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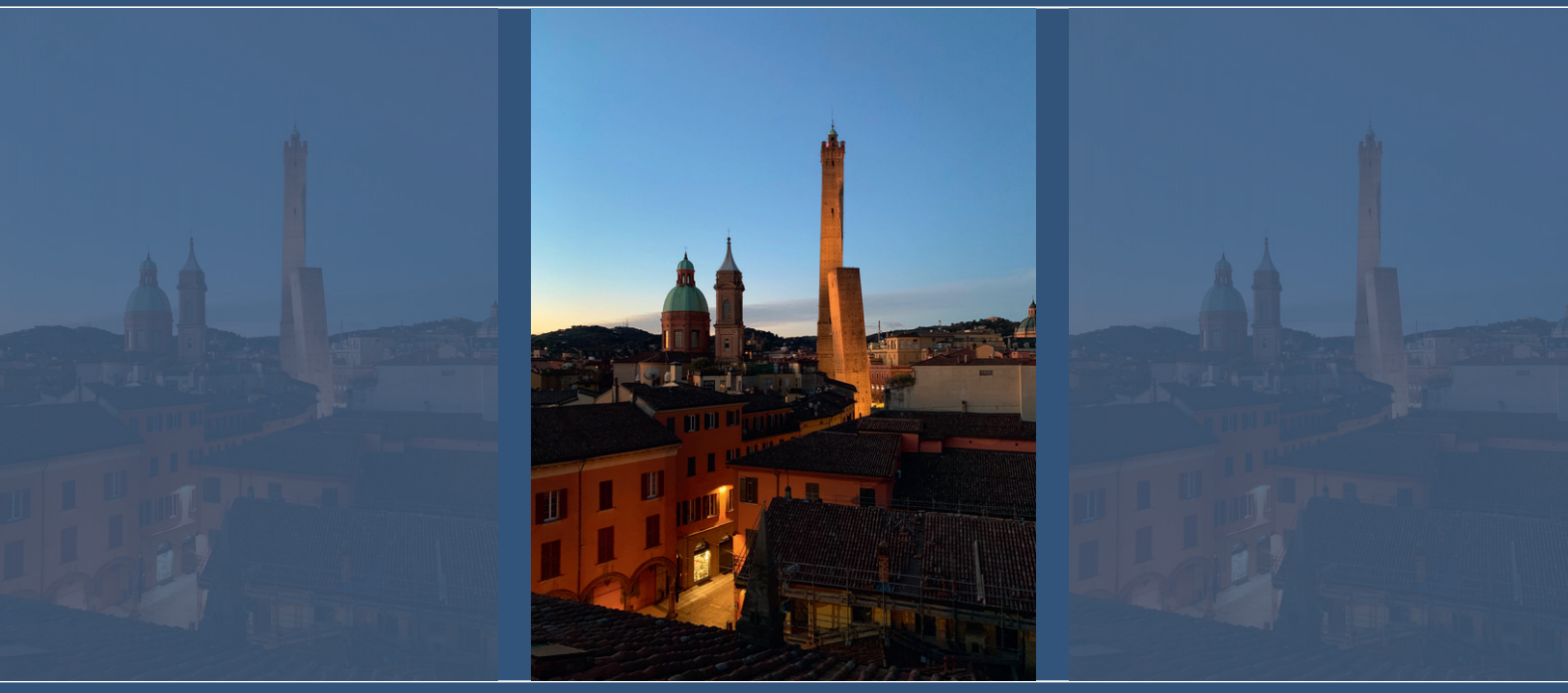

FEPS 2019 – BOLOGNA (ITALY)

Joint Meeting of the Federation of European Physiological Societies (FEPS) and the Italian Physiological Society (SIF)
Bologna (Italy), September 10th – 13th 2019

Abstracts of the Joint Meeting

A Joint International Meeting celebrating the 70th Anniversary of the Italian Physiological Society

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COVER

View of the two medieval lining towers in center town Bologna, at the sunrise. The tallest is called Asinelli, the smallest and more leaning tower is called Garisenda, from the names of the families that wanted their construction at the beginning of the XII century (1109-1119). Mentioned by Dante in the Divine Comedy, by Goethe in the Italian Journey, and by other poets and writers in the following centuries, the Two Towers are the landmark of Bologna and located in center town, at the intersection of the most ancient streets (including via Zamboni, the main artery of the University district). The Alma Mater Studiorum University of Bologna had been founded some years before, in 1088.
The photo has been taken by Prof. Jaroslav Pokorný, President of the Czech Physiological Society from a terrace at the beginning of via Zamboni during his stay in Bologna for the Meeting of the International Scientific Committee in preparation of the FEPS-SIF 2019 Congress.

FEPS 2019 – BOLOGNA

JOINT Meeting of the Federation of European Physiological Societies (FEPS) and the Italian Physiological Society (SIF)

With participation of the
Austrian, Croatian, Czech, French, Slovak, Slovenian, Spanish, Swiss and Turkish Physiological Societies

Bologna (Italy), September 10th – 13th 2019

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ACTA PHYSIOLOGICA

Opening FEPS Lecture

L.01

Hormonal Regulation of Secretion in Collecting Duct Intercalated Cells

Leipziger J

Department of Biomedicine, Physiology, Aarhus University, 8000 Aarhus C, Denmark

The collecting ducts of the kidney organise the final regulation of the excretion of electrolytes, water and acid/base equivalents from our body. A unique population of interspersed cells that partially protrude into the tubular lumen mediate the handling of either acid or base. These cells also appear critically involved in electrolyte and water homeostasis and display immune cell functions. The -intercalated cells (-IC) are able to secrete protons and are central for urine acidification. Defects of the -IC cause distal renal tubular acidosis. The β-intercalated cells mediate active HCO3- secretion and cause urine alkalisation. Both cell types show very abundant expression of hormone and paracrine factor receptors rendering them to be target of acute functional regulation. I will present an array of surprising and unpublished data highlighting the vivid biology of the "intercalated cells system". A part will address the hormonal regulation of the β-IC. The human genetic disease Cystic Fibrosis will be reviewed, and the term renal tubular alkalosis will be suggested and argued for.

Rising Star Award Lecture

L.02

Roads, cars and trailers: How the microtubule network regulates β-adrenoceptor relaxation in arteries.

Jepps T

University of Copenhagen, Denmark

In arterial smooth muscle cells, changes in availability of integral membrane proteins influence the regulation of blood flow and blood pressure, which is critical for human health. However, the mechanisms that regulate the expression and coordination of specific receptors and ion channels in vascular smooth muscle are understood poorly. In the vasculature, very little is known about microtubules, which form a perfect network upon which proteins can be transported to and from the cell membrane. Our laboratory have revealed a novel and critical role of microtubules in regulating β-adrenoceptor relaxations in arteries, through the regulation of Kv7.4 channel levels in the membrane (Lindman et al., 2018). Further investigation has revealed the mechanisms behind this microtubule-dependent regulation of the β adrenoceptor-mediated response in vascular smooth muscle cells. Dynein is a motor protein bound to the microtubule network that transports "cargo", including membrane proteins, along the cellular road network created by microtubules. Our work not only provides the first evidence of dynein expression in vascular smooth muscle, but also identifies an original dynein-dependent process coordinating an important aspect of vascular function, namely the regulation of β adrenoceptor-mediated relaxations. These findings advance our understanding of an important physiological pathway, namely β adrenoceptor-mediated relaxation, which is known to be compromised in hypertension.

Plenary SIF Lecture "Fabio Ruzzier"

L.03

Control of calcium in the heart: free and beyond

Eisner D

Unit of Cardiac Physiology, The University of Manchester, Manchester, United Kingdom

Calcium is the master controller of cardiac function. Its concentration ($[Ca^{2+}]i$) needs to increase on each beat to trigger the heart to contract to pump blood and must fall to low enough levels between beats so that the heart can relax and refill. Heart disease, the major killer world-wide, is associated with abnormal calcium signaling. Calcium binding to proteins allows precise and rapid control of their function and two consequences will be considered. (i) This binding means that only about 1% of Ca^{2+} is free with the rest bound to buffers. Therefore changes of buffering may have as much effect as those of Ca2+ fluxes in determining the levels of $[Ca^{2+}]i$. I will discuss the implications in health and disease. (ii) Ca^{2+} ions cannot be destroyed; regulation requires that $[Ca^{2+}]i$ be controlled by movements across membranes (surface and sarcoplasmic reticulum). Therefore, in the steady state, over one cardiac cycle, the calcium fluxes must be balanced. I will demonstrate how this flux balance helps control both diastolic and systolic $[Ca^{2+}]I$ as well as setting the Ca^{2+} content of the sarcoplasmic reticulum, an important determinant of contractility.

Closing SIF Lecture

L.04

The Human Brain Project: insight into brain physiology

D'Angelo E

Department of Brain and Behavioral Sciences, University of Pavia, Italy

The Human Brain Project (HBP) joins the efforts of scientists around the main theme of understanding the brain and, in order to do this, is leveraging on the most sophisticated techniques of neuronal modeling. The HBP brain model reflects the need of giving a coherent quantitative framework to brain phenomena, which typically range over multiple scales, from molecules to neurons to circuits and behavior. The HBP brain model, once generated, is expected to promote itself a broad set of technologies and applications in neurorobotics, neuromorphic computing and medicine. In these years, the main microcircuits of the brain have been modeled in detail, accounting for synaptic properties and for phenomena like dendritic and axonal computation and revealing the inner structure of microcircuit functioning. On the other hand, large-scale simplified network models of the whole brain have been constructed and tested to analyze brain signals generated during mental processing, in health and disease. It is now the time to integrate the two approaches into a unified modeling framework and to disclose the much awaited new perspectives for future brain research. The work is supported by the Human Brain Project of EU (GA 720270 and 604102) and by the CNL grant, Centro Fermi (Rome, Italy)

European Young Physiologists Symposium (EYPS)

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Oral presentations

OP.01

Dissecting the physiological function of amyloid-β peptide at the synapse

Gulisano W[1], Melone M[2,3], Ripoli C[4,5], Tropea MR[1], Li Puma DD[4,5], Giunta S[1], Cocco S[4], Marcotulli D[2], Origlia N[6], Palmeri A[1], Conti F[2,3,7], Grassi C[4,5], Puzzo D[1,8]

[1]Dept. Biomedical and Biotechnological Sciences, University of Catania, Italy; [2]Dept. Experimental and Clinical Medicine, Università Politecnica delle Marche, Italy; [3]Center for Neurobiology of Aging, IRCCS INRCA, Italy; [4]Institute of Human Physiology, Università Cattolica del Sacro Cuore, Italy; [5]Fondazione Policlinico Universitario A. Gemelli IRCCS, Italy; [6]Neuroscience Institute, Italian National Research Council, Italy; [7]Foundation for Molecular Medicine, Università Politecnica delle Marche, Italy; [8]Oasi Research Institute-IRCCS, Italy.

The increase of oligomeric amyloid-beta (oAβ) is considered one of the earliest events in Alzheimer's disease (AD) pathophysiology, leading to synaptic dysfunction and memory loss. However, the peptide is produced in the healthy brain where it exerts several physiological functions. Previous studies have demonstrated that endogenous oAβ42 is needed for synaptic plasticity and memory and, when at picomolar concentrations, it enhances long-term potentiation (LTP) and memory. Based on these findings, here we investigated the pre- and post-synaptic mechanisms underlying the neuromodulatory role of picomolar oAβ42 at the synapse in male and female mice. Dual patch-clamp whole-cell recordings of hippocampal CA1 pyramidal neurons showed an increase of miniature excitatory postsynaptic current frequency. This, together with the decrease of paired-pulse facilitation and the increase of docked vesicles in axon terminals suggested that the peptide enhanced neurotransmitter release. At postsynaptic level, oAβ42 induced an increase of postsynaptic density length, accompanied by a higher expression of plasticity-related proteins such as phospho-CREB (Ser133), phospho-CaMKII (Thr286), and BDNF. These changes resulted in the conversion of early- into late-LTP and of short- into longterm memory through the nitric oxide/cGMP/protein kinase G intracellular cascade. We also demonstrated that the oAβ42-induced effects were present upon extracellular but not intracellular application and depended upon 7 nicotinic acetylcholine receptors. Taken together, our findings clarified the mechanisms underlying the physiological role of oAβ42 on synaptic function and memory formation and might be useful to better understand the events leading to the abnormal increase of Aβ in the brain of AD patients.

OP.02

Altered dopamine metabolism leads to a unique impaired αSynuclein proteostasis in Parkinson's Disease.

Masato A[1], De Lazzari F[1], Madany M[2], Thor A[2], Bisaglia M[1], Greggio E[1], Beltramini M[1], Boassa D[2], Bubacco L[1].

[1]Department of Biology, University of Padova, Italy; [2]Department of Neurosciences and National Center for Microscopy and Imaging Research, University of California San Diego, California, USA.

Parkinson's Disease (PD) is pathologically characterized by the progressive loss of nigrostriatal dopaminergic neurons and aberrant accumulation of the presynaptic protein Synuclein (Syn). Several factors have been proposed to trigger Syn aggregation, resulting in Syn-induced neurotoxicity. One of them is 3,4-dihydroxyphenylacetaldehyde (DOPAL), a toxic dopamine metabolite, which covalently modifies lysine residues of proteins. In vitro and cellular studies demonstrated that DOPAL triggers Syn oligomerization, prevents Syn association to synaptic vesicle membranes and affects synapse physiology. On this ground, our aim is to investigate the consequences of DOPAL modification of Syn on overall Syn proteostasis, in terms of aggregation, degradation and trafficking. By combining different biochemical and imaging approaches, we first assessed that DOPAL treatments in catecholaminergic BE(2)-M17 cells resulted in Syn build-up, oligomerization and decreased clearance, together with a general impairment of protein degradation systems. In parallel, live time-lapse imaging on rat primary cortical neurons revealed impaired Syn trafficking between the soma and the periphery in the presence of DOPAL. Finally, we conducted ultrastructural analysis by using markers for correlated light and electron microscopy (CLEM) to localize Syn and Syn-DOPAL oligomers in the neuronal compartments, both in the soma and at the pre-synaptic terminals. Interestingly, we found that DOPAL treatment promoted Syn loading into multi-vesicular bodies, suggesting an upregulation of Syn trafficking along the endo-lysosomal pathway. Concluding, our work will contribute in unravelling the link between Syn-induced neurotoxicity and the preferential vulnerability of nigrostriatal dopaminergic neurons in PD.

OP.03

Decoding visuospatial properties and movement intentions from macaque posterior parietal cortex.

Filippini M[1], Morris AP[2], Hadjidimitrakis K[2,1], Breveglieri R[1], Fattori P[1].

[1]DIBINEM University of Bologna, Italy; [2]Neurosci. Program, Biomedicine Discovery Institute, Department of Physiology., Monash Univ., Clayton, Australia.

Neural prostheses represent a promising approach to restore basic motility in patients affected by spinal cord lesions. Intact signals recorded from cerebral cortex can be decoded and used to drive neural prostheses. Understanding how the brain codes information and how different cortical areas could contribute to prosthesis operation is still a critical point. Neurons in the dorsomedial area V6A of macaque mediate sensory-motor transformations and show sensitivity to different spatial variables. To asses if these signals are adequate to drive a full capable neural prosthetic arm, we recorded spiking activities from 145 V6A neurons in two macaques while they performed either an instructed delayed reaching task and a fixation-only task. Targets were 9 LEDs arranged in the 3D space in front of the animal. A Maximum Likelihood Estimation decoding algorithm was used to get a metric estimation of reach/fixation target location in both tasks. A code generalization approach showed that visuospatial and motor preparation codes were similar during the delay period, the two codes diverged shortly after the cue to execute the required motor response. Despite the apparent similarity between tasks during the initial period, it was still possible to correctly detect the task executed throughout the trial duration. Finally, a Bayesian classifier was used to decode the sequence of task phases. These results show that V6A signals can be used to reliably decode visuospatial properties, information about type of intended movement (its presence or absence) and task progress. Combined, these properties could support prostheses that extract, from a single area, the target of a movement and respond as the intention to move is formed.

