action, HP was filtered and administered both intrathecally (i.t.) and intracerebroventricularly (i.c.v.). Antinociceptive effects of HP extract were evaluated using formalin and tail-flick pain models. **Results:** Peripheral effects of HP extract were assessed in three doses (200, 400 and 800 mg kg-I, i.p.) and compared with the antinociceptive effect of Sodium Salicylate (SS) as a positive control. Administration of 300 mg/kg of SS i.p. had no effect on tail-flick latency, while all doses of extract increased it. In both phases of formalin test, all doses of extract alleviated the animal's nociception, but SS (300 mg kg-I) produced antinociception only in the second phase of formalin test. In central administration, the HP (1 and 2 mg/rat, i.t.) induced analgesia in the tail-flick and both phases of formalin tests. The i. c. v. administration of HP (2 mg/rat) produced analgesia in both phases of formalin test, but not on tail flick latency. **Conclusions:** The results showed that peripheral and central administration of HP has antinociceptive effect and its spinal effect seems to be more potent than its cerebral effect.

PF19-157

Differences in modality and localization of cutaneous threshold sensations under alternating current stimulation

Bogdanov V., Gorlov D., Gorgo Y.
Human and Animal Physiology Department, Biological Faculty, Taras Shevchenko National University of Kyiv, Ukraine
vlabogd@yahoo.com

Aims: Different excitable tissues of skin react specifically to stimulation by electrical current with different properties (Kajimoto et. al., 1999). Our aim was to compare modality and localization of threshold sensations under cutaneous stimulation by alternating current (AC) of different frequencies. **Methods:** Healthy volunteers (n= 180) of both sexes (18–22 years) were studied. The method of limits was used to detect absolute thresholds somatosensory sensations from electrical stimulation of left forearm skin by alternating current (AC) 0.1-50 mA at 30 Hz – 3 kHz AC frequencies (Bogdanov et al., 2004). The skin was stimulated through two flat 4 * 5 cm square silver electrodes separated by 10 cm on the left ventral forearm. The electrodes were applied to the skin with a gauze pad moistened with normal saline. Subjects reported the onset of the smallest detectable sensations and their modality and localization in the forearm. **Results:** Under 0.03-0.10 kHz AC stimulation the probability of a nociceptive sensation (48.3-80.7 %) and distal localization (50.3 %) was the highest among other frequencies ($p<0.05$ by t-test for each property). Under 0.3 kHz we observed the highest frequency of numbing effect in the palm (14.6%). Under 1- 3kHz the occurrence vibro-tactile modality (49.2-58.1%) and proximal localization (65.6%) were the highest among other frequency ranges. **Conclusion:** The frequency of AC cutaneous stimulation affects both modality and localization of the sensations, that can be explained by a selective effect on either sensory endings or sensory nervous pathways.

PF19-158

Immobilization, cold and hot stresses – temperature and nociceptive changes in rats: comparative study

Bocheva A.I., Djambazova E.B.
Department of Pathophysiology, Medical University of Sofia, Bulgaria

Various stress models have been reported to induce analgesia due to stress. This is a phenomenon, referred to as stress-induced analgesia. Exposure of an animal to stressful stimuli, perceived by the animal as a threatening, emergency condition, except nociception, induces a transient increase in core temperature. This response is often called stress-induced hyperthermia. There is literature data that decrease of pain sensitivity often affects thermoregulatory mechanisms in the threatened organism. The purpose of the present study was to compare changes in pain thresholds and core temperature after three models of acute stress: immobilization (IS), cold (CS) and hot (HS). Antinociceptive effects were evaluated using the paw pressure test. The changes in the mechanical nociceptive threshold of the male Wistar rats were measured by analgesimeter. Colonic temperature was measured using a digital thermometer. The obtained results show that after IS, CS and HS there are significant increase in pain thresholds. The most pronounced was the effect in nociception after HS, but this effect was very short. Effects of IS and CS was not so powerful, but they were observed during the whole investigated 30th min period. After three models of stress increase in core temperature was observed during 15th, 30th and 45th min of the experiment. Only in the beginning of the experiment CS elicits hypothermic effect, while IS and HS significantly increased core temperature, most pronounced for HS. In conclusion IS, CS and HS induced nociceptive and temperature changes in rats, which are with different intensity and continuance.

Key words: stress-induced analgesia, core temperature, nociception
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PF19-159

Cord dorsum potentials evoked by stimulation of hindlimb peripheral nerves in anaesthetised mice

Sears T.A., Cibert-Goton V.
The Wolfson CARD, King's College London, Guys Campus, London, UK

Aim: To further in vivo studies of spinal cord function in WT and KO mice we needed to measure averaged cord dorsum potentials evoked by electrical stimulation of hindlimb nerves as a guide to somatotopy. 33 CD1- mice (30-40g) were given 1.8 gm/kg Urethane, i.p. and after 15min, 0.4 gm/kg for sustained anaesthesia. Mice were supported prone by clamps at T13 and L3/4, the L1-2 laminae removed, dura opened and tissues covered by 4% agar. The sciatic nerve was stimulated in-continuity (mid-thigh) and the sural, medial and lateral popliteal nerves freed for stimulation at ankle level. Surface and depth recordings were amplified and processed by a CED 1401 and Spike 2 software. **Results:** Sciatic stimulation at 1.0 -1.3 xT evoked a synchronous, triphasic CAP (mean latency to $^{+ve}$ peak 2.0ms) followed at intensities >1.3T by a negative field potential. In 8 WT mice, systematic rostro-caudal recordings made 200µ apart at 5T gave a maximal (averaged) CAP potential of 2.15 mV; SEM +/- 0.22) and a mean field potential of -0.98mV; SEM +/- 0.1 centered 1.6-2.0mm caudal to T13; in some mice T13 was removed to determine this maximum. Stimulation of the other nerves gave partial overlapping distributions with the field potential amplitude then dominating. Depth recordings showed phase reversal of the field potentials at different depths and the procedures aided the location of naturally evoked unit activities. **Conclusion:** These results provide data for comparison with those from current studies on EphB1-KO mice exhibiting altered pain behaviour.

PF20-160

Zinc content in stomach mucosa cells of cold stressed animals

Bogdanova O., Bogdanov V., Lukashov D., Ostapchenko L.
Biochemistry department, Taras Shevchenko National University of Kyiv, Ukraine
basilevsis@yahoo.com

Aims: Zinc is essential for the normal growth and the reproduction (Frassinetti S., 2006). The role of zinc deficiency in gastrointestinal pathology is widely discussed. One of the main causes of stomach ulcer is acute and chronic stressing. We measured zinc content in stomach mucosa cells of rats with stomach lesions, induced by cold stress, during the recovering period. **Methods:** Male rats (200-250g) of both control and experimental groups (n=6), kept separately in controlled condition, were fasted for 24 h with water ad libitum. Animal experiments were carried out following the guidelines of the local ethics committee. Gastric lesions were provoked as described in (Biswas K., 2003). Zinc content was measured using atomic absorption spectroscopy. All of the data were expressed as the means ± S.E. The significance was calculated using One-Way ANOVA and „t"test in STATISTICA 5.0. **Results:** A decrease in zinc content was observed immediately after stress and on 5th day after that (2.3 and 2.1 fold respectively, $P< 0.05$). In animals treated with Omeprazole (antiulcer drug, 0.8 mg/kg daily), zinc content dropped immediately after cold stress (1.6 times) and on 2nd and 3rd day after that (1.6 and 1.8 times respectively, $P< 0.05$). In unstressed animals Omeprazole injections caused a significant decrease in the parameter. The most pronounced decreases were observed on 2nd, 3rd and 4th days of treatment (1.9, 1.8 and 2.4 times respectively, $P< 0.05$). **Conclusion:** These results suggest the involvement of zinc in recovering of gastric lesions.