OP.04

Specific activation of raphe pallidus-projecting neurons from dorsomedial hypothalamic nucleus at torpor onset in mice

Squarcio F[1], Amici R[1], Bastianini S[1], Berteotti C[1], Chiavetta P[1], Hitrec T[1], Lo Martire V[1], Luppi M[1], Martelli D[1], Occhinegro A[1], Stanzani A[2], Tupone D[1], Zoccoli G[1], and Cerri M[1].

[1]Department of Biomedical and Neuromotor Sciences-Physiology, [2]Department of Veterinary Medicine, University of Bologna

Torpor is an energy-saving strategy, which is characterized by an active inhibition of metabolism leading to a reduction in body temperature. The mechanism underlying torpor onset is still unknown. However, reasonably, in order to enter torpor, Raphe Pallidus (RPa), the key area in the control of thermogenic sympathetic outflow, has to be inhibited. The aim of this study was to assess the role of RPa-projecting brain areas in torpor induction in mice. Twenty-eight C57BL/6J female mice, adapted to an ambient temperature (Ta) of 28 C, underwent surgery for the injection of the retrograde tracer Cholera Toxin-b subunit (CTb) within the RPa. Afterwards, mice were assigned to one of the following experimental groups: i) Torpor (n=5): torpor was induced by a 36-h fasting, followed by an acute exposure to low Ta (15 C); ii) Cold Exposure (n=5): mice were only acutely exposed to Ta

15 C; iii) Fasting (n=10): mice were only fasted; iv) Control (n=8): no changes in the ambient conditions were made. Mice from the Torpor group were sacrificed 90 min. after torpor onset, while the sacrifice of other animals was time-matched. Animals were transcardially perfused and their brains extracted for immunohistochemical detection of cFos expression and CTb localization. The number of double stained (CTb+/cFos+) neurons was significantly ($p<0.05$) larger in the Torpor group vs. each of the three control groups in the dorsomedial hypothalamic nucleus (DMH); vs. either the Control or the Fasting groups in the paraventricular hypothalamic nucleus (PVH); and vs. Control group only in the ventrolateral periaqueductal gray (VLPAG). The large and specific activation of DMH neurons at torpor onset suggests a role for this nucleus in triggering torpor in mice.

OP.05

The impact of constitutive GABA-A receptor-mediated depolarization in peripheral Cfibers axons.

Bonalume V.[1], Caffino L.[1], Castelnovo L.F.[1], Liu S.[2], Hu J.[2], Schmelz M.[3], Fumagalli F.[1], Carr R.W[3]*, Magnaghi V[1]*

[1]Department of Pharmacological and Biomolecular Sciences, University of Milano, Italy; [2]Institute of Pharmacology, Heidelberg University, Mannheim, Germany; [3]Experimental Pain Research, Heidelberg University, Germany.

Mature neurons of central nervous system are characterized by maintenance of a low intracellular chloride concentration leading to GABAA receptor (GABAAR) mediated hyperpolarizing currents. However, immature neurons as well as mature olfactory and somatosensory neurons maintain an elevated intracellular chloride concentration whereby inward GABAAR currents depolarize neurons. We report that unmyelinated sensory fibers in adult mice are depolarized by GABA. Depolarizing axonal C-fiber responses to GABA (1μM-1mM) were mimicked by GABAAR specific ligands and absent in conditional KO mice, lacking GABAA-β3 subunit in NaV1.8 expressing neurons, thus confirming the presence of functional GABAAR along C-fibers axons. Combined with qRT-PCR analysis of DRG explants, these data suggest that the most prominent peripheral GABAAR composition is $2\beta3\gamma2$. We demonstrated that the physiological role of GABAAR in peripheral C-fibers axons is activity-dependent and secondary to a shift in ECl, mediated by NKCC1 activation. NKCC1 increases its activity during sustained electrical stimuli, resulting in a reinforcement of GABA depolarizing currents, able to modulate C-fiber conductance velocity. Unmyelinated fibers are characterized by conduction slowing during sustained activity. Cfibers slowing was more pronounced in GABA-β3 null mice and reduced by exogenous GABA application, while the modulation mediated by the application of GABAAR specific competitive antagonist bicuculline, and the allosterical agonist allopregnanolone demonstrate the presence of endogenous GABAAR activation. In conclusion, our data suggest that GABAAR is constitutively active along C-fiber axons, and that during sustained activity it limits axonal excitability loss, allowing the physiological perception of pain. (This study was supported by a grant from MIUR "Progetto Eccellenza").

OP.06

Receptor architecture of the macaque monkey superior parietal lobule

Impieri D[1], Zilles K[2,3,4], Niu M[2], Rapan-Jankovic L[2], Schubert N[2], Gamberini M[1], Galletti C[1], Palomero-Gallagher N[2,3]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2]Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Germany; [3]Department of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen, Germany; [4]JARA-BRAIN, Jülich-Aachen Research Alliance, Jülich, Germany.

The macaque monkey superior parietal lobule (SPL) is part of a neuronal network involved in the integration of visual and somatosensory information for execution of reaching and grasping movements. The cytoarchitecture of areas V6, V6Ad, V6Av, PE, PEc, PEci and PGm of the SPL has been described, but little is known about their receptor architectonic organization, which could give crucial insights related to its functional organization. We applied quantitative in vitro receptor autoradiography to analyze the distribution patterns of fifteen different receptors for glutamate, GABA, acetylcholine, serotonin, dopamine, and adenosine in the SPL cortex of three adult male Macaca fascicularis monkeys. The mean (averaged over all cortical layers) receptor densities were visualized as a receptor fingerprint for each area. Multivariate analyses were conducted to detect clusters of areas according to the degree of (dis)similarity of their receptor organization. Glutamate and GABAergic receptor families are the maximally expressed in all the areas analysed. Receptor densities are higher in supra- than in infragranular layers of SPL areas, with the exception of kainate, M2, and adenosine receptors. Differences in regional and laminar receptor distribution patterns confirm the location and extent of areas V6, V6Ad, V6Av, PE, PEc, PEci and PGm as found in cytoarchitectonical and functional studies, but also enable the definition of three subdivisions within area

PE and suggest that PEc is part of Brodmann's area 7 instead of area 5, as previously supposed. Hierarchical cluster analyses demonstrate that SPL areas are organized in two groups, an organization that corresponds to the visual or sensory-motor characteristics of those areas.

Short Oral Presentations

OP.07

Neural correlates of spatial attention shifts in the medial superior parietal lobule of the macaque.

De Vitis M[1,2,3], Balan P[1,2], Galletti C[3], Fattori P[3], Vogels R[1,2], Vanduffel W[1,2,4,5]

[1]Laboratory of Neuro- and Psychophysiology, KU Leuven, Belgium; [2]Leuven Brain Institute, KU Leuven,Belgium; [3]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [4]MGH Martinos Center, Charlestown, MA, USA; [5]Harvard Medical School, Boston, MA USA.

Human fMRI and TMS showed the involvement of superior parietal lobule (SPL) in covert shifts of attention. Despite hints from monkey electrophysiology (Galletti et al 2010) and fMRI studies (Caspari et al 2015), still little is known about the underlying neuronal mechanisms. Guided by monkey fMRI maps, we recorded single and multi-unit activity from shift-selective regions in medial SPL using laminar probes. Our experimental paradigm was similar to that of human (Molenberghs et al 2007) and monkey fMRI experiments (Caspari et al 2015), and allowed us to dissociate attentional shift, stay and motor events (used to probe the allocation of attention). Stimuli consisted of 2 pairs of shapes, each containing a relevant and irrelevant stimulus (one located in the receptive field (RF), and the other diametrically opposed). A replacement of the first stimulus pair by the second could induce a spatial attention shift when the relevant stimulus position changed to the opposite visual hemifield (shift event). Alternatively, when the latter was replaced by the relevant stimulus of the second pair this corresponded to a stay event. We recorded from 192 multi-units in areas V6/V6Av of one rhesus monkey. We found that the average population activity of all recorded neurons was higher for shift than for stay events (Kruskal-Wallis test, FDR corrected) when the direction of the shifts pointed towards the RF. Shift-related population activity peaked around 40-60 ms after event onset, where 66.7% of cells showed significant shift-selective activity (contrast shift vs. stay, Wilcoxon test, p=10-5). Consistent with thehuman and monkey data, these preliminary results show a strong correlate of shifting spatial attention at neuronal level within areas V6/V6A in the absence of overt behaviour.

OP.08

Functional analysis, properties and kinetics of a PepT2-type di/tripeptide transporter of the Atlantic salmon (Salmo salar) highly expressed in midgut and hindgut

Vacca F[1], Bossi E[1], Gomes AS[2], Cinquetti R[1], Barca A[3], Verri T[3], Murashita K[2,4], Rønnestad I[2].

[1]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [2]Department of Biological Sciences, University of Bergen,Norway; [3]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [4]Research Center for Aquaculture Systems, National Research Institute of Aquaculture, Japan Fisheries Research and Education Agency, Minamiise, Mie, Japan.

The SoLute Carrier 15 (Slc15) family includes H+-dependent transporters that play a key role in the cellular uptake/reuptake of di/tripeptides and peptidomimetics. In mammals, in the epithelial cells of intestine and renal tubules two di/tripeptide transport systems have been characterized: the low-affinity/high-capacity system Slc15a1 (PepT1) and the highaffinity/low-capacity system Slc15a2 (PepT2). While PepT1 transporters have been studied in many teleost species, data on PepT2 is still lacking, except for zebrafish (Danio rerio). Here, we performed basic functional and expression analyses of a newly cloned Atlantic salmon (Salmo salar) PepT2. In Xenopus laevis oocytes, transient currents analysis showed that both total amount of charges moved (Q) and decay time ( ) vs membrane voltage shifted to more positive potential values when extracellular pH decreased, highlighting the role of H+ in the first step of transport cycle. Transport current vs voltage relations, as from Gly-L-Gln dose-response experiments, allowed kinetic parameters to be determined as a function of potential (from 140 to +20 mV) and external pH (5.5, 6.5, 7.6). Salmon PepT2 showed the higher apparent Gly-L-Gln affinity (K0.5) at pH 5.5 and 6.5 at the physiological membrane potential and an increase of maximal relative current (Imax) for more negative potentials and more acidic conditions. Notably, mRNA tissue expression analysis revealed that it is highly expressed in midgut and hindgut. Similar to zebrafish, this salmon PepT2 is a high-affinity/low-capacity transporter (K0.5 for Gly-Gln 4,4 μM, Imax -10 nA at -40 mV at pH 6.5), but its specific expression in the mid-to-distal portions of the gut opens to distinct and not yet known roles for a PepT2-type protein in fish physiology.

OP.09

Role of the genetic variants of bitter taste receptor TAS2R38 in disease aetiology and attainment of longevity

Melis M[1], Sollai G[1], Errigo A[2], Pes GM[2], Cossu G[3], Hummel T[4], Morelli M[1], Crnjar R[1], Barbarossa IT

[1]Department of Biomedical Sciences, University of Cagliari, Italy; [2]Department of Clinical and Experimental Medicine, University of Sassari, Italy; [3]Neurology service and Stroke Unit, A.O. Brotzu, Cagliari, Italy; [4]Department of Otorhinolaryngology, Dresden University of Technology, Germany.

Bitter-taste receptors play crucial roles in detecting bitter compounds not only in the oral cavity, but also in extraoral tissues including the brain. The immune and nervous systems communicate with each other by using a common chemical language and by sharing receptors and ligands. Bitter-taste receptors serve immune roles in some of the tissues in which they are expressed, and an efficient immune response is needed in the longevity attainment. We evaluated the TAS2R38 receptor genetic variants as a genetic risk factor for the development of taste disorders (TD), idiopathic Parkinson's disease (PD) or attainment of longevity (AL). Subjects were genotyped for the TAS2R38 gene. PTC or PROP bitterness were assessed in TD, PD patients and healthy controls (HC). PAV/PAV TD patients gave high PTC ratings, PAV/AVI reported lower values like those determined in AVI/AVI or rare genotypes, while PAV/PAV and PAV/AVI HC gave higher ratings as compared to AVI/AVI or rare genotypes HC. Most PD patients, which showed PROP taste disruption, had the AVI form, a high number had a rare variant and only 5% of them had the PAV/PAV genotype, and so did 25% of HC. TD and PD patients did not meet the Hardy-Weinberg equilibrium at TAS2R38 locus and HC were in equilibrium. Centenarian individuals of an isolated cohort from Sardinia showed a higher frequency of PAV form and lower of the AVI one, as compared to three HC cohorts. Our findings identify, in the genetic constitution at the TAS2R38 locus, a risk factor for the development of the two diseases and suggest that the disease-associated taste disruption may represent a marker able to identify patients. Our data also provide suggestive evidence on the association between human longevity and TAS2R38 variants.

OP.10

Depressant effects of adiponectin on the smooth muscle cell excitability in the mouse gastric fundus: a possible peripheral signal in the hunger-satiety cycle?

Idrizaj E[1], Garella R[1], Castellini G[2], Francini F[1], Ricca V[2], Baccari MC[1], Squecco R[1]

[1]Department of Experimental and Clinical Medicine, University of Florence, Italy; [2]Department of Health Sciences, University of Florence, Italy.

Evidence exists that some white adipose-tissue derived hormones that act centrally to regulate food intake also influence the activity of the gastrointestinal smooth muscle, whose motor phenomena represent a source of peripheral signals involved in the control of feeding behavior. In this view, adiponectin (ADPN) too has been shown to influence food intake and we recently reported its ability to affect the mechanical responses and to induce a decay of the basal tension in preparations from the mouse gastric fundus. On this ground, the present electrophysiological study aims to investigate, for the first time, the possible action of the hormone on the smooth muscle cell (SMC) excitability and membrane phenomena, which can affect the primary events leading to the mechanical responses. Experiments were performed by the technique of the microelectrodes in SMCs from the gastric fundus of female mice. ADPN caused evident effects on SMCs, hyperpolarizing the resting membrane potential and affecting the passive membrane properties. Moreover, ADPN induced changes on the main ion currents amplitude by enhancing the outwardly delayed rectifier K+ currents (Kv and Ks) and decreasing that of the voltage and Ca2+ activated (BK) K+ currents as well as the T- and L-type Ca2+ currents. All these results are suggestive of an inhibitory action of ADPN on gastric SMCs excitation-contraction coupling. Experiments are in progress to identify the possible signalling pathways through which the hormone exerts its effects. The newly observed depressant action of ADPN on the gastric SMC excitability, together with our previous mechanical results, might lead to consider these effects of ADPN also as a peripheral signal in the hunger-satiety cycle and thus in feeding behaviour.

OP.11

Pharmacological targeting of VTA dopaminergic neurons in a mouse model of Alzheimer's disease

La Barbera L.[1,2,3], Nobili A.[1,3], Latagliata E.C.[1], Cutuli D.[1], Krashia P.[1,2], Vedele F.[1,2], D'Amelio M.[1,3]

[1]Department of Experimental Neurosciences, IRCCS Santa Lucia Foundation, Rome, Italy; [2]Department of Systems Medicine, University of Rome 'Tor Vergata', Italy; [3]Department of Medicine, University Campus Bio-medico, Rome, Italy.