PF20-161

Effect of the form of nutrition on gastrointestinal tract in juvenile and adult rats

Hubkova Z.[1], Mravec B.[1,2], Bernadic M.[1], Hulin I.[1], Hubka P.[1]
[1]Institute of Pathological Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic; [2]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic
zuzana.hubkova@fmed.uniba.sk

Aim: The character of nutrition was shown to substantially affect the overall healthy status of an organism. The purpose of the study was to test the influence of the liquid nutrition and intermittent fasting on the gastrointestinal tract. **Methods:** Two control groups of rats – adult (AC) and juvenile (JC) were fed by normal pellet diet. Two groups of rats – adult (AL) and juvenile (JL) were fed by normocaloric liquid diet. One group of adult rats (AF) was fed every other day by normal pellet diet (intermittent fasting). All animals had free access to water. After 10 weeks, weights of the body, stomach, liver, as well as electrogastrogram and systolic blood pressure were evaluated. **Results:** Body weights of AF group were significantly smaller than in AC and AL groups ($p<0.01$). All other groups did not differ significantly in body weights. Stomach weights were significantly smaller in AL group when compared with both AC and AF groups ($p<0.01$). Similarly, the stomach weights in JL group were significantly smaller in comparison to JC group ($p<0.01$). Relative liver weights did not differ significantly among analyzed groups. Systolic blood pressure was significantly lower in AF group when compared with both other adult groups. Functional measures of electrical gastric activity showed no substantial changes. **Conclusion:** These results demonstrate that changes in form of nutrition (without any change in composition and quantity of nutrition) cause significant plastic changes in gastrointestinal tract and in other systems in the juvenile animals as well as in adult ones.

PF20-162

Discriminating function of the pylorus in the normal state and after transection in dogs

Bosyuk I.Y., Beregova T.V., Kharchenko N.M.
Kyiv National Shevchenko University, Ukraine
ibosyuk@mail.ru

Aim: to investigate the influence of transection of branches of ramus pyloricus nervi vagi (RPNV), which innervates pylorus in regulation of gastric emptying. **Methods:** the investigations were carried out in chronical experiments on 2 groups (7 dogs) of dogs with fistulas of fundic part of stomach and duodenum. The dogs of first groups (4 dogs) were with intact nervous system (INS). The dogs of the second groups (3 dogs) were after operation of cutting of RPNV. Evacuation from stomach was investigated by the method of draining the duodenal fistula with 100 gram of bread mixed with 600 rubber corpuscles and red rubber corpuscles (3x3x4 mm), which were markers. In every 30 minutes sample of chyme we established amount of rubber corpuscles in it. Amount of corpuscles shows the part of food which left the stomach for each 30 minutes. **Results:** The vagotomy of RPNV lead to the disturbance of proportion of the exit of the big (red) and small (black) corpuscles from the stomach. In the first and second half an hour of the evacuation process we observed the same exit of all corpuscles from the stomach. Thus 40-50% from the general amount of small (black) and big (red) corpuscles have left the stomach. In the third and fourth half an hour the amount leaving decreased to the 12-14% of the total amount. **Conclusion:** The obtained results must be taken into account when performing such operations like pancreato-duodenal resection, duodenectomy.

PF20-163

About the role of glutamate NMDA-receptors in realisation gastric acid secretion in rats

Dziubenko N., Beregova T.
Kyiv National Taras Shevchenko University, Kyiv, Ukraine
dziubenko@rambler.ru

Aim: to compare the influence of channel blocker MK-801 and the blocker of polyamine site arcaine of NMDA-receptors on stimulated gastric acid secretion (GAS). **Methods:** The investigations were carried out on white male rats in acute experiments by method of perfusion of isolated stomach by Ghosh and Shild. The GAS was stimulated by intraperitoneal (i.p.) injection of carbacholin (0.01 mg/ kg) and pentagastrin (0.026 mg/ kg). MK-801 was injected in dose 0.1 mg/ kg, i.p., arcaine – in dose 10 mg/ kg, i.p. One experimental group of animals was vagotomised and treated with MK-801. **Results:** It was established that blockade of polyamine site of NMDA-receptors by arcaine suppressed carbacholin GAS by 79% ($P<0.001$) and pentagastrin GAS by 36% ($P<0.001$). MK-801 diminished carbacholin GAS by 37% ($P<0.01$) and did not influence pentagastrin GAS. Increasing effect of MK-801 on carbacholin GAS is abolished by vagotomy. So, in realisation GAS are involved central glutamate receptors that are localised on the neurons of nucleus tractus solitarii (NTS). Parietal cells have own receptors for gastrin, but realisation of GAS, stimulated by pentagastrin are controlled by strong cholinergic stimulus of the vagus. We suggest that excitation of polyamine site of central NMDA-receptors by endogenous glutamate by vagus passes to intrinsic mucosa and increases GAS, stimulated by pentagastrin. **Conclusion:** Excitation of ion channel and polyamine site NMDA-receptors by endogenous glutamate are important for realization of GAS stimulated by carbacholin. But for realisation of pentagastrin GAS is necessary stimulation only polyamine site by endogenous glutamate.

PF20-164

Time-dependent changes in epithelial secretion during murine experimental colitis

Hock M.[1], Sotak M.[1], Kment M.[2], Pacha J.[1]
[1]Department of Epithelial Physiology, Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic; [2]Department of Clinical and Transplantation Pathology, Institute of Clinical and Experimental Medicine, Czech Republic; miroslav_hock@yahoo.com

Aims: This study evaluated the time course of disturbances of colonic chloride secretion during experimental colitis. **Methods:** Colitis was induced by treatment of Balb/c mice with 2% dextran sodium sulphate (DSS) in drinking water for 5 days. At different time points during or after induction of colitis the effect of secretagogues, histamine (5.10^{-4} M), carbachol (10^{-4} M) and 5-hydroxytryptamine (10^{-4} M), was studied under short-circuit conditions in the absence or presence of tetrodotoxin (10^{-6} M). In addition, histological scores were evaluated and transcript levels of some inflammatory markers were measured using real-time quantitative RT-PCR. **Results:** Whereas, the markers of inflammation were elevated, the TTX-sensitive (ENS mediated) pro-secretory tonus has not been increased. In inflamed tissue the cholinergic agonist carbachol was either without any effect on chloride secretion or even decreases it (-5.0 ± 3.0 $\mu A.cm^{-2}$, resp. -5.6 ± 4.0 $\mu A.cm^{-2}$, vs. 25 ± 3.6 $\mu A.cm^{-2}$ in control). In healthy animals, the stimulatory effect of carbachol was higher in the absence of TTX (25 ± 3.6 $\mu A.cm^{-2}$ vs. 10.8 ± 0.6 $\mu A.cm^{-2}$). In contrast, DSS-treated mice showed higher responsiveness to carbachol in the presence of TTX but only during the first two days, especially the second day (36.0 ± 8.9 $\mu A.cm^{-2}$ vs. 20.2 ± 3.1 $\mu A.cm^{-2}$). The effect of 5-HT was higher in the presence of TTX in mice exposed to DSS within the first four days, but 2 and 4 weeks later the response to 5-HT was independent of TTX similar to control animals. **Conclusion:** This data suggests changes of the gut nervous system in the early phase of experimental colitis.
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PF20-165

The influence of histamine on hepatic portal vessels and blood filling of liver

Goloborodko J., Komarenko V., Ianchyk P.
Kyiv National Taras Shevchenko University, Kyiv, Ukraine

Aims: In a common blood circulation river-bed histamine shows vasodilatation properties. The purpose of the study was to discover effects of histamine on hepatic portal vessels and hepatic blood filling. **Methods:** Experiments registered the system arterial and portal pressure changes. For this purpose were cannulation of general carotid and portal vein done. One more catheter was used for intraportal conduction of the explored preparations. Hepatic blood filling was registered by the method of impedance reography. Research was made in sharp experiments on white laboratory rats (weight 200 – 300 g). Rats were anaesthetized by intracavitary conduction of urethan solution (1 g/kg). **Results:** Intraportal conduction of histamine did not draw the reactions on system arterial pressure. At the same time were observed vasomotor effects of histamine in the vascular river – bed of liver. Actually, the blood pressure in portal vein increased on 53.4 ± 15.7% in relation to an initial level. The latent period of pressorial reaction in the portal vessel was 9.6 ± 2.0 s, and the maximum of reaction amounted in 17.5 ± 3.7 s after the beginning of conduction of preparation. At the same time with the changes of pressure in portal vein, hepatic blood filling decreased on 18.7 ± 6.6% ($p < 0.05$).
Conclusion: In the hepatic portal system histamine shows vasoconstrictor effects, which are accompanied by the increase of tone of portal vein and decrease of hepatic blood filling.