We recently demonstrated that in the Tg2576 mouse model of Alzheimer's Disease (AD) the Ventral tegmental area (VTA) dopaminergic neurons

degenerate. Degeneration occurs prior to Aβ-plaque deposition, tau tangles or any sign of cortical or hippocampal neuronal loss. The resulting dopamine deprivation in projection areas leads to memory and reward deficits reminiscent of AD, whereas levodopa could rescue these deficits in Tg2576 mice. Here, we investigate the hypothesis that the degeneration of dopaminergic neurons in Tg2576 is related to increased activity of the tyrosine kinase c-Abl. Our aim was to examine the ability of Nilotinib, a c-Abl inhibitor, to block/delay degeneration in Tg2576 dopamine neurons, with the aim of eventually preventing cognitive and noncognitive deficits. Nilotinib was earlier shown to reverse the loss of dopaminergic neurons in Parkinson's disease (PD) models, to improve cognitive/motor performances via autophagic degradation of β-amyloid and -synuclein in models of AD and PD, respectively, and to reduce disease-related biomarkers in PD patients. We chronically treated Tg2576 mice with Nilotinib (1 mg/kg, on alternate days), starting before the onset of VTA neuronal loss. We analyzed Nilotinib efficacy by stereological cell count of dopaminergic neurons, hippocampal microdialysis of dopamine outflow and behavioral testing. Nilotinib treatment: i) reduced VTA neuronal loss, ii) increased dopamine outflow in the hippocampus and iii) improved cognitive abilities. These data suggest that the neuroprotection of VTA dopaminergic neurons in Tg2576 mice can postpone the onset of AD, demonstrating their importance as novel therapeutic targets.

OP.12

A BDNF mimetic can rescue trisomy-linked neurodevelopmental alterations

Stagni F, **Uguagliati B**, **Giacomini A**, **Emili M**, **Bartesaghi R**, **Guidi S**

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy.

Down syndrome (DS) is a genetic condition characterized by intellectual disability (ID). Widespread neurogenesis impairment starting from the fetal phases of brain development is considered a major determinant of ID. In spite of numerous efforts, no therapies currently exist for ID in DS. The brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays a key role in neurogenesis through its binding to the tropomyosin-related kinase receptor B (TrkBR). Evidence for reduced BDNF levels in the DS fetal brain suggests that this defect may play a role in neurogenesis disruption. Therapy with BDNF is impracticable due to its poor blood-brain barrier (BBB) penetration. However, the flavonoid 7,8-Dihydroxyflavone (7,8-DHF) can cross the BBB, binds to TrkBR and activates its signaling cascade, thereby mimicking BDNF. The goal of our study was to establish whether prenatal therapy with 7,8DHF restores neurogenesis in the Ts65Dn mouse, a widely-used model of DS. Pregnant Ts65Dn females received 7,8-DHF from embryonic day 10 until delivery. On postnatal day 2, the pups received an injection of BrdU in order to label neural progenitor cells (NPCs) and were killed after 2h. An evaluation of the number of NPCs in the subventricular zone of the lateral ventricle (the major forebrain neurogenic niche), striatum, frontal neocortex and hippocampus showed that in embryonically-treated Ts65Dn mice the number of NPCs underwent full restoration. This study provides novel evidence that prenatal treatment with the BDNF mimetic 7,8-DHF, a natural substance usable in humans, rescues trisomy-linked neurogenesis impairment. This finding provides new prospects for the rescue of intellectual disability in Down syndrome and, possibly, other BDNF-linked neurodevelopmental disorders.

OP.13

BRomoDomain-containing protein 4 (BRD4) regulates oxidative stress and autophagy in skeletal muscle

Segatto M[1], **Fittipaldi R**[2], **Szokoll R**[2], **Filippakopoulos P**[3], **Caretti G**[2]

[1]Department of Biosciences and Territory, University of Molise, Italy; [2]Department of Bioscience, University of Milan, Milan, Italy; [3]Nuffield Department of Clinical Medicine, University of Oxford, UK.

BRomoDomain-containing protein 4 (BRD4) is an epigenetic reader that regulates gene transcription by recognizing acetylated lysines of histones. BRD4 has a critical function in the transcriptional modulation of genes involved in cell cycle progression, cell growth and differentiation. Even though the role of BRD4 is poorly characterized in skeletal muscle, emerging evidence suggests its involvement in different aspects of muscle physiology. In this work, we assessed the role of BRD4 in the modulation of oxidative stress and autophagy in skeletal muscle, in physiological conditions and during homeostasis loss. To reach this aim, we used the small molecule JQ1 as a pharmacological approach to inhibit BRD4. Our data highlight that BRD4 affects autophagy in differentiated myotubes by regulating oxidative stress. Importantly, BRD4 inhibition restores autophagy alterations caused by oxidative stress in the mdx mouse model of Duchenne muscular dystrophy, leading to an overall improvement of the pathological hallmarks of the dystrophic phenotype. Notably, BRD4 inhibition significantly decreases the expression of NADPH

oxidase subunits, and this event is accompanied by the induction of autophagic proteins. Taken together, these results demonstrate that BRD4 influences muscle autophagy by controlling oxidative stress, suggesting that BRD4 targeting may represent a novel therapeutic strategy to manage neuromuscular disorders.

OP.14

Ghrelin Has Protective Effect on Synaptic Transmission and Neuronal Damage in Sepsis

Ates G[1], Ozkok E[2], Yorulmaz H[3], Gundogan GI[4], Tamer S[5]

[1]Department of Physiology, Istanbul Yeni Yuzyil University, Turkey; [2]Department of Neuroscience, Istanbul University, Turkey; [3]School of Nursing, Halic University, Istanbul, Turkey; [4]Department of Histology and Embryology, Yeni Yuzyil University, Istanbul, Turkey; [5]Department of Physiology, Istanbul University, Istanbul, Turkey.

Ghrelin is known to have anti-inflammatory and anti-oxidant effects. The presence of its receptor and expression in hippocampus is demonstrated as a possible therapeutic agent in septic encephalopathy. We aimed to investigate the effects of Ghrelin on neuronal damage and synaptic transmission in hippocampus of rats with sepsis induced with lipopolysaccharide (LPS). Adult Wistar albino male rats were divided into 4 groups as the control, LPS (5 mg/kg i.v., 5 mg/kg i.p after 12 hours), Ghrelin (10 nmol/kg i.v.), LPS+Ghrelin (Local Ethic Committe for Animal: 2013/123). The brain tissues were decapitated under anesthesia 24 hours after the first injection were taken in 10% formaldehyde. NeuN was used for alive neurons, S100-ß was used for degenerated neurons, and the immunoreactivity of synaptophysin antibodies were investigated for synaptic vesicles. Statistical analysis was performed using the one-way variant analysis, and Tukey test. In LPS group, S-100ß involvement increased, and synaptophysin and NeuN involvement decreased compared to other experimental groups ($P<0.01$). No significant difference was observed in the control, Ghrelin, and LPS+Ghrelin groups ($P>0.05$). In LPS, we detected a decrease in the number of alive neurons, and an increase in the number of damaged neuron in the hippocampus. Also, a decrement was found in synaptic vesicular proteins. It was obtained that Ghrelin administration preserved alive neuron, and prevented the occurence of neuronal damage, and caused no reduction in the involvement of synaptophycin. In conclusion, we suggest that the exogenous administration of Ghrelin has protective effect on synaptic transmission and neuronal damage in sepsis.

OP.15

Crosstalk between p21 Activated Kinase 6 (PAK6) and transcription factor EB (TFEB) to regulate neuronal autophagy

Plotegher N, Agostini F, Cogo S, Tessari I, Bubacco L, Civiero L, Greggio E

Department of Biology, University of Padova

PAK6 belongs to the family of p21-activated kinases, which play a central role in actin cytoskeleton dynamics. Despites its high expression in neurons, the physiological function of PAK6 in the brain is still poorly characterized. We recently showed that PAK6-mediated phosphorylation of 14-3-3 proteins at Ser58 is key to regulate the interaction of 14-3-3a with client proteins, including the Parkinson's disease-kinase LRRK2. 14-3-3s, which are highly enriched in the brain, bind phosphorylated motifs on target proteins, regulating their activity and cellular localization. The role of PAK6 kinase activity in regulating 14-3-3 interaction with its binding partners suggested us that PAK6 may be involved in the activation of TFEB, the master regulator of lysosomal biogenesis and autophagy, whose translocation to the nucleus is negatively regulated by interaction with 14-3-3s. Thus, the aim of our work is to test the hypothesis that PAK6 regulates TFEB activation in neurons. By combining biochemical and imaging approaches, we found that PAK6 kinase activity drives TFEB nuclear translocation. Furthermore, PAK6 overexpression affects TFEB expression levels and activation of autophagy by inhibition of mTOR increases PAK6 cellular activity, suggesting that PAK6 acts downstream of mTOR and upstream of TFEB. Ongoing experiments are elucidating the precise molecular mechanisms by which PAK6 activates TFEB. In particular, we are testing 1) the impact of PAK6-dependent phosphorylation of 14-3-3s on 14-3-3/TFEB interaction and 2) whether PAK6 directly phosphorylates TFEB. Because autophagy is operated by virtually every cell in the body, the neuronal-enriched expression of PAK6 may confer the necessary level of tissues specificity to finely tune brain autophagy.

TEACHING PHYSIOLOGY

Symposium

Changing from Traditional Lectures to Interactive Classrooms and Digital Based Learning

Organizer: Bayram Yilmaz (Istanbul, Turkey)

Oral presentations

OP.16

MyMi.mobile - a Personalized Adaptive Approach to Digital Learning in Microscopic Anatomy

Langer-Fischer K[1], Eichner B[4], Brandt D[1], Bertsch A[3], Dietrich M[3], Schmucker M[3], Igel C[3], Heimrich B[2], Britsch S[1]

[1]Institute of Molecular and Cellular Anatomy, Ulm University, Ulm,Germany; [2]Institute of Anatomy and Cell Biology, Albert-Ludwigs-University Freiburg, Germany; [3]German Research Center for Artificial Intelligence (DFKI Berlin), Germany; [4]Competence Center eLearning in Medicine Baden-Württemberg, Ulm University, Germany

The population of German medical students, as in many other countries, is characterized by the growing diversity and individualization of their educational biographies, their learning talents and preferences. There is emerging evidence that one-fits-all approaches to university teaching are no longer sufficient to efficiently meet individual needs and expectations of students. To better address the demands of a modern curriculum in medical training and to promote individual success of our students we have developed in collaboration with the Anatomical Institute of the University of Freiburg and the German Research Center for Artificial Intelligence (DFKI) a web-based digital learning App for microscopic anatomy, MyMi.mobile (https://mymi.uni-ulm.de). Upon login onto a secure personal account MyMi.mobile systematically observes and traces the individual learning behavior of the user. Anonymized data sets are processed using methods of artificial intelligence (AI) in order to identify specific strenghts and deficiencies of the individual user. Moreover, patterns of learning behavior that are associated at a higher level with study success or with non-success are determined. We expect such analyses to benefit from the availability of large data sets obtained from two independent medical training programs (Ulm and Freiburg). Finally, we hope that MyMi.mobile will help to better identify and address individual needs of students and to efficiently increase their individual learning success.

OP.17

Scientific visualisation: medical illustration and animation in physiology education

Evren M

Visuluma Scientific Visualisation, Ege University Technology Devolopment Zone, Turkey

Advances in medical education require use of visual materials instead of classical methods to improve education quality. This requirement has led to a new art-science branch called medical illustration. Medical illustration can be defined as the visual transfer of knowledge with an emphasis on complete accuracy and ease of comprehension. Scientific visualition finds a wide range of applications from informing patients and raising public understanding to undergrad/specialist education and academic research. It utilizes a variety of methods and forms of presentation including the oldest form of visual communication: drawings and illustrations as well as 3D animations, 3D printed personalized models and virtual reality. Physiology is one of the most complicated fields in medical education and requires extensive use of scientific visualization. Visual materials and training are essential for accurate teaching of anatomic structures and physiological processes as well as for effective on-going education at later clinical training stages. In this presentation we will cover the following topics: (i) introduction to medical illustration and animation, (ii) applications of visualization in physiology education, (iii) hardware and software used to produce visual material in the field of physiology, (iv) developing your own visual designs.

OP.18

Evidence-based teaching, flipped classrooms, and resistance to change

Silverthorn D

Medical Education, University of Texas at Austin, USA

Calls for reform in higher education science teaching go back more than 20 years in both Europe and the United States, but change has been slow, despite a growing body of evidence showing that active, student-centred learning enhances understanding and retention of content as well as problem-solving skills. Research from educational psychology and cognitive neuroscience has provided evidence that supports the use of formative assessments and teaching strategies such as interleaving and retrieval practice. Flipped classroom, team-based and problem-based learning, and gamification are all successful approaches for

changing traditional lectures into student-centred sessions. So why relatively few higher education teachers adopted these innovative methods? A review of the literature on resistance to change reveals three main barriers to change that are international: institutional or cultural roadblocks, student resistance, and the academic staff themselves. Some barriers, such as lack of staff training in how to teach and reliance on student evaluations, can be overcome with resources and policy changes. Other barriers, like institutional attitudes that value teaching less than bench research, will be harder to break down

OP.19

Tough Topics in physiology (made easy) – a peer reviewed, interactive online resource to enhance student learning

Wallace H

Medical Education, University of Liverpool, UK

Year one medical students must understand fundamental physiological concepts, before they can move on to pathology, diagnosis and management. We have observed that certain topics are repeatedly challenging to students and are difficult to teach in the large lecture environment. This has led to the development of a novel, peer reviewed digital based learning resource, consisting of different physiological topics based on recurrent poor student assessment performance. Each topic is accessed via the university's virtual learning environment, and is set up to provide data on the effectiveness of the resource on student learning. The resource provides different learning approaches with a common theme, including learning through acquisition, investigation, practice and assessment. Each topic is linked to a workshop adopting a flipped classroom approach, and is available as a revision aid. The topic is developed further for year two to include pathophysiology, more complex assessment and clinical scenarios. The accessibility and progression of the Tough Topics series enables students to return to fundamental topics as their spiral of knowledge grows.

BLOOD PHYSIOLOGY

Symposium

Platelets beyond hemostasis

Organizer: Alice Assinger, Vienna, Austria

Invited Oral Presentations

OP.20

Platelets mediate ischemia-induced revascularization through C5aR1-induced secretion of CXCL4

Nording H[1], Emschermann F[1], Patzelt J[1], Knoep K[2], Mezger M[1], Borst O[4], Feil R[5], Chavakis E[6], von Hundelshausen P[7], Kohl J[8,9], Gawaz M[3], Langer HF[1,4]

[1]Section of Cardioimmunology, Medical Clinic II, University Heart Center Lübeck, Germany. [2]Department of Cardiology and Angiology, Hannover Medical School, Germany. [3]University Hospital, Department of Cardiovascular Medicine, Eberhard Karls University, Tuebingen, Germany. [4]University Hospital, Medical Clinic II, University Heart Center Lübeck, Germany. [5]Institute for Biochemistry, Eberhard Karls University, Tuebingen, Germany. [6]Department for Internal Medicine III/Cardiology, University Hospital of the Johann Wolfgang Goethe University, Frankfurt am Main, Germany. [7]Institute for Cardiovascular Prevention, Ludwig Maximilians University Munich, Germany. [8]Institute for Systemic Inflammation Research, University of Lübeck, Germany. [9]Division of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Platelets contribute to tissue neovascularization. The specific factors underlying this function, however, are unknown. Here, we identified the complement anaphylatoxin C5a- mediated activation of C5a receptor 1 (C5aR1) as one critical platelet mediator. We showed that platelets expressing C5aR1 exert an inhibitory effect on endothelial cell functions like migration and 2D and 3D tube formation. Furthermore, we demonstrated the abundance of platelets expressing complement receptors and complement activation in environments where neovascularization takes place in vivo. Growth factor- and hypoxia-driven vascularization were markedly increased in C5ar1-/- mice. Platelet-specific deletion of C5aR1 resulted in a proangiogenic phenotype with increased capillarization and improved pericyte coverage. Mechanistically, we found that C5a induced the preferential release of CXC chemokine ligand 4 (CXCL4, PF4) from platelets as an important antiangiogenic paracrine effector molecule. Interfering with the C5aR1-CXCL4 axis down to the levele fo the CXCL4 receptor CXCR3 reversed the antiangiogenic effect of platelets both in vitro and in vivo. In conclusion, we have determined a novel mechanism for neovascularization suppression via the activation of the C5a/C5aR1 axis in platelets and the induction of anti-angiogenic factor CXCL4.