PF20-166

Salmonella mediated antioxidative and anti-inflammatory gene therapy in dextran sodium sulphate treated female Wistar rats

Palffy R., Behuliak M., Celec P.
Institute of Pathophysiology, Department of Molecular Biology, Institute of Physiology, Comenius University, Bratislava, Slovakia
palffyroli@yahoo.co.uk

Aim: To analyze the effects of Salmonella-mediated gene therapy using Cu-Zn superoxid dismutase (SOD1) and a mutant of monocyte chemoattractant protein-1 (7ND-MCP1) on chemically induced colitis. **Methods:** Dextran sodium sulphate (DSS)-induced colitis in female Wistar rats was treated with SOD1 and 7ND-MCP1 encoded by plasmids carried by *Salmonella typhimurium* SL7207 applied daily during one week. Faecal consistency, clinical status and body weight were monitored during the whole experiment. Malondialdehyde (MDA) and advanced oxidation protein products were measured as markers of oxidative stress. Interleukin 1, inteleukin 6 and tumour necrosis factor alpha were quantified in colon and plasma samples using ELISA. **Results:** Faecal consistency and MDA showed a slight improvement in SOD1 and 7ND-MCP1 + SOD1 groups, but not in the 7ND-MCP1 group. Surprisingly, no differences were found in body weight increase, markers of oxidative stress in plasma and even in inflammatory markers in colon homogenates. Plasma concentrations of IL1, IL6 and TNFalpha were lower in the treatment groups than in the DSS group. However, DSS induced inflammation could only be confirmed by plasma IL1 and TNFalpha but not IL6. **Discussion:** Although some parameters showed a tendency to be improved by the bacteria-mediated antioxidative and anti-inflammatory gene therapy, the inflammation in the experimental DSS group was rather minor in comparison to other studies. Whether this is caused by the choice of gender and strain of the experimental animals will be studied.

PF20-167

The influence of omeprazole-induced hypergastrinaemia of different terms on the morphofunctional state of the colonic mucosa

Hurlenko T., Beregova T., Voronina O., Ovcharik T.
Department of Pharmaco-Physiology and Department of Cytology, Histology and Developmental Biology, Biological Faculty, Kyiv National Taras Shevchenko University, Kyiv, Ukraine; g_tanya@mail.ru

The implication of gastrin as a trophic agent for colonic mucosa (CM) in arising and development of neoplasm in colon is undoubted. But its role in maintenance of the colonic epithelial transport function is almost not studied. The aim of the study was to examine the effect of omeprazole-induced (OM) hypergastrinemia (HG) of different terms on the colonic morphofunctional indices. **Methods:** Adult male Wistar rats were used under urethane anaesthesia according to a method of isolated colonic loop perfusing technique *in vivo* for determining of net water and electrolyte (Na^+, K^+, Cl^-) movements (Jnet). The 1st studied group included animals (n=20) receiving a vehicle (i.p.) for 1, 2, 3 and 4 weeks – baseline group (BG); the 2-5 groups (n=27) – animals receiving OM (Dr.Reddy's, India) (14 mg/kg; i.p.) injections for 1-4 weeks respectively to induce HG. Plasma gastrin level (GL) was determined by radioimmunoassay. Mucosa thickness (MT), crypt depth (CD) and nuclear profound area of epitheliocytes (NPA) indices were measured. **Results:** Administration of OM for 1 week increased GL by 207.05% and decreased Jnet NaCl compared to BG. After 2 weeks of OM administration no significant changes of studied MFI were observed. Prolongation of OM influence up to 3 weeks entailed significant increase of GL by 131.10%, $JnetCl^-$, CD and MT and decrease of $JnetNa^+$. HG for 4 weeks (GL increased by 189.27%) Jnet water, Na^+ and NPA decreased and $JnetCl^-$, CD, MT increased significantly. **Conclusions:** The short-term OM-induced HG alters NaCl absorption. Prolongation of HG up to 3 weeks displays the trophic effect of gastrin on CM along with altered electrogenic $JnetNa^+$ absorption. OM-induced HG for 4 weeks causes appearance of hyperplastic features and provokes decreasing of Jnet water and NaCl absorption.

PF20-168

Activation of pepsin by Al^{3+} ions *in vitro*

Pavelkic V.[1], Gopcevic K.[2], Krstic D.[2], Djuric D.[2], Ilic M.[3]
[1]Vinca – Institute of Nuclear Science, Department of Physical Chemistry; [2]School of Medicine, University of Belgrade, Visegradska 26; [3]Institute of General and Physical Chemistry, 11000 Belgrade, Serbia

Aims: Aluminium does not belong to essential elements, and as a non-regulatory ion can be toxic to many organisms. Average daily dietary aluminium intake is approximately 2-6 mg Al/day in children and 6-14 mg Al/day in adults. An additional source of aluminium is food additives and Al-containing antacids. The purpose of this study was the investigation of the *in vitro* effect of Al^{3+} ions in strong acid conditions on pepsin (E.C.3.4.23.1.) activity and its electrophoretic mobility. **Methods:** The Worthington method based on enzyme-catalyzed measured rate of hydrolysis of denatured haemoglobin substrate was used for evaluation of enzyme activity in the absence (control) and presence of Al^{3+} ions. Kinetic analysis was carried out according to a slightly modified method of Anson. The data analyzed by the software package Origin 6.1 and the results were recalculated using EZ FIT. Native electrophoresis of pepsin on 10% polyacrylamide gel carried out at 4°C according to the Laemmli procedure. **Results:** It was demonstrated that Al^{3+} ions stimulate the pepsin activity on concentration dependent manner. Kinetic analysis showed that Al^{3+} ion (10^{-2} – 10^{-6} M) increases the maximal velocity (V_{max}) rather than the apparent activity (K_m) implying the noncompetitive nature of activation. Native PAGE electrophoresis shows the decrease in electrophoretic mobility of pepsin which is proportional to the concentrations of Al^{3+} ions. **Conclusion:** The present kinetic analyses indicated that the activation by Al^{3+} ions was of partial non-competitive type, which classifies this phenomenon as a case of non-essential activation. The decrease in the electrophoretic mobility of pepsin molecule additionally confirms the conformational changes in pepsin induced by Al^{3+} binding.

PF20-169

The influence of the antagonists of the H_3-histamine receptors on the duration of the phases of rest and work of periodical motility of the stomach and duodenum

Savchenko Y., Beregova T., Tsyryuk O.
Kyiv National Taras Shevchenco University, Biological Faculty, Kyiv, Ukraine
ysavchenko@mail.ru

Aim: to investigate the action of the antagonists of the H_3-histamine receptors on fast motility of stomach and duodenum in dogs. **Methods:** the investigations were carried out in chronic experiments on dogs with fistulas of fundal part of stomach and duodenum. By ballonographic method we recorded the periodical motility of stomach and duodenum and studied the influence on it the antagonists of H_3-histamine receptors: thioperamide (0.07 mg/kg, intravenously), betahistine (1.2 mg/kg, in duodenum). **Results:** in the control experiments the phase of rest in stomach lasted 46.8±12.5 min and in duodenum 29.14±7.2 min. The phase of work in stomach and in duodenum lasted 44.3±14.6 and 61.3±10.1 min, consequently. Thioperamide evoked the lengthening the phase of work of periodical motility in the stomach on 93.5% ($p<0.01$) and in duodenum on 61.3 % ($p<0.01$). The phase of rest in the stomach decreased on 10.8 % ($p>0.05$) and in duodenum on 28.9 % ($p<0.01$). Another antagonist betahistine had the analogical effect on the duration of the phases of periodical motility. Betahistine increased the duration of the phase of work in stomach and duodenum on 100.5 and 72.3 % ($p<0.01$), accordingly. Whereas the duration of the phase of rest were diminished in stomach on 39.7 % and in the duodenum 39.3 % ($p<0.01$). **Conclusion:** 1. Endogenous histamine acting on H_3-histamine receptors determine the duration of the phases of rest and work of periodical motility. 2. Antagonists of H_3-histamine receptors may be useful in patients with disturbances of periodical motility of gastrointestinal tract.