OP.21

Platelet ITAM receptors: new regulators of inflammation and vascular integrity

Rayes J

University of Birmingham, UK

Platelets are best known for their role in hemostasis and thrombosis. They also play crucial roles in host defense, inflammation and tissue repair. Many of these roles are regulated by the immune-like receptors GPVI and CLEC-2, which signal through an immunoreceptor-tyrosine-based-activation-motif (ITAM). GPVI is activated by collagen in the sub-endothelial matrix and by fibrin and fibrinogen in the thrombus. CLEC-2 is activated by the transmembrane protein podoplanin which is found outside of the vasculature and is up-regulated in inflammation and cancer. We have recently shown that CLEC-2 and GPVI regulate immune cell recruitment and activation in septic mice. CLEC-2 deletion inhibits macrophage recruitment to the site of infection and exacerbate the cytokine storm during sepsis. Despite sharing a common signaling pathway leading to platelet activation, CLEC-2 and GPVI play opposite roles in inflammation. CLEC-2-podoplanin axis is anti-inflammatory and GPVI is largely pro-inflammatory. During immune complex-mediated dermatitis, CLEC-2 and GPVI maintain vascular integrity at the site of inflammation. This form of inflammatory hemostasis limits the bleeding in the inflamed organs inflicted by immune cell infiltration. However, this form of bleeding is beneficial in wound repair leading to accelerated skin wound healing. My talk will address our recent findings on the role of CLEC-2 and GPVI in inflammatory, infection and tissue repair. I will also present and discuss recent evidences on the beneficial role of impaired vascular integrity-mediated bleeding in tissue repair.

OP.22

Platelets in inflammation and infection

Schrottmaier W

Centre of Physiology of Pharmacology, Medical University of Vienna, Austria

Platelets do not only get activated during inflammation and infection but are sensitive fine- tuners of immune responses. In both acute and chronic inflammation such as pulmonary injury and atherosclerosis platelets exacerbate cytokine release, thereby boosting recruitment of innate leukocytes and their host-damaging activities including neutrophil phagocytosis and monocyte/macrophage differentiation into foam cells. However, platelets walk a fine line between thrombosis and immunoregulation, while also maintaining vascular integrity and promoting tissue repair. Platelets can get directly activated by bacterial and viral stimuli, launching rapid and direct responses and thus representing an important first line of defence against invading pathogens. These leukocyte-activating functions of platelets are beneficial for pathogen clearance, as platelet inhibition accelerates bacterial and viral burden. However, upon excessive immune stimulation, e.g. in severe sepsis, the immunomodulatory functions of platelets appear to be quickly overshadowed by their haemostatic functions, and while capture of pathogens in microthrombi can locally curtail pathogen dissemination, systemic thrombosis leads to vessel occlusion and tissue hypoxia. Chronic or re-occurring inflammatory conditions, however, seem to affect platelet function in a different way. Platelets seem to be less responsive and potentially exhausted in patients with chronic inflammatory disease. While persistent inflammation directly affects megakaryocytic development and platelet production, also intrinsic factors generated during inflammatory diseases such as adipositas or re-occurring endotoxaemia render platelets to a hypo-reactive phenotype. This mechanism could provide an evolutionary advantage by diminishing the risk of thrombotic complications in inflammation and add one more layer of complexity to the multifaceted role of platelets in immune responses and immunothrombosis. Hence, even though platelets are only fine-tuners of the immune system, they represent double-edged swords and depending on the underlying condition they may tip the balance in inflammation and infection to either counteract or exacerbate disease severity.

Oral presentations

OP.23

Platelet-specific expression of the inflammatory NFkB activator IKK_2 reduces atherosclerosis and protects mice from hepatosteatosis

Mussbacher M[1], Salzmann M[1], Pereyra D[2], Schrottmaier WC[1], Kral-Pointner JB[1], Marak R[1], Basilio J[1], Kuttke M[1], Ketelhuth D[3], Starlinger P[2], Assinger A[1], Schmid J[1]

[1]Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, Austria; [2]Center of Molecular Medicine, Karolinska Institute, Sweden; [3]Department of Surgery, General Hospital of Vienna, Austria

Platelets have been identified as important modulators of atherosclerosis as they facilitate infiltration of leukocytes via formation of platelet-leukocyte aggregates and contribute to the generation of an inflammatory environment by the secretion of cytokines and chemokines from their respective granules. Unexpectedly, mice expressing constitutive active IKK2, a main activator of NF-kB signaling, specifically in megakaryocytes and platelets (caIKK2PLT mice),

showed a hypo-reactive platelet phenotype with prolonged bleeding times and impaired arterial thrombus formation. To characterize the role of these platelets in the context of atherosclerosis, we crossed caIKK2PLT mice on an ApoE KO background and fed them a hypercholesteremic diet. *En face* preparations of isolated aortas revealed significantly decreased plaque areas in caIKK2PLT mice, which furthermore showed a lower degree of hepatosteatosis and reduced levels of plasma cholesterol. Livers of these mice exhibited decreased immune cell infiltration and oxidative stress, which was in line with a lower number of platelet-monocyte aggregates. In summary, we were able to show that platelets with persistent activity of an inflammatory molecule reduce the development of atherosclerosis presumably via regulation of liver inflammation and cholesterol metabolism.

OP.24

Selective inflammatory activation of megakaryocytes and platelets by transgenic expression of ikk2 results in a hypo-reactive platelet phenotype

Salzmann M, Mussbacher M, Schrottmaier WC, Kral-Pointner JB, Resch U, Hoesel B, Bleichert S, Moser B, Basílio J, Assinger A, Schmid JA

Institute of Vascular Biology and Thrombosis Research, Medical University of Vienna, Austria

Inflammatory conditions favor thrombotic processes and are the main driving force of diseases like atherosclerosis. NF-κB, one of the key pathways regulating inflammation, is activated by IκB-Kinase 2 (IKK2). Recently non-genomic roles of IKK2 have emerged in platelets, the central cells orchestrating hemostasis and thrombosis. While most studies analyzed the effects of IKK2 inhibition, we are the first to investigate the effect of persistent activation of IKK2 in platelets, thus mimicking a state of chronic inflammation. To our surprise, platelet reactivity was not increased but diminished. Mice with persistently active IKK2 in platelets had prolonged bleeding and deficiencies to form a thrombus, including reduced platelet GPIIb/IIIa activation and degranulation. This reduced response was accompanied by reduced Akt phosphorylation, a key molecule in platelet activation. Our data sheds new light on IKK2 activity and/or persistent inflammation on platelet activation. Inflammatory conditions drive thrombotic processes, but platelets may have developed strategies to counteract this prothrombotic stimulus through a yet unknown IKK2-mediated feedback mechanism, thereby potentially compensating adverse thrombotic events. Understanding the underlying mechanism of this hypo-responsive platelet phenotype could help us to develop novel strategies to prevent or treat thrombotic complications in patients with persistent inflammation.

CARDIOVASCULAR PHYSIOLOGY

Symposium 1

The role of intrinsic and environmental factors in microvascular regulation: impact of nutrients and life style

Organizer: Helena Lenasi , Ljubljana, Slovenia

Invited Oral Presentations

OP.25

Increased oxidative stress underlies attenuated flow-induced dilation in cerebral resistance arteries of Sprague-Dawley rats on a high salt diet

Drenjančević I, Matić M, Jukić I, Mihaljević Z, Stupin A

Faculty Medicine University of Osijek, Croatia

High salt (NaCl) dietary intake is well known risk factor for the development of hypertension and microvascular dysfunction, leading to organ damage and increasing the risk of cardiovascular events. Endothelial function is impaired with HS dietary intake prior to increases in blood pressure. Novel studies suggest that this impairment is related to suppressed levels of angiotensin II, which has permissive, modulatory effect on the mechanisms of endothelial function via various vasodilatory and vasocontrictory pathways. Recently we demonstrated that short-term HS diet (4%NaCl in regular rat chow for 7 days) leads to attenuated flow-induced dilation (FID) of middle cerebral arteries (MCA) of normotensive Sprague Dawley rats, related to increased vascular oxidative stress (decreased cerebral vessels antioxidant enzymes' expression) (J Physiol, 2016; 594:4917-31). Increased superoxide/ reactive oxygen species levels affect the vascular NO levels which are decreased in HS fed rats, assessed by direct fluorescence. HS intake changes FID mechanisms to, albeit reduced, entirely NO dependent, in contrast to the low-salt diet-fed group, where FID is NO, prostanoid, and epoxyeicosatrienoic acid dependent. Plasma lipid peroxidation products are increased in HS diet-fed rats, together with increased

expression of transcription factor HIF-1α and VEGF. In vivo ROS scavenging by TEMPOL restores FID in HS diet-fed rats and ameliorates HS-induced increases in the HIF-1α and its downstream target genes (AJP 2018;315:H718-H730). Finally, HS diet causes increased oxidative stress in leukocytes of peripheral lymph organs suggesting the ongoing low-grade inflammatory processes (J Physiol 2016; 594(17): 4917-31). Support: Croatian Science Fundation grant #IP-2014-09-6380.

OP.26

Exploring the short-term, physiological, micro-circulatory effects of regional diets in sedentary, older, adult populations

Klonizakis M[1], Rogerson D[2], Milner M[1], Könönen H[1], McNeill S[2], Liu Y[1]

[1]Centre for Sport and Exercise Science, Sheffield Hallam University, UK; [2]Academy of Sport and Physical Activity, Sheffield Hallam University, UK

Cardiovascular Disease (CVD) is the biggest preventable cause of mortality in the Western world. Lifestyle interventions based on the Mediterranean (MD) and New Nordic (NND) diets have been proposed to provide cardiovascular benefits in clinical and healthy-but-at- risk populations. Although the benefits are undeniable, it is not known as of whether their physiological effects are greater in the long-term to those observed following a short-term consumption. This is important, as such knowledge will influence clinical recommendations and public health planning involving regional diets, as it will determine their optimal implementation duration. It will also help determining as of whether there are limitations to the benefits offered by them alone and if additional lifestyle arms (e.g., exercise, sedentary behaviour reduction etc.), would be necessary to achieve a greater CVD risk-reduction target. Therefore, we explored the physiological, micro-circulatory effects of MD and NND diets, following a 4-week implementation period, in sedentary, older, adult populations. We conducted a series of studies, involving the consumption of the MD and NND in previously unaccustomed, sedentary populations. We observed a statistically-significant, short-term improvement on axon-mediated microvascular vasodilation and endothelial- mediated nitric oxide synthesis, following the consumption of NND, but not with MD. Our findings suggest that different regional diets offer physiological benefits at different timelines. Therefore, clinicians and policy-makers should not recommend identical durations when making dietary prescriptions. Further work is required to identify the optimal implementation periods for different age and clinical groups, as expectations in physiological improvement differ.

OP.27

Exercise can reverse hypertension-induced microvascular dysfunction

Koller A

Department of Morphology and Physiology, Semmelweis University, Sport-physiology Res Center, University of Physical Education, Budapest, Hungary, Department of Physiology, New York Medical College, Valhalla, NY, USA

Hypertension elicits changes in the function and structure of small arterial vessels, which contribute to the development of increased systemic blood pressure. The functional changes include reduced release and action of dilator factors (nitric oxide, prostaglandins) and increased production of constrictor molecules (endothelin, thromboxane A2, reactive oxygen species, vascular angiotensin II). These changes functionally increase the basal vascular tone of microvessels and constrictor responses to a variety of stimuli. Persisting high blood pressure leads to morphological remodelling of vascular wall, such as thickening of wall, due to increased smooth muscle amount, deposition of collagen, elastin and fibrotic tissues, making the lumen smaller and less elastic. Most of these changes are assumed to be due to the increased intraluminal pressure and most likely to disturbed wall shear stress, thus it was logical to hypothesize that exercise will alleviate these remodelling, because during exercise hemodynamic forces changes and release of vasodilator factors is increased as indicated by post-exercise hypotension. Indeed, it has been shown that daily aerobic exercise upregulates the release of nitric oxide and prostaglandins, reduces the release of endothelins and increases the expression of the antioxidant enzymes, such as superoxide dismutase and catalase. As a result, the balance between the dilator and constrictor factors and the basal microvascular tone can be restored reducing total peripheral vascular resistance. Correspondingly, the 2018 ESC/ESH Guidelines for the management of arterial hypertension suggests that treatment of hypertension should be started by regular exercise and changes in life style.

Oral Presentations

OP.28

Glutamate induces intracellular Ca^{2+} signals and nitric oxide release in human brain microvascular endothelial cells

Negri S[1], Faris P[1,2], Pellavio G[3], Orgiu M[1], Forcaia G[4], Botta L[1], Sancini G[4], Laforenza U[2], Moccia F[1]

[1]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Italy; [2]Research Centre, Salahaddin University-Erbil, Iraq; [3]Department of Molecular Medicine, University of Pavia, Italy; [4]Department of Experimental Medicine, University of Milano Bicocca, Italy

Neurovascular coupling (NVC) is the mechanism whereby an increase in neuronal activity causes an increase in local cerebral blood flow (CBF) to ensure local supply of oxygen and nutrients to the activated areas. Recent work suggested that endothelial Ca^{2+} signals could underpin NVC by recruiting the endothelial nitric oxide NO (NO) synthase (eNOS). Herein, we sought to assess whether also glutamate elicits metabotropic Ca^{2+} signals and NO release in hCMEC/D3 cells, a widely employed model of human brain microvascular endothelial cells. Glutamate induced a dose-dependent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that was blocked by α-methyl-4- carboxyphenylglycine and phenocopied by trans-1-amino-1,3-cyclopentanedicarboxylic acid, which, respectively, block and activate Group 1 metabotropic glutamate receptors (mGluRs). Accordingly, and hCMEC/D3 expressed both mGluR1 and mGluR5 and the Ca^{2+} response to glutamate was inhibited by their pharmacological blockade with, respectively, CPCCOEt and MTEP hydrochloride. The Ca^{2+} response to glutamate was initiated by endogenous Ca^{2+} release from the endoplasmic reticulum (ER) and endolysosomal (EL) Ca^{2+} store through inositol-1,4,5-trisphosphate receptors and twopore channels, respectively, and sustained by store-operated Ca^{2+} entry (SOCE). Additionally, glutamate induced robust NO release that was suppressed by pharmacological blockade of the accompanying increase in $[Ca^{2+}]_i$. These data demonstrate for the first time that glutamate may induce Ca^{2+} signals and NO release by activating Group 1 mGluRs in human brain microvascular endothelial cells. The Ca^{2+} response to glutamate is likely to support NVC during neuronal activity, thereby reinforcing the emerging role of brain microvascular endothelial cells in the regulation of CBF. Keywords: glutamate, brain microvascular endothelial cells, nitric oxide

OP.29

$TRPV_4$ channels involvement in the response of lymphatic vessels intrinsic contractility to temperature

Solari E, Marcozzi C, Giaroni C, Baj A, Bistoletti M, Negrini D, Moriondo A

Department of Medicine and Surgery, University of Insubria, Italy

Rhythmic, spontaneous contractions of the lymphatic muscle in the vessel wall are one of the two propelling mechanisms that a subset of collecting lymphatic vessels can deploy to drain liquids from the interstitial spaces to the bloodstream. In *ex vivo* experiments, diaphragmatic lymphatic vessels (located in the thermal core) showed a very steep contraction frequency change and an unusual high Q10 response to temperatures in the 32- 39°C range. TRPV4 channels are known for their temperature-dependent gating with a range well within the steepest portion of the lymphatic vessel response to temperature. Real-time PCR analysis of spontaneously contracting diaphragmatic lymphatics revealed a 6-fold higher TRPV4 expression and an almost equal TRPV3 expression compared to the one in dorsal root ganglion neurons taken as positive controls. Whole-mount immunostaining of spontaneously contracting lymphatic vessels showed TRPV4 positivity scattered along vessels walls. Ruthenium red application (10-20 μM, not specific TRPVs blocker) greatly reduced the temperature-dependent modulation of lymphatic vessels contraction frequency in a dose-dependent manner, whereas HC067047 (2.5-5 μM, specific TRPV4 blocker) completely abolished temperature-dependent changes in contraction frequency. Overall, present data support the hypothesis that TRPV4 channels might be the temperature sensor of lymphatic vessels

Symposium 2

Genetic and epigenetic control of cardiac rhythm

Organizer: Dario Di Francesco , Milan, Italy

Invited Oral Presentations

OP.30

Night bradyarrhythmias: circadian control of heart rate via a sinus node clock and the funny channel

Boyett M, D'Souza A, Wang Y

Cardiovascular Sciences, University of Manchester, UK

In the human, there is a circadian rhythm in the resting heart rate and it is higher during the day in preparation for activity. Conversely, bradyarrhythmias occur primarily at night. The same occurs in rodents, although the changes occur in reverse (because mice are nocturnal rather than diurnal). Although the lower heart rate at night is widely assumed to be the result of high vagal tone, it is still not known if there is an intrinsic change in heart rate driven by a local circadian clock. In the mouse, we have shown a circadian rhythm in the heart rate not only *in vivo*, but also in denervated *ex vivo* preparations (e.g. the Langendorff-perfused heart). Experiments (e.g. qPCR) have revealed functioning canonical clock genes, e.g. Bmal1, in the sinus node (the pacemaker of the heart); therefore, there is a functioning circadian clock in the sinus node. In the sinus node, we have identified a circadian rhythm in the expression of some key ion channels. Most notably, we have identified a circadian rhythm in the pacemaker ion channel, HCN4, and the corresponding ionic current (the funny current, *If*). In the isolated sinus node, block of HCN4 and *If* by 2 mM Cs+ abolished the circadian rhythm in the beating rate. Disruption of the circadian clock in the sinus node (by cardiac-specific knockout of Bmal1) also abolished the circadian rhythm of HCN4 and *If*. In conclusion, there is a circadian rhythm in intrinsic cardiac pacemaking as a result of a local circadian clock in the sinus node that drives rhythmic expression of HCN4. The data reveal a novel regulator of sinus node function and mechanistic insight into the occurrence of bradyarrhythmias at night

OP.31

A heart in a dish: 3D microtissues with cardiomyocytes and non-myocyte cells derived from human iPSCs

Campostrini G

Dept of Anatomy and Embryology, Leiden University Medical Center, The Netherlands

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) obtained by in vitro differentiation are structurally and functionally immature, limiting their potential as human cardiac model. To better reproduce the human heart physiology, we generated three-dimensional microtissues including hiPSC-derived cardiomyocytes and non-myocyte cardiac cells. Using just 5000 cells per microtissue, we were able to induce upregulation of sarcomeric genes and improved electrophysiological properties in hiPSC-CMs, supporting increased maturation compared with monotypic two-dimensional cardiac cultures. This system supports the role of cardiac non-myocyte role in CM function. These microtissues represent an advanced human stem cell-based platform for cardiovascular disease modelling and testing of relevant drugs.

OP.32

Understanding arrhythmogenic cardiomyopathy using human iPSCs and primary stromal cells.

Rossini A

Institute for Biomedicine, Eurac Research, Italy

Arrhythmogenic cardiomyopathy (ACM) is an inherited genetic disorder, characterized by the fibro-fatty substitution of heart muscle causing heart failure, ventricular arrhythmias and sudden cardiac death. ACM is a genetic disorder but because of the low penetrance observed in patients, the involvement of both additional genetic and environmental factors has been proposed to contribute to its aetiology. We are using human induced pluripotent stem cell (hiPSC) and primary cardiac stromal cells derived from ACM patients to understand cellular and molecular alterations responsible for the onset of the pathology.

Oral Presentations

OP.33

Human iPSC modeling of familial forms of atrial fibrillation in patient-derived cardiomyocytes

Benzoni P[1], Giannetti F[1], Rocchetti M[2] Bucchi A[1], Baruscotti M[1], Di Francesco D, Olesen MS[3], Dell'Era P[4], Barbuti A[1]

[1]Department of Biosciences, Università degli Studi di Milano, Italy; [2]Department of Biotechnology and Biosciences, Università degli Studi di Milano Bicocca, Italy; [3]The Heart Centre, Rigshospitalet Universit, Denmark, [4]Department of Molecular and Translational Medicine, Università degli Studi di Brescia, Italy

Atrial Fibrillation (AF) is the most common type of cardiac arrhythmias whose incidence increase with age. Sometimes AF develops in young people with no evidence of other diseases. The molecular causes of AF are poorly understood. Here we used iPS cells-derived cardiomyocytes (iCM), obtained from two sisters who share more than one hundred mutated genes, one patient with a mutation in *PITX2* and three healthy controls. Patch-clamp analysis was used to record and compare action potentials and ion currents in 30 day-old iCM. iCM from the AF siblings display a higher beating rate than those of controls (CTRL). Stimulated action potentials are significantly prolonged

(+74% of action potential duration at 90% repolarization, APD90) in AF- than CTRL-cardiomyocytes and, under stressful conditions, generate more ectopic beats. AF cells show a significantly larger pacemaker (If) and calcium (ICaL) currents than controls, compatible with the faster rate and longer APD. PITX2-iCM instead showed a delay in maturation, a reduced firing rate (-41.2%), a shorter action potential duration (-41.7% of rate-corrected APD90), a larger amplitude (96.3±2.2 mV, n=32 vs 82.7±1.8 mV, n=61) and a steeper fast depolarization (dV/dTmax 14.6±1.9 mV/ms, n=32 vs 9.0±1.4 mV/ms, n=61) than CTRL-cells, suggesting alterations in sodium and potassium currents. These results show that AF car arise from different ion channels alterations. This approach may lay the basis for understanding the mechanism responsible for the onset of specific form of genetic AF.

OP.34

Effects of a calmodulin kinase II inhibitor, KN-93, on the electrophysiological modifications produced by local stretch of ventricular myocardium. Experimental study

Parra G[1], Zarzoso M[2], Such-Miquel L[2], Brines L[1], Muñoz M[1], Del Canto I[3], Goran R[1], Soler C[1], Genovés P[3], Arias O[4], Alberola A[1], Such L[1], Chorro FJ[4]

[1]Department of Physiology, Universitat de València, Spain; [2]Department of Physiotherapy, Universitat de València, Spain; [3]INCLIVA, Spain; [4]CIBERCV, Instituto de Salud Carlos III, Spain

Ventricular stretch occurs in pathophysiological processes and triggers hyperphosporilation of the cardiac ryanodine receptor (RyR2) produced by calmodulin kinase II (CaMKII). This elicits diastolic Ca^{2+} leakage mediated by the RyR2 and it can trigger life-threatening arrhythmias. We have investigated the effects of the CaMKII inhibitor, KN93, on ventricular myocardium refractoriness and electrophysiological heterogeneity modifications by acute local stretch on a model of isolated and perfused rabbit heart. Seventeen adult male New Zealand White rabbits were heparinized and euthanized by sodium thiopental injection (European Ethic Guidelines). The hearts were excised, isolated and perfused in a Langendorff system. A pacing electrode and a recording multielectrode were placed on the left ventricle epicardium submitted to stretch. The stretching was induced by an intraventricular device. Ventricular fibrillation (VF) was triggered by pacing and VF analysis in the time domain was performed to determine the mean fibrillatory cycle length (VV), and 5th percentile (p5), as an estimation of the functional refractory period. The coefficient of variation of mean VV (CV of VV), as a heterogeneity index, was calculated. Determinations were performed, previously, during and after stretch in both control (n=10) and KN-93 (n=7) groups. An ANOVA test was used for comparisons. Significance when $p<0.05$. The p5 decreased and the CV of VV increased, after local stretch in control group. No modifications were observed in treated group, although p5 was lower and CV of VV higher in treated than in control group before stretching. Thus, KN-93 prevents the refractoriness and electrophysiological heterogeneity alterations by the acute stretch, although it modifies these properties at baseline.

Symposium 3

Walking the last mile: current strategies and challenges in the maturation of novel cardiomyocytes for myocardial repair

Organizer: Nina Ullrich , Heidelberg, Germany

Invited Oral Presentations

OP.35

Patient-specific human hearth-on-chip to study mechanotransduction

Elvassore N

Department of Industrial Engineering University of Padova Via Marzolo 9 35131 Padova, Italy.

Physical cues are major determinants of cellular phenotype and evoke physiological and pathological responses on cell structure and function. Cellular models aim to recapitulate basic functional features of their in vivo counterparts or tissues in order to be of use in in vitro disease modeling or drug screening and testing. Understanding how culture systems affect in vitro development of human pluripotent stem cell (hPSC)-derivatives allows optimization of cellular human models and gives insight in the processes involved in their structural organization and function. We show involvement of the mechanotransduction pathway RhoA/ROCK in the structural reorganization of hPSC-derived cardiomyocytes resulting in the intracellular localization of SERCA2 pumps and concurrent improvement in calcium cycling. By integrating the culture substrate with a macroscopic stretching setup able to accurately apply cyclic uniaxial elongation, we show how response to mechanical loads in hPSC-derived cardiomyocytes deviates from the canonical mechanical-stress response observed in healthy- cardiomyocytes. In particular, we focused on that cell junctions play an important role in coordinating intercellular communication and intracellular

ultrastructures, with desmosomes representing the mechanical component of such intercellular connections. Mutations to desmosomal component proteins compromise both inter- and intracellular signalling and correlate with severe diseases like arrhythmogenic cardiomyopathy (AC), with pathological phenotypes in tissues subjected to intense mechanical stimuli (skin and heart). We explore the consequences of dysfunctional desmosomes in one line of induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) derived from an AC patient with a homozygous pathogenic mutation in desmosomal component protein plakophilin-2 (PKP2). We specifically aim at investigating the response to mechanical stress in an AC-pathological setting.

OP.36

Cardiovascular diseases and drugs: where are we with hiPSC models?

Mummery C, Bellin M, Giacomelli E, van Meer V, Orlova V, Sala L, Tertoolen L

Dept. Anatomy and Embryology, Leiden University Medical Centre, The Netherlands

Derivation of cardiovascular cell types from human pluripotent stem cells derived from patients or introducing targeted mutations is an area of growing interest as a platform for drug discovery and toxicity. Our lab has been investigating organs on chip and microtissue solutions in which cardiomyocytes and cardiac vascular and stromal cells are present. This promotes cardiomyocyte maturation and in combination with new methods for functional phenotyping, we have been able to quantify the outcomes of drug and disease mutation responses in situ. The use of isogenic pairs has proven very important since variability between “healthy control” hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. hiPSC derived cardiomyocytes with mutations in ion channels and other genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients.

OP.37

Engineered human myocardium for enhanced maturation of pluripotent stem cell-derived cardiomyocytes

Tiburcy M[1,2], Liaw N[1,2], Schlick S[1,2], Zimmermann W-H[1,2]

[1]Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Germany; [2]DZHK - German Center for Cardiovascular Research, Germany

Tissue engineering of heart muscle from human pluripotent stem cells holds great potential for *in vitro* studies, disease modeling, and cardiac replacement therapy. Here, we aimed to identify cues that drive maturation of cardiomyocytes in engineered human myocardium (EHM). EHM was generated by casting highly purified human pluripotent stem cell-derived cardiomyocytes (>92% Actinin+) and stroma cells into a collagen 1 hydrogel. After tissue consolidation, (electro)-mechanical conditioning on flexible holders (facilitating auxotonic contractions) was applied to enhance tissue maturation. This protocol results in a highly functional human heart muscle with a contractile force of up to 10 mN/mm2, typical Frank-Starling as well as Bowditch responses (positive force frequency), a physiological active to passive force ratio and clear positive inotropic responses to drugs like isoprenaline. This is paralleled by morphological and molecular maturation of mostly elongated, rod-shaped cardiomyocytes. As EHM without stroma cells does not form, we dissected the role of stroma cells in the formation of functional tissue and investigated biophysical properties. Interestingly, even in the first 90 mins of tissue formation, stroma cells impact stiffening of the developing tissue with evidence of cardiomyocyte/stroma cell cross talk. EHM with stroma cells acquire a near-physiological stiffness of 1-2 kPa. Adding electrical pacing to mechanical conditioning further improved specific EHM functions like calcium handling and metabolism. Cardiomyocyte-stroma cell interactions and electromechanical conditioning are two major drivers of cardiomyocyte maturation in EHM.

Oral Presentations

OP.38

The functional expression of the Lamin A mutant $Q_{517}X$ leads to nuclear and cytoskeleton remodeling with reduced action potentials frequency in HL_1 cardiomyocytes

Gerbino A[1], De Zio R[1], Forleo C[2], Milano S[1], Procino G[1], Favale S[2], Svelto M[1], Carmosino M[3]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Italy; [2]Department of Emergency and Organ Transplantation, University of Bari "Aldo Moro", Italy ; [3]Department of Sciences, University of Basilicata, Italy

Mutations in the LMNA gene, which encodes A-type nuclear Lamins, are among the most frequent genetic cause of dilated cardiomyopathy (DCM). We identified a novel LMNA nonsense mutation (Q517X) co-segregating with DCM with left ventricular dysfunction and conduction defects. Aim of this study is to gain insights into the unknown pathogenic mechanism induced by the expression of Q517X at cellular level. HEK293 cells and HL-1 cardiomyocytes were transiently transfected with either Lamin A or Q517X both tagged with mCherry. Q517X expression, localization and its effects on diverse cellular mechanisms were evaluated by western blotting, laser-scanning confocal microscopy and FRET analysis in single cell. Also, patch clamp experiments were used to evaluate the effect of Q517X expression on HL-1 spontaneous action potentials (APs). When expressed in both cell types, Q517X mislocalized within the nucleoplasm where it clustered in aggregates of different sizes. Nuclear pores and emerin were also irregularly distributed along the nuclear envelope probably indicating a general impairment of the nuclear structural organization upon Q517X expression. In addition, cells expressing Q517X underwent a cytoskeleton remodeling not dependent by cAMP levels/PKA activity, likely contributing to impaired trafficking of ion channels at the plasma membrane. Of note, HL-1 cardiomyocytes expressing the mutant showed a significant reduction in APs frequency induced by an increased duration of both single APDs (APD100) and the cell cycle (i.e. the distance between to sequential APDs' thresholds). So far, these results suggest the involvement of aberrant ion channels trafficking/expression/activity as the pathogenic mechanism for the conduction defect associated to this LMNA truncating alteration.

OP.39

Loss of full-length dystrophin alters calcium handling in response to substrate stiffness during maturation of human induced pluripotent stem cell-derived cardiomyocytes

Pioner JM

Department of Experimental and Clinic Medicine, University of Florence, Italy

Cardiomyopathy invariably affects teenage patients with Duchenne Muscular Dystrophy (DMD). In humans, developmental consequences of loss of full-length dystrophin (Dp427) are poorly understood. We used induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) generated from a DMD patient (DMD-ΔExon50) compared to healthy control- hiPSC-CMs and its isogenic cell line with CRISPR-Cas9 genome edited deletion of a G base at position 263 of the DMD gene (c.263delG-CMs). We combined nanopatterned substrates with long-term culture to improve maturation of hiPSC-CMs. We studied hiPSC-CM Ca- handling at multiple time points (60-75-90 days). In Control- and DMD-CMs, we simultaneously measured action potentials (AP) and Ca^{2+} transients (Ca-T), with Fluovolt and Cal630 dyes respectively. In control CMs we observed over time a prolongation of action potential duration (APD), increased Ca-T amplitude, and faster Ca-T rise (time to peak) and Ca-T decay (RT50). The contribution of the SR to Ca release (estimated by a post-rest potentiation protocol or Caffeine-induced Ca-T) increased over time and appeared as the main determinant of the progressive rise of CaT amplitude. Compared to controls, at all stages of maturation the DMD-CMs showed: shorter APD, reduced CaT amplitude and faster Ca-T rise and RT50. Caffeine-CaT amplitude was reduced with slower CaT suggesting lower SR calcium content and NCX function respectively. We then tested the response to surfaces with increased stiffness. Notably, increased substrate stiffness led to larger CaT amplitude in both control and DMD-CMs but the latter developed slower RT50. C.263delG- CMs recapitulated DMD-CM CaT responses indicating that calcium-handling abnormalities are driving alterations due to loss of Dp427 in human cardiomyocytes.

CELL PHYSIOLOGY

Symposium 1

Targeting metabolic modulation and mitochondrial dysfunction for cardioprotection

Organizer: Tatiana Ravingerová (Bratislava, Slovakia)

Invited Oral Presentations

OP.40

PPARβ/δ at the cross talk between cardiac metabolism and mitochondrial function

Lazou A

School of Biology, Aristotle University of Thessaloniki, Greece

Impaired mitochondrial biogenesis and function linked with derangement of cardiac metabolism play a vital role in the pathogenesis of cardiac diseases. Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor transcription factor

superfamily, are critical regulators of cardiac metabolism and function in health and disease. Although PPARβ/δ is the most prevalent subtype in the myocardium, it is the least studied member of the PPAR subfamily. Accumulating evidence indicates the role of PPARβ/δ in many physiological functions, ranging from enhanced fatty acid catabolism and improved insulin sensitivity, to inflammation inhibition and mitochondrial biogenesis, and highlights its protective role in the improvement of cardiac function under diverse pathological settings. Selective agonism in rodent heart provided evidence of the protective potential of PPARβ/δ against post-ischemic cardiac dysfunction and failure. PPARβ/δ controls oxidative metabolism and fuel preference, with its target genes involved in fatty acid and glucose utilization, whereas it is also implicated in the regulation of cardiac redox balance through effects on transcriptional regulation of antioxidant enzymes or other effectors that could modulate oxidative stress. Furthermore, PPARβ/δ activation modulates mitochondrial dynamics and autophagy and attenuates cardiac remodeling and inflammation in animal models of heart failure or diabetic cardiomyopathy. Thus, PPARβ/δ might serve as a therapeutic target to improve cardiac function in several cardiac pathologies.

OP.41

New cardiokines involved in the control of cardiac metabolism

Planavila A, **Rupérez C**, **Ferrer-Curriu G**, **Villarroya F**

Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona and CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Spain

Although a considerable effort has been devoted to improve therapy, heart failure remains a critical health problem; thus, the identification of underlying molecular mechanisms is crucially important for improving the efficacy of therapeutic strategies. Experimental data suggest that control of metabolic pathways is involved in the pathophysiology of cardiac hypertrophy and failure by modulation of crucial cellular processes. Hypertrophy is one of the main ways in which cardiomyocytes respond to mechanical and neurohormonal stimuli and is characterized by a shift in the source of energy from fatty acids to glucose. Therefore, the study of the mechanisms controlling metabolism in the heart represents a major challenge for the future in terms of therapeutic applications to treat cardiac hypertrophy and failure. The term "cardiokine" refers to the proteins secreted by the cardiac tissue required for the maintenance of normal cardiac function and for the control of pathological remodeling of the myocardium in response to injury through their ability to act in a paracrine/autocrine manner to modulate cardiomyocyte hypertrophy; survival and apoptosis; fibroblast activation; and inflammation. Recently, we have demonstrated that the heart locally generates FGF21 which acts in an autocrine manner preventing hypertrophy, metabolic dysregulation and the activation of pro-inflammatory pathways in cardiac tissue.

OP.42

Mitochondrial potassium channels and cardioprotection

Szewczyk A

Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Poland

Lecture will present the most interesting issues regarding function, regulation and pharmacology of the mitochondrial potassium channels. There are eight potassium channels known to contribute to the potassium permeability of the inner mitochondrial membrane: ATP-regulated channel, calcium-regulated channels of large (large, intermediate and small conductance), voltage-regulated Kv1.3 and Kv7.4 channels, two- pore-domain TASK-3 channel and SLO2 channel. The primary function of the mitochondrial potassium channels is regulation of the mitochondrial membrane potential. Additionally, mitochondrial potassium channels alter cellular respiration, regulation of the mitochondrial volume and ROS synthesis. The focus of the presentation will be on cardioprotective role of mitochondrial potassium channels induced by drugs, kinases and gases such as carbon monoxide, nitric oxide and H_2S. Additionally, an interaction with respiratory chain of mitochondrial channels will be reviewed. This study was supported by a grant 2015/17/B/NZ1/02496) from the National Science Centre, Poland.

Oral Presentations

OP.43

The Thyroid Hormone activating enzyme, Type 2 deiodinase, increases myogenic differentiation by regulating mitochondrial metabolism and reducing oxidative stress

Cicatiello AG[1], Sagliocchi S[1], Di Cicco E[1], Ambrosio R[2], Miro C[1], Nappi A[1], Mancino G[1], Raia M[3], Paladino S[4], Salvatore D[5], Dentice M[1]

[1]Department of Clinical Medicine and Surgery, University of Naples "Federico II", Italy; [2]IRCCS SDN, Italy; [3]CEINGE–Biotecnologie Avanzate Scarl, Italy; [4]Dipartimento di Medicina

Molecolare e Biotecnologie Mediche, University Federico II, Italy; [5]Department of Biomedical Advanced Sciences, University of Naples "Federico II", Italy

Thyroid hormone (TH) is a key metabolic regulator that acts by coordinating short-term and long-term energy needs. Accordingly, significant metabolic changes are seen with variations in thyroid status in humans. Although it is established that hyperthyroidism enhances metabolic state, the net effects on cellular respiration and generation of reactive oxygen species (ROS) are still unclear. To elucidate the function of augmented TH signal in muscle cells, we generated a doxycycline-inducible cell line, in which the expression of the TH-activating enzyme, type II deiodinase (D2) is reversibly turned on by the "Tet-ON" system. Interestingly, the increased intracellular TH resulted in a net shift from Oxidative Phosphorylation (OXPHOS) to glycolysis and a consequent increase in extracellular acidification rate. As a result, both the basal and the doxorubicin-induced cellular ROS production was reduced. Importantly, the expression of a set of antioxidant genes was up- regulated, and, among them, the mitochondrial scavenger SOD2 was specifically induced at transcriptional level, by D2-mediated TH activation. Finally, we observed that the attenuation of the oxidative stress and increased levels of SOD2 induced by D2 are essential components of the differentiating cascade of muscle cells triggered by the TH and D2. Indeed, TH activation via D2 induction increases muscle cells differentiation and triggers the intracellular cascade leading to SOD2-mediated myogenic process. In conclusion, our findings indicate that TH has a key role in oxidative stress dynamics through regulation of ROS generation. The role of TH and its intracellular metabolism as mitochondrial detoxifying agents is a novel finding, shedding light on metabolic processes relevant to muscle physiology.

OP.44

High fat diet and environmental pollutants exposure: mitochondrial uncoupling as protective mechanism toward hepatic injury in rats

Migliaccio V[1,2], Di Gregorio I[1], Sica R[2], Scudiero R[2], Putti R[2], Lionetti L[1]

[1]Department of Chemistry and Biology, University of Salerno, Italy; [2]Department of Biology, University Federico II, Naples, Italy

Liver is the main organ involved in dietary lipid handling and xenobiotic detoxification. Mitochondrial dysfunction and oxidative stress play a key role in hepatic injury. This study aimed to investigate oxidative stress, mitochondrial dysfunction and uncoupling in response to exposure to high fat diet (HFD) and/or non-toxic dose of the environmental pollutant dichloro diphenyl dichloroethylene (DDE, the first metabolite of DDT). Groups of rats were so treated: 1- standard diet (N group); 2- standard diet plus oral administration of DDE (10 mg/kg b.w.) (N+DDE group); 3- HFD (D group); 4- HFD plus DDE (D+DDE group). Oxidative stress, mitochondrial fatty acid oxidation and uncoupling protein 2 (UCP2) expression/content were analyzed. D rats showed increased hepatic lipid accumulation and fatty acid oxidation associated with increased oxidative stress and UCP2 content. Enhanced fatty acid oxidation leads to increased ROS production which can be controlled by mitochondrial uncoupling that, by increasing proton leak back to the matrix, contributes to regulate the excess superoxide production by the electron transport chain. DDE treated- groups also showed increases in hepatic injury, fatty acid oxidation and oxidative stress, but with a higher increase in hepatocytes UCP2 content vs. D group, mainly when associated with a normal diet. Noteworthy, UCP2 in liver of control rats was expressed only in immunocompetent cells, whereas the oxidative stress, produced by HFD and/or DDE, induced mRNA synthesis and protein translation in hepatocytes too. Present findings confirmed that both HFD and xenobiotic exposure induced hepatic oxidative stress and showed that the UCP2 induction could be an adaptive response to limit excessive ROS damage, mainly in condition of xenobiotic exposure.

Symposium 2

Physiopathology of signaling transmission and membrane transport

Organizer: Giovanna Valenti, Bari, Italy

Invited Oral Presentations

OP.45

Regulation of renal urea and water transport: implications for therapy of nephrogenic diabetes insipidus

Sands JM, Klein JD

Department of Medicine and Department of Physiology, Emory University, USA

Nephrogenic diabetes insipidus (NDI) is an inability of the kidney to respond to vasopressin, resulting in production of very large quantities of dilute urine. Congenital NDI results from mutations in the type 2 vasopressin receptor (V2R) in 90% of families. Since these patients do not have mutations in the aquaporin-

2 (AQP2) water channel or the UT-A1 urea transporter, we tested the effect of AMP-activated protein kinase (AMPK). AMPK directly phosphorylated AQP2 and UT-A1 in vitro. An AMPK activator, metformin, increased AQP2 and UT-A1 phosphorylation in rat inner medullary collecting ducts (IMCDs). Metformin also increased osmotic water permeability and urea permeability in perfused rat terminal IMCDs. Next, we studied whether metformin could improve urine concentration in two rodent models of congenital NDI. Rats were gavage fed with tolvaptan (10 mg/kg/d) to block the V2R, and half were given metformin (800 mg/kg/d). Tamoxifen-induced V2R knockout mice were given metformin (600 mg/kg) or vehicle. Urine osmolality in tolvaptan-treated rats was restored to control levels by metformin within 3 days and sustained for 10 days. Metformin increased the protein abundance of AQP2 by 44% and UT-A1 by 61% and in tolvaptan-treated rats. In V2R knockout mice, metformin increased urine osmolality within 1 hour; the increase persisted for 12 hours and AQP2 protein abundance increased. These results suggest that AMPK activators might provide a promising treatment for congenital NDI.

OP.46

Targeting calcium-sensing receptor signaling as novel therapeutic for asthma

Riccardi D

University of Cardiff, UK

Asthma is a chronic pro-inflammatory lung disorder that affects 340 million people, worldwide. It is characterised by airway inflammation, hyperresponsiveness (AHR) and remodelling. Current treatments are only palliative and do not address the disease root-cause and are associated with unwanted side effects. In addition, ~10% of the patients are steroid-resistant and do not respond to even oral corticosteroid tretament, resulting in significant morbidity and mortality and substantial healthcare costs. Therefore, there is need for novel asthma therapeutics, particularly for steroid-resistant asthma. Recently we have show that the calcium-sensing receptor, CaSR, is expressed in the airways, and it is activated by polycations whose expression is increased during allergic asthma (eosinophil cationic proteins, major basic proteins, polyamines. Polycation-induced CaSR activation in human airway smooth muscle cells leads to an increase in intracellular Ca2+ as well as Akt, p38 MAPK and ERK phosphorylation, effects prevented by calcilytics. Prophilactic calcilytic administration appears to be as good as inhaled fluticasone propionate at suppressing inflammation in murine allergic asthma models. Therapeutic administration of calcilytics, delivered topically, prevents AHR, inflammation and remodelling in murine models of both allergic and non-allergic asthma. 15 month old mice with targeted CaSR ablation from smooth muscle cells are protected from age- related lung fibrosis. Thus, CaSR activation by polyvalent cations and polycations drives inflammation, AHR and remodelling. Blocking CaSR-mediated signalling in the airways with topically delivered calcilytics represents a promising new treatment for asthma.

OP.47

Adipose and liver aquaglyceroporins: functional relevance and (dys)regulation in metabolic disorders

Calamita G

Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari “Aldo Moro”, Italy

Aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) encompass a subfamily of aquaporin channels (AQPs) that facilitate the movement of water and non-charged small solutes, especially glycerol, an important metabolite for the control of fat accumulation and for glucose homeostasis, across cell membranes. Adipose tissue represents a major source of lipolytic glycerol released via AQP7 and AQP3. Plasma glycerol is imported by hepatocytes mainly through AQP9 and converted into glycerol-3-phosphate (G3P) by the glycerol kinase for *de novo* synthesis of glucose (gluconeogenesis) and triacyglycerols (lipogenesis). Fat tissue and liver also express AQP10 whose functional significance in the related cells is unclear. Although with distinctions between rodents and humans insulin and leptin act as modulators of aquaglyceroporins through the PI3K/Akt/mTOR signaling pathway. Estrogens exert a negative regulation on adipose and hepatic aquaglyceroporins likely explaining the sexual dimorphisms seen in health and metabolic diseases. Considerable dysregulation of fat AQP7 and hepatic AQP9 is found in energy balance disorders. AQP7 deficiency has been linked to abnormal triglycerides accumulation in adipose tissue and adult onset obesity while alterations in hepatic AQP9 expression have been shown in animal models and patients with diabetes, obesity and fatty liver disease. AQP9 is involved in the lipid-lowering activity of silybin, a nutraceutical phytocompound, on hepatocytes through modulation of autophagy (see communication by Vergani et al. at this meeting). Pharmacological and nutraceutical modulation of adipose and liver aquaglyceroporins may offer promise for the management of a spectrum of clinical disorders including metabolic and energy balance diseases.

Oral Presentations

OP.48

Honeybee products and wound healing: an AQP3, H_2O_2 and Ca^{2+} signaling affair

Martinotti S[1], Pellavio G[2], Patrone M[1], Laforenza U[2], Moccia F[3], Ranzato E[1]

[1]Dipartimento di Scienze e Innovazione Tecnologica (DiSIT) University of Piemonte Orientale Italy; [2]Department of Molecular Medicine, University of Pavia, Italy; [3]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Italy

Wound healing is of countless significance for skin medicine and a precise focus is set on natural compounds. In spite of a wide amount of literature about honeybee products, such as propolis and honey, in clinical practices, the subjacent mechanisms of action are still largely unclear. We have recently elucidated the modulatory effects of different honey types in an in vitro model of keratinocytes and fibroblasts. Our data confirmed that honey could be used safely not only for external applications on healthy skin, but also as a dressing on wounds. The high concentrations of sugars (about 80%) combined with less than 1% of water causes osmotic stress preventing microorganism growth. Moreover, hydrogen peroxide (H_2O_2) was recognized as a major antibacterial compound present in honey. Specific aquaporins (AQPs) facilitate the passive diffusion of H_2O_2 across the biological membranes. AQP-mediated transmembrane transport of H_2O_2 is of physiological importance for further downstream signaling events, such as the onset of intracellular Ca^{2+} signals, which activates multiple Ca^{2+}-dependent processes associated to tissue regeneration. We found that honey produces H_2O_2 in the extracellular space, AQP3 mediates the H_2O_2 entry in to the cytosol, and in turn, H_2O_2 activates TRPM2 and Orai1 determining Ca^{2+} entry from the outside that induces wound closure. Intriguingly, we also revealed the involvement of H_2O_2 as a main mediator of propolis regenerative effects. We observed that the propolis-extracellularly released H_2O_2 could pass across the plasma membrane through AQP3 modulating intracellular responses. Our observations open up a new horizon for the use of the honeybee products in the management of skin disorder by the modulation of aquaporin expression.

OP.49

Two novel *SCN5A* loss-of-function mutations affect patients with severe arrhytmogenic syndromes

Murano C[1], Binda A[1], Lucano D[1], Micaglio E[2], Ciconte G[2], Ghiroldi A[3], Anastasia L[3,4], Pappone C[2], Rivolta I[1]

[1]School of Medicine and Surgery, University of Milano Bicocca, Italy; [2]Arrhythmology Department, Scientific Institute for Research, Hospitalization, and Health Care (IRCCS) Policlinico San Donato, Italy; [3]Laboratory of Stem Cells for Tissue Engineering, Scientific Institute for Research, Hospitalization, and Health Care (IRCCS) Policlinico San Donato, Italy; [4]Department of Biomedical Sciences for Health (L.I.T.A.), University of Milano, Italy

SCN5A gene encodes for the α subunit of the cardiac isoform of the voltage gated sodium channel. Mutations in this gene have been correlated with several arrhytmogenic syndromes among which Long QT Syndrome (LQTS) type 3 and Brugada Syndrome (BrS) are the most studied. Here we describe two novel mutations related to these phenotypes. The c.86_87delinsTG causes the substitution of Ala 29 in the N-terminus of the protein with a Val and was found in a 36 years old man diagnosed with BrS. The mutation c.5089T>C substitute the Phe 1697 with a Ser in the intramembrane pore forming region and was found in a 45 years old woman diagnosed with LQTS. Whole cell patch-clamp studies on HEK293 cells revealed that both the mutations are responsible for a loss-of-function of the protein. In particular the A29V completely abolished the inward current. The F1697S mutation reduced the current density (-183.1±37.1 pA/pF, n=37 in WT vs -107.3±33.7 pA/pF, n=29 in F1697S; $p<0.01$) and caused a significant negative shift in the half-maximal voltage (V1/2) of steady- state inactivation curve and a positive one in the V1/2 of the activation curve (-8 and +6.4 mV, respectively). These gating changes caused a shift and a reduction in the window current that may markedly modify the action potential duration. Moreover, F1697S substitution slowed down the recovery from inactivation, being 8.1±0.2 ms (n=27) in WT and 12±0.4 ms (n=17) in F1697S the time necessary for the recovery of the 50% of the channels. These findings suggest that both the mutations strongly reduce the sodium inward current anticipating a higher risk of arrhythmogenesis for the patients. Still more has to be done in order to explain the clinical phenotype of LQTS in light of a loss-of-function of the F1697S Nav1.5 channel.

ENDOCRINE PHYSIOLOGY

Symposium

Oxytocin, vasopressin and related peptides: novel functions and therapeutic potentials

Organizer: Sara Arrowsmith and Susan Wray , Liverpool, UK

Invited Oral Presentations

OP.50

Novel analogues of oxytocin and vasopressin to modulate human myometrial contraction

Arrowsmith S

Harris-Wellbeing Preterm Birth Research Centre, Institute of Translational Medicine, University of Liverpool, UK

Oxytocin (OT) and vasopressin (AVP) are closely related, neurohypophysial nonapeptide hormones. They share high chemical similarity and their receptors share high (~80%) extracellular binding domain sequence homology which leads to significant cross talk: OT can activate the AVPRs and AVP the OTR. Oxytocin is one of the most frequently used drugs in obstetrics. It can induce and augment uterine contractions in cases of labour dystocia and prevent postpartum haemorrhage, whilst OTR antagonists are used as tocolytics for halting preterm labour. Vasopressin may also have a physiological role in the myometrium as it can also induce uterine contractions. There is pressing need to develop better tocolytics for preterm birth and uterotonics for labour dystocia. Barriers for progress however, include the complex hormone-receptor interplay and lack of receptor-selective ligands to target them. Differences in receptor expression between species is also complex. The aims of this talk are to discuss the role of these hormones and their receptors in modulating human myometrial contraction. Data will be presented in which novel analogues of OT and AVP displaying targeted receptor selectivity have been used to determine the importance of the individual receptor subtypes in human myometrium. Receptor expression studies will also be discussed. The second aim of this talk is to highlight effective collaborations between the fields of medicinal and peptide chemistry and physiology in facilitating translational research from bench to bedside.

OP.51

Oxytocin in pain and associated disorders

Charlet A

Centre National de la Recherche Scientifique, and University of Strasbourg Institute for Advanced Study (USIAS), France

Oxytocin, the “great facilitator of life”, is a nonapeptide mainly synthesized in the paraventricular, supraoptic and accessory nuclei of the hypothalamus. OT has become over the last several years a center of attention for the regulation of functions in emotional behavior, among which social recognition and partner choice, aggression and maternal care, addiction, as well as pain integration and anxiety. In particular, our lab recently shed light on the oxytocin- induced modulation of both sensory and emotion processing. Indeed, we described for the first time a small population of ~30 oxytocin neurons that forms intra-hypothalamic and hypothalamo-spinal projections to coordinates central and peripheral oxytocin release in order to significantly dampen pain. Besides, we have shown direct hypothalamic oxytocin neurons axonal projections to the central nucleus of the amygdala that are relevant for anxiety relief. Recently, we demonstrated that an oxytocin neuron engram is recruited during fear learning and facilitates fear extinction in a context-depended manner via glutamate release in the CeA. Therefore, the study of oxytocin-induced regulation of neuronal circuits constitute a potent track to address the clinicians’ questions regarding chronic pain and associated affective disorders. However, the oxytocin short half-life and numerous side effects make it a poor candidate for clinical trials. In this regards, we propose the development of new, non- peptidergic, agonists, to open the therapeutical window.

OP.52

Septal oxytocin signaling and the regulation of social fear

Menon R, **Grossmann C**, **Neumann I**

Department of Behavioural and Molecular Neurobiology, University of Regensburg, Germany

Intense fear and avoidance of social situations are symptomatic of maladaptations like social anxiety disorder (SAD). Using the social fear conditioning (SFC) paradigm, we have identified lateral septum as a key brain structure wherein the pro-social and anxiolytic neuropeptide oxytocin (OXT) is involved in the extinction of social fear. Considering our previous results with synthetic OXT, here we studied endogenous OXT mediated regulation of social fear expression using models of activated OXT-system like lactation in female mice and sexual activity in male mice. Social fear conditioned (SFC+) lactating mice did not show any SFC-induced fear in comparison to virgin females. Similarly, mating before social fear extinction reduced fear expression in male mice. c-Fos immunohistochemistry revealed increased LS-activation in SFC+ virgin female and male mice whereas LS-activity remained dampened throughout SFC in lactating mice. Lack of social fear could be reinstated by LS infusion of OXTR antagonist (OXTR-A) in lactating mice. Conversely, LS infusion of OXT reversed SFC-induced social fear in virgin female and male mice. Using chemogenetics we implicated a subpopulation of LS-projecting OXTergic neurons

within the hypothalamus in reduced social fear expression during lactation. Current experiments focus on the identification of (i) similar circuits, which mediate the social fear reducing effects of sexual activity in male mice and (ii) downstream targets of OXTR-expressing neurons within the LS of male and female mice. Taken together, our data shows that enhanced OXT signaling within the LS during lactation and mating strongly prevents social fear expression in female and male mice respectively.

Oral Presentations

OP.53

Functional characterization of gain-of-function mutations of the V_2 vasopressin receptor leading to nephrogenic syndrome of inappropiate antidiuresis (NSIAD)

Ranieri M[1], Tamma G[1], Pellegrini T[1], Vezzi V[2], D'Ambrosio C[2], Di Mise A[1], Venneri M[1], Costa T[2], Cotecchia S[1], Valenti G[1]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy; [2]Department of Pharmacology, Istituto Superiore di Sanità, Italy

Nephrogenic Syndrome of Inappropriate Antidiuresis (NSIAD) is a chromosome X-linked disease associated to gain-of-function mutations of the V2 vasopressin receptor (V2R), a G protein-coupled receptor. NSIAD can be quite severe in affected male children. It is characterized by inability to excrete a free water load, hyponatremia, and undetectable vasopressin circulating levels. In this study, we have expressed the wild type V2R and three constitutively active V2R mutants, the R137L, R137C and the F229V in MCD4 cells, a cell line derived from renal mouse collecting duct, stably expressing the vasopressin- sensitive water channel Aquaporin-2 (AQP2). In cells expressing each active mutant, AQP2 was constitutively localized to the apical plasma membrane, in the absence of vasopressin stimulation. Conversely, in cells expressing the wild type V2R, AQP2 was localized in intracellular vesicles and redistributed to the apical membrane in response to vasopressin. In line with these observations, under basal conditions, osmotic water permeability of each constitutively active mutant was significantly higher compared with that of cells expressing the wild type V2R. Interestingly, specific inhibition of PKA reduced the basal osmotic water permeability only in F229V expressing cells, indicating the activation of a PKA-dependent pathway. Conversely, for the R137L and R137C mutants a PKA-independent signalling leading to redistribution of AQP2 and consequent increase in osmotic water permeability is predicted. Our findings demonstrate, for the first time, a direct link between the activating mutations of the V2R and the alteration of water permeability in cells expressing V2R mutants providing a rationale for the water balance disturbance observed in NSIAD.

OP.54

Sexual differences in the adrenal structure and morphometry in the Saharan gerbil *Gerbillus tarabuli* (Thomas, 1902)

Zatra Y[1,2], Aknoun Sail N[2], Kheddache A[2], Benmouloud A[2], Charallah S[2], Khammar F[2], Amirat Z[2]

[1]Faculty of Nature and Life Sciences, University of Blida 1, Algeria; [2]Research Laboratory of Arid Lands, University of Science and Technology Houari Boumediene (USTHB), Algeria

In the desert mammals, seasonal reproduction allows births only under favourable conditions and the adrenal glands show significant adaptive changes ensuring survival in arid areas. In order to elucidate the gonad-adrenal interrelationships, we compare in both sexes of *Gerbillus tarabuli*, a Sahara Desert rodent, the effects of castration performed during the breeding season on the adrenal weight and structure. So, we used 14 male and 14 female adult free-living gerbils divided into control and castrated groups. Fifty days after castration, animals were euthanized, the right adrenal gland was fixed in 10% formalin and 5µm thick sections stained with Masson Trichroma. Morphometric measurements were performed in the adrenal cortex and the cell and nuclear areas of each cortical zona. In control group, body weight of males and females are similar. However, males have heavier adrenal glands than females. Height of the adrenal cortex is also greater in the male due to that of zona fasciculata. The gonadectomy does not modify the body weight but it reduces the left/right adrenal weight dimorphism and increases the paired adrenal weight in both sexes. The adrenal hypertrophy occurred mainly in the zona reticularis (tissue thickness and cell and nuclear areas) of both sexes while zona fasciculata undergoes a slight decrease in the male but increases in the female. The zona glomerulosa remains unchanged in the male whereas it is hypertrophied in the female. These structural changes which differ between the two sexes suggest a gonadal dependent regulation of the adrenal gland with different modulating effects of sex steroids. These effects are probably involved in the development of anatomical and physiological strategies of adaptation of this desert rodent.

EXERCISE PHYSIOLOGY

Symposium

The pathophysiology of exertional dyspnoea: from physiology to clinical applications

Organizer: Pierantonio Laveneziana (Paris, France) & Georges Leftheriotis (Nice, France)

Invited Oral Presentations

OP.55

The mechanisms of dyspnoea: from physiology to clinical applications

Morelot-Panzini C

UMRS 1158 and Unité ambulatoire d'Appareillage Respiratoire de Domicile, Service de Pneumologie, Médecine Intensive et Réanimation, Hôpital Pitié-Salpêtrière, Paris, France

Dyspnoea is a complex, multifaceted and highly personalised sensory experience, the source and mechanisms of which are incompletely understood: there is no unique central or peripheral source of this symptom. Dyspnoea is a debilitating symptom, whose impact is second only to that of pain; it is estimated that up to a quarter of the general population and half of severely ill patients are affected by it. Dyspnoea is also an important predictor of quality of life, exercise tolerance and mortality in various conditions. In patients with chronic obstructive pulmonary disease (COPD), it has been shown to be a better predictor of mortality than forced expiratory volume in 1 s (FEV1). In patients with heart disease referred for clinical exercise testing, it is a better predictor of mortality than angina. Dyspnoea is also associated with decreased functional status and worse psychological health in older individuals living at home. It is also a factor in the low adherence to exercise training programmes in sedentary adults and in patients with COPD. The mechanisms of dyspnoea are still unclear. Recent studies have emphasised the multidimensional nature of dyspnoea in the sensory–perceptual (intensity and quality), affective distress and impact domains. The perception of dyspnoea involves a complex chain of events that depend on varying cortical integration of several afferent/efferent signals and coloured by affective processing. This talk aims to provide state-of-the-art advances on the multidimensional and multidisciplinary aspects of dyspnoea, by addressing three different themes: 1) the neurophysiology of dyspnoea, 2) exercise and dyspnoea, and 3) the clinical impact and management of dyspnoea.

OP.56

Back to basics: the fundamental principles of exercise physiology

Ward S

Human Bio-Energetics Research Centre, UK

Exercise tolerance is determined by minimizing the rate at which metabolic acidaemia and related contributors to fatigue develop. Minimizing H+ production depends on a high-capacity, rapidly-responding aerobic energy-transfer system. Constraining the acidaemia depends on ventilation (V'E) clearing additional CO2 released by bicarbonate-buffering and effecting compensatory hypocapnia (respiratory compensation). An appropriate exercise V'E response is determined by pulmonary CO2 output (V'CO2) (metabolic component), arterial PCO2 (PaCO2) (control set-point), and the dead space fraction of the breath (VD/VT) (gas-exchange inefficiency): $V'E=863 \cdot V'CO2/[PaCO2 \cdot (1-VD/VT)]$. The role of V'E and therefore dyspnoea in limiting exercise tolerance requires consideration of: whether arterial blood-gas and acid-base requirements are met; the cost of meeting these requirements; whether V'E is limited; and (d) the associated dyspnoea. Sources of exercise limitation may include: expiratory air-flow limitation (high demands for CO2 clearance imposing V'E requirements that approach, or even exceed, respiratorymechanical limits); inadequate pulmonary capillary transit-time for oxygenation (high cardiac outputs; a compromised pulmonary-capillary bed); intolerable dyspnoea (low or absent airflow- and/or volume-reserve; peripheral-chemoreceptor sensitization by arterial hypoxaemia); and interstitial pulmonary oedema (high intra-pulmonary vascular pressures). Therefore, in ventilatory-limited situations (e.g. lung disease; highly-fit endurance athletes), factors which reduce ventilatory demand (e.g. reducing arterial hypoxaemia and/or metabolic acidaemia; improving VD/VT; ameliorating dynamic hyperinflation via altered breathing-pattern) have the potential to improve exercise tolerance.

OP.57

The relative strength of common synaptic input to motor neurons is not a determinant of the maximal rate of force development in humans

Felici F[1], Bazzucchi I[1], Casolo A[1,2], Falla D[3], Farina D[2], Del Vecchio A[1,2]

[1]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy; [2]Department of

Bioengineering, Imperial College London, SW7 2AZ, London, UK; [3]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, UK

Correlation between motor unit discharge timings, often referred to as motor unit synchronization, is determined by common synaptic input to motor neurons. Although it has been largely speculated that synchronization should influence rate of force development, the association between the degree of motor unit synchronization and rapid force generation has not been determined. In this study, we examined this association by both simulations and experimental motor unit recordings. The simulation model demonstrated that the relative proportion of common synaptic input received by motor neurons, which determines motor unit synchronization, does not influence the rate of force development ($R = 0.03$, $P>0.05$). Nonetheless, the estimates of correlation between motor unit spike trains were significantly correlated with force speed ($R>0.8$, $P<0.0001$). Moreover, the analysis of motor unit discharges experimentally identified from the tibialis anterior muscle of 20 healthy individuals during explosive isometric contractions revealed that the strength of the neural drive to muscle was associated with the rate of force development. However, the estimates of correlation between motor unit spike trains were entirely determined by the average motor unit discharge rate ($R > 0.7$, $P<0.0001$). These results indicate that the strength of the neural drive to muscle, but not the degree of motor unit synchronization, contributes to most of the variance of human explosive force among individuals. In addition, estimates of correlation between motor unit discharge timings depend strongly on the number of identified discharges and is not indicative of the strength of common input.

Oral Presentations

OP.58

Respiratory muscle activity as a potential mechanism of adaptation to high altitude hypoxia.

Matytsin VO, Avdiushenko SA, Ganapolsky VP

Research Center, Military Medical Academy named after S.M. Kirov, St. Petersburg, Russia

It is well known that periodic hypoxic trainings can activate hypoxia inducible factor pathway that stimulates hematopoiesis, angiogenesis and oxidative metabolism. The aim of the study was to investigate whether an increase of respiratory muscle activity could significantly affect the early adaptation to high altitude hypoxia. The study involved 15 healthy male athletes, aged between 23-30 years. To determine the pulmonary function and the maximum oxygen uptake, athletes performed a spirometric and cycle ergometer tests before and after the course of periodic hypoxic trainings. Periodic hypoxic trainings were performed in the barocomplex Tabay (Japan) daily for 8 days, where the duration of a single training was 1 hour. The altitude of the first ascent was 1500 m above the sea level, of the second - 2000 m and of the successive ones - 2500 m. After a course of hypoxic trainings, the maximum oxygen uptake increased in 14 out of 15 subjects, while the minute ventilation increased in 13 athletes. The spirometric test revealed significant increase in forced vital capacity (FVC) and forced expiratory volume (FEV) of lungs at time intervals of 0.5 s, 1s and 3s.The results of the present study suggest that periodic hypoxic trainings can potentially improve the performance of the athlete's muscular system. Thus, it is assumed that an increased working capacity of the respiratory muscles after a course of hypoxic training contributes to the early adaptation to hypoxia.

OP.59

Effects of recovery interval positioning on the work above critical power in humans

Vinetti G[1], Taboni A[1,2], Ferretti G[1,2]

[1]Department of Molecular and Translational Medicine, University of Brescia, Italy; [2]Department of Anaesthesiology, Pharmacology and Surgery Intensive Care, University of Geneva, Switzerland

In the two-parameter model of critical power (CP), the amount of work performed above the CP in an exhausting exercise bout (W') is a constant. The introduction of a recovery interval (Tr) affects the shape of the relationship between power (P) and time to exhaustion (Tlim) mainly thanks to a partial reconstitution of W', with conflicting results regarding CP. Moreover, the effects of positioning the same Tr in different temporal locations of the effort is unknown. To clarify this issue, eight subjects performed five different constant-P trials to exhaustion at the cycle ergometer (90-110% of the maximum aerobic power) for Tlim, CP and W' determination. Each trial was followed by a 3-min passive Tr, after which exercise was resumed at the same P until exhaustion (double square-wave test). Then, a series of five analogous double square-wave tests were performed, with the difference that Tr was placed at half of the measured Tlim (½Tlim). Control CP as determined from five trials to Tlim was 218 ± 40 W. CP was marginally affected by adding a post-Tr exhausting bout either after Tlim (213 ± 37 W) or after ½Tlim (215 ± 40 W). The fraction of recovered W' was higher when Tr was placed at Tlim than at ½Tlim (42%

vs 31%, p = 0.04). Moreover, the amount of recovered W' was positively related to P in both conditions (R2=0.95 and R2=0.76, respectively). In conclusion, W' recovery is enhanced by prior exhaustion and shows a positive dose-response relationship with trial's P. These findings have implications for training and racing strategies and their underlying physiology.

NEUROPHYSIOLOGY

Symposium 1

Experimental epilepsy models

Organizer: Erdal Agar (Samsun, Turkey)

Invited Oral Presentations

OP.60

Cannabinoid CB_1 receptors in epilepsy

Agar E

Department of Physiology, University of Ondokuz Mayis Samsun-Turkey

Cannabinoid system involves in several physiologic and pathophysiologic conditions such as epilepsy. The cannabinoid CB1 receptors are the primary site action for cannabinoid-induced effects on the brain. The effects of agonist and antagonist of CB1receptors were studied on the penicillin-induced epileptiform activity, PTZ- induced kindled and genetic absence models of epilepsy by using multiple analysing methods. The agonist (ACEA) and antagonist (AM-251) of CB1 receptors, at doses of 7.5 μg and 0.50 μg, were administered intracerebroventricularly, respectively. ECoG activity was recorded for three hours. The brain was taken out for the analysis by using immunohistochemical, immunofluorescence staining, western blot and real time PCR methods. ACEA reduced the severity of epileptic activity while AM-251 enhanced the epileptic activity by causing status epilepticus-like activity in all experimental models. Down regulation of CB1 receptors have been seen in the cortex and hypothalamus of absence epileptic WAG/rij rats in the presence of both ACEA and AM-251. CB1 receptor gene expression increased in the hypothalamus of absence epileptic rats. Epileptic activity caused a down regulation of CB1 receptor in the cortex and posterior hypothalamus of kindled rats. The administration of ACEA and AM-251 decreased protein expression only in hypothalamus of kindled rats. The results reveal evidence that cannabinoid CB1 receptors plays an important role in regulating the severity of epileptic activity, at least in the cortex and hypothalamus of brain of epileptic rats.

OP.61

Structural and functional reorganization of the hippocampal network in epilepsy

Haas CA

Experimental Epilepsy Research, Dept. of Neurosurgery, Medical Center, University of Freiburg, Germany

Mesial temporal lobe epilepsy (MTLE) represents the most frequent form of drug- reistant epilepsy in adults and is characterized by spontaneous, recurrent epileptic seizures. The occurrence of MTLE is highly correlated with an initial precipitating insult (IPI) in childhood which is assumed to trigger pathological network reorganization that evolves over years. The most common pathology in MTLE is hippocampal sclerosis (HS), characterized by neuronal loss, reactive gliosis, granule cell dispersion and synaptic rearrangement. We used the intrahippocampal kainate (ihpKA) mouse model for MTLE, which recapitulates the major pathological hallmarks of the human disease, including unilateral HS and spontaneous recurrent seizures to address the following questions: How is the entorhinal-hippocampal network reorganized under epileptic conditions? What is the relationship between synaptic remodeling and epileptic activity? Can early hippocampal changes predict later epilepsy severity? Our key findings are: (1) Input from the entorhinal cortex (EC) to the epileptic hippocampus is strengthened, due to enlargement and de novo formation of synapses with dentate granule cells (2) These synapses are established mainly in the sclerotic region before the emergence of high amplitude seizure activity. (3) The extent of early changes in hippocampal microstructure and molecular composition is directly correlated with the severity of subsequent HS and seizure frequency, and (4) corresponding imaging biomarkers also apply to human MTLE. In conclusion, we present evidence for a novel circuit mechanism in MTLE involving profound rearrangement of entorhinal synapses in the sclerotic hippocampus, and that hippocampal changes during early epileptogenesis predict later disease severity. Financial support: Cluster-of-Excellence „BrainLinks-BrainTools“ (DFG grant EXC1086)

OP.62

The strategies of drug targeting into the brain through the blood-brain barrier in drug-resistant epilepsy

Uğur Yilmaz C[1,2], Temizyürek A[3], Emik S[4], Orhan N[5], Ahishali B[6], Akcan U[3], Atiş M[3], Arican N[7], Gürses C[8], Kaya M[3]

[1]Dept of Pharmaceutical Biosciences, Uppsala University, Sweden; [2]Dept of Laboratory Animals Science, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkey; [3]Dept of Physiology, Koç University School of Medicine, Turkey; [4]Dept of Chemical Engineering, Faculty of Engineering, Istanbul University-Cerrahpaşa, Turkey; [5]Dept of Neuroscience, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkey; [6]Dept of Histology and Embryology, Koç University School of Medicine, Turkey; [7]Dept of Forensic Science, Istanbul Faculty of Medicine, Istanbul University, Turkey; [8]Dept of Neurology, Koç University School of Medicine, Turkey

Epilepsy is a pathological condition characterized by recurrent and unprovoked epileptic seizures, affecting almost 1% of the world's population. Drug-resistant epilepsy as a subgroup of the disease is still considered as a significant worldwide public health problem owing to the limitations in the efficacy of therapeutic approaches. Temporal lobe epilepsy (TLE) involves approximately one-third of epilepsy patients, and along with some cases of absence epilepsy accounts for intractable epilepsy, which currently cannot be cured with the available antiepileptic drugs. One of the main reasons for the failure of antiepileptic drugs in refractory epilepsy is their inability to cross the intact blood-brain barrier (BBB) which is majorly constituted by the endothelial cells of brain microvessels. Recently, effective brain delivery systems have been described to overcome the BBB resistance in experimental models. In a recent study, lacosamide (LCM)-conjugated gold nanoparticles (GNPs) were shown to decrease the amplitude of EEG-waves in an animal model of TLE induced by kainic acid. In WAG/Rij rats, which are genetically predisposed to absence seizures, LCM-conjugated GNP injections caused significant decreases in EEG amplitudes and anxiety levels in behavioral experiments. The above-mentioned observations suggest that GNP is an effective neuro-nanocarrier facilitating the passage of LCM across the BBB thereby enabling an anti- epileptogenic activity. In conclusion, neuro-nanocarrier-conjugated antiepileptic drugs account for a promising BBB-targeted modality in the treatment of intractable epilepsy owing to their enhanced transport into the brain. Acknowledgement: This study was supported by TÜB TAK (Project # 115S327).

Oral Presentations

OP.63

Astrocytes and their role in epilepsy?

Zorec R

Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; Celica BIOMEDICAL, Lab Cell Engineering, Ljubljana, Slovenia

To understand mechanisms of epilepsy, one needs to study astrocytes, a heterogeneous neuroglial cell type. These cells can get excited when neurotransmitters bind to their membrane receptors and feed-back by releasing their own signals to the neural network. This involves vesicles, which store chemicals termed gliotransmitters or more generally gliosignaling molecules. In the former case chemical messengers get released from astrocytic sites proximal to the synapse, which defines communication to occur in the nano-space of contact between the synapse and the astrocyte. In contrast gliosignaling molecules may also be released into the extracellular space and get transported to locations far away from the active astrocyte. This mode of release resembles the endocrine system. Hence astrocytes are considered to be part of the gliocrine system in the brain, where the glymphatic system mediates the convection of released molecules. This complex system not only plays a role in vesicle-based cell-to-cell communication, but also synchronizes the provision of energy for neural networks. Astrocytes contain glycogen, a form of energy store. Excitation of astrocytes by volume transmitters, such as noradrenaline (NA), released by locus coeruleus neurons, activates adrenergic receptors and stimulates glycogenolysis, providing lactate. This lecture will discuss elements of vesicle-based communication and excitation-energy coupling by astrocytes and how this mechanisms get impaired in neurologic diseases including epilepsy, focussing into the delivery of ion channels (Kir4.1) and glucose transporters, both playing a role in epilepsy.

Symposium 2

Shaping integrative physiology during wakefulness and sleep by the hypocretin/orexin neurons

Organizer: Giovanna Zoccoli (Bologna, Italy)

Invited Oral Presentations

OP.64

Thermoregulation and REM sleep expression: The energy allocation hypothesis and the role of hypocretins

Schmidt MH

Department of Neurology, Bern University Hospital, Switzerland

The hypocretin (Hcrt) system has been hypothesized to integrate sleep-wake neurophysiology with energy metabolism. We propose that this integration plays a role in optimizing global shifts in whole organism resource allocations. Although Hcrt neurons promote waking and feeding, for example, they are also sensors of energy balance through their receptivity to glucose, leptin and ghrelin. Intermingled with Hcrt, the lateral hypothalamus (LH) contains numerous other cell types, including melanin concentrating hormone (MCH) neurons that show a reciprocal firing pattern with Hcrt and are known to promote REM sleep. In this presentation, emphasis is placed on the role of the LH as an integrative circuit that optimizes the expression of REM sleep versus waking in the setting of their competing thermoregulatory demands. Data are presented suggesting that the LH integrates numerous input variables, including ambient temperature, energy balance, sleep pressure and circadian time, for behavioral state output expression. Thermoregulation is the most energetically expensive biological function shared by endotherms, yet REM sleep is characterized by loss of thermoregulatory control in association with generalized muscle atonia. Hcrt neurons play a functional role in brown adipose tissue activation, muscle tone and thermoregulatory responses for waking behavior. Optimization of energy expenditures at the whole organism level necessitates a top-down network responsible for coordinating metabolic operations in a state-dependent manner across organ systems. We propose that this LH integration system is responsible for global shifts in state-dependent resource allocations, ultimately promoting resource optimization and an energy conservation function of sleep-wake cycling.

OP.65

The hypocretin/orexin neurons as part of the central autonomic network: implications for cardiovascular control during wakefulness and sleep

Silvani A

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

Orexin A and B (also called hypocretin 1 and 2; H/O) are neuropeptides released by neurons in the hypothalamus. The loss of H/O neurons, possibly due to an autoimmune process, is the hallmark of narcolepsy type 1 (NT1), which entails periods of irrepressible need to sleep and sudden losses of muscle tone during wakefulness. Studies in patients and mouse models have also associated NT1 with alterations in cardiovascular (CV) control during wakefulness and sleep. In particular, the physiological decrease in arterial blood pressure (ABP) from wakefulness to non-rapid-eye-movement (non-REM) sleep is often blunted in NT1, with a non-dipper ABP profile, whereas the physiological increase in ABP from non-REM sleep to REM sleep is often enhanced. Moreover, NT1 may entail increases in heart rate, and decreases in ABP and muscle sympathetic nerve activity during relaxed wakefulness. Whether these CV anomalies increase the risk of CV morbidity and mortality in patients with NT1 is still debated (doi: 10.1007/s10286-017- 0473-z). On the other hand, neuroanatomical and functional evidence supports the biological plausibility of the link between NT1 and anomalies in CV control. In particular, the H/O neurons, which are lacking in NT1, are a key component of the central autonomic network (doi: 10.1177/2514183X18789327), an interconnected set of brain structures that are critical for CV control as well as for the control of neuroendocrine, respiratory and sphincter motor neurons. Enhanced research interactions between physiology and clinical sciences are needed to fully exploit the implications of the H/O neuron involvement in CV control, both to optimize the care of patients with NT1 and to identify possible H/O-related druggable targets for CV disorders.

OP.66

The hypocretin/orexin circuit shapes wake behavior and EEG activity: repercussions for NREM sleep quality

Vassalli A[1], Li S[1], Franken P[2], Tafti M[1]

[1]Department of Physiology, University of Lausanne, Switzerland; [2]Center for Integrative Genomics, University of Lausanne, Switzerland.

Signaling by Hypocretins (HCRT) plays a formidably wide array of functions, which globally adjust physiology and behavior to environmental demands. We used EEG analysis to monitor brain network activity in mice with various genetic alterations in HCRT circuits. We found that cataplexy and REM sleep of mice lacking all HCRT signaling are characterized by stereotyped high-amplitude EEG theta hypersynchronies, that surprisingly are confined to frontal cortices, and also appear in the EEG of

narcoleptic children during cataplexy and REM sleep. In waking, we found that HCRT is critical to stabilize the powerful theta and fast-gamma oscillations that characterize theta-dominated wake (TDW), the waking substate which underlies motivated behavior, and triggers cataplexy in narcolepsy. We further found that time spent in TDW predicts delta power, a proxy of sleep depth, in subsequent slow-wave-sleep (SWS). Therefore TDW acts as the principal driver of the sleep homeostat, and theta-deficient waking leads to a shallower sleep. We generated several conditional