PF20-170

The influence of desmopressin on bile acid secretion

Spivak L.S., Gorenko Z.A., Parchami Ghasaee S., Samonina H.O., Veselsky S.P.
Peter Bogach Institute of Physiology, National Taras Shevchenko University of Kyiv, Ukraine
ludish@ukr.net

Aims: The neurohypophysal peptide vasopressin (AVP) is involved in diverse function, including smooth muscle contraction, stimulation of liver glycogenesis, modulation of ACTH release, inhibition of diuresis. However, the role of this peptide in the regulation of bile formation is not clear yet. **Methods:** Biliary duct cannulated rats were used in acute experiment. Desmopressin (1-Desamino-8D-arginin vasopressin, dDAVP) (1 ng/100 g b.w.) and antagonist of $V1_a$ vasopressin receptors ([β-Mercaptoβ, βcyclop entamethylenepropionyl[1], O-me-Tyr[2], Arg8]-Vasopressin) (1μg/100 g b.w.) were administered i.v. Secreted bile volume was measured. The quantitative content of bile acid in each probe taken was determined using the thin-layer chromatography method. **Results:** Obtained data demonstrate that dDAVP considerably increases volume of bile secretion. Moreover, absolute content of conjugated bile acids in bile, such as taurocholates (+65.7%, $p<0.01$), glycocholates (+49.0%, $p<0.05$) was significantly increased. Administration of $V1_a$ vasopressin receptors antagonist was followed by the slow increase bile secretion rate. $V1_a$ antagonist caused very similar to dDAVP injective effects alterations of taurocholates (+46.6%, $p<0.05$), glycocholates (+58.1%, $p<0.01$) output. Interestingly that during blockade of $V1_a$ recepors by $V1_a$ vasopressin receptors antagonist dDAVP do not show fully the choleretic action and total content of bile acids was decreased respectively, taurocholates (-27.3%, $p<0.01$) and glycocholates (-20.1%, $p>0.05$) as compared to dDAVP effect. **Conclusion:** These results demonstrated that dDAVP elevates bile flow and absolute content of conjugated bile acid in bile drowing $V1_a$ vasopressin receptors.

PF20-171

The influence of pioglitazone on basal gastric acid secretion in omeprazole-treated rats

Tsyryuk O., Kukharskyy V., Beregova T.
Kyiv National Taras Shevchenko University, Pharmaco-Physiology Department, Kyiv, Ukraine
tsyryuk@univ.kiev.ua

Aim: of the study was to investigate the influence of pioglitazone (PPARgamma ligand) on basal gastric acid secretion in omeprazole – treated rats. **Methods:** The study was carried out on 50 white rats. They were divided into three groups: I (control) – were injected with 0,2 ml H2O (per os); II – were injected with omeprazole (OM) (14mg/kg, i.p.); III – were injected OM and pioglitazone (30mg/kg, per os). All drugs were injected during 28 days. Functional state of parietal cells and gastric mucosal hypertrophy was evaluated by basal gastric acid output (BGAO). BGAO was determined after 24 hours after last injection. BGAO was investigated under urethane anaesthesia (1.1 g/kg, i.p.) by method of isolated stomach perfusion by Ghosh and Shild. Also in rats was measured blood plasma gastrin concentration by radioimmunoassay method. **Results:** It was established that in 28 days of OM injection BGAO and gastrin plasma level increased by 283.7% and 189.3% in comparison to control accordingly. After 28 days of pioglitazone and OM treatment BGAO was diminished by 53.4% in comparison to rats after 28 days of OM injection. But pioglitazone did not influence on augmentation of gastrin plasma level evoked by OM. **Conclusion:** 1) hypergastrinaemia evoked by OM lead to the general hyperplasia and hyperplastic mucosa has an increased capacity to produce acid; 2) compensatory effect of pioglitazone on BGAO may indicate for retardation of mucosal hypertrophy process evoked by hypergastrinaemia. Therefore PPARgamma receptor is a novel target for the development of new and effective anticancer therapies.

PF20-172

Increased expression of Hsp70 and iNOS in experimental atherosclerosis is reversed back by lacidipine

Yetik-Anacak G., Oktem G., Ustunes L.
Ege University, Faculty of Pharmacy, Department of Pharmacology, Turkey

Background: Recently high amount of NO has been shown to induce hsp70 expression in SMC. There are not enough data on increased expression of both iNOS and heat shock protein 70 (hsp70) in atherosclerotic vessels. Lacidipine, may affect several physiological and biochemical pathways in atherosclerosis. **Aim:** The purpose of this study is to investigate the production of hsp70 and iNOS in atherosclerotic carotid arteries and the effect of lacidipine on these expressions. **Methods:** White rabbits were either received lacidipine (5 mg/kg/day) or vehicle (%1 carboxymethylcelulose p.o.). On the 8th day, a non occlusive, soft silicon collar was positioned around the left carotid arteries. The right carotid arteries were sham-operated. Both carotid arteries were removed on the 22nd day. Expression and distribution of hsp70 and iNOS of the arteries were determined by immunohistochemistry. Intimal thickening were measured by computer based morphometric analysis. **Results:** Lacidipine significantly inhibited the intimal thickening and index (intima/media) in collared arteries. Both hsp70 and iNOS expression were increased in smooth muscle layers of atherosclerotic carotid arteries and reversed back by lacidipine treatment. **Conclusions:** We suggested that increased levels of iNOS, by producing high amount of NO, may also induce hsp70 expression in atherosclerotic carotid arteries. Lacidipine may have beneficial effect on pathogenesis of atherosclerosis by overcoming increased levels of these inflammatory proteins.

PF20-173 EYPS Keynote Lecture

Role of uPAR and plasmin in bone marrow stem/progenitor cell retention and mobilization

Marc Tjwa
Center for Transgene Technology and Gene Therapy, VIB, K.U.Leuven, Belgium

The mechanisms of bone marrow (BM) haematopoietic progenitor cell (HPC) retention, engraftment and mobilization remain incompletely identified. Though a family member of the established HPC marker Sca-1, a role of membrane-anchored urokinase receptor (uPAR) in HPC biology remains unknown. We therefore studied its importance in HPC retention and mobilization by phenotyping mice lacking uPAR.

We found that membrane-anchored uPAR marks various subpopulations of HPCs and that, in steady-state conditions, cell-autonomous loss of uPAR partially depletes the HPC pool in the BM. Loss of uPAR on HPCs impaired their cell cycle quiescence, chemoprotection, and short-term homing and engraftment in the BM niche, indicating that uPAR is a cell-autonomous retention signal for HPCs. Importantly, in response to 5-FU myeloablation or G-CSF stimulation, the membrane-anchored uPAR retention signal on HPCs is inactivated by plasmin via proteolytic cleavage and converted into a soluble uPAR cleavage product (suPAR). Further studies highlighted that suPAR is not merely a waste product, but actively amplifies the mobilization of HPCs. Consistent herewith, mobilization of HPCs was impaired in the absence of uPAR or plasminogen. Membrane-anchored uPAR enhanced, while suPAR inhibited alpha4-beta1 integrin-dependent adhesion on BM stromal cells, thus suggesting that plasmin converts uPAR from a retention to a mobilization signal. Interestingly, membrane-anchored uPAR is also expressed on $Lin^{-}Sca\text{-}1^{+}cKit^{+}$ BM cells while cell-autonomous loss of uPAR on donor BM cells reduces their long-term engraftment and multilineage repopulation of primary and secondary myeloablated recipients, suggesting that uPAR might also play a role in haematopoietic stem cells (HSCs).

Hence, our findings indicate that expression of membrane-anchored uPAR on HPCs functions as a novel retention signal promoting their maintenance, cell cycle quiescence, chemoprotection, homing, engraftment and mobilization, but they also unveil an unprecedented mobilization pathway, involving the inactivation of the membrane-anchored uPAR retention signal on HPCs by plasmin.

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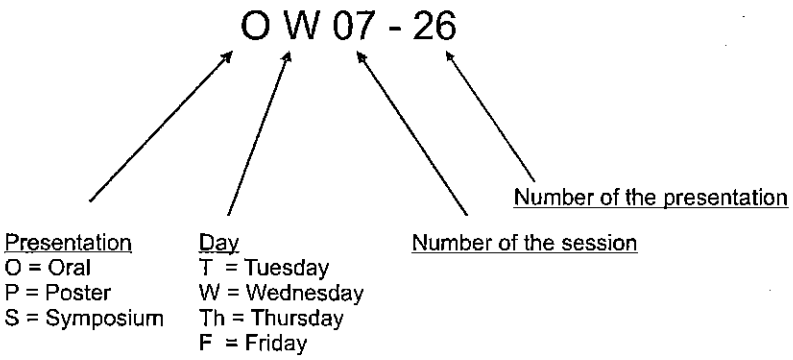

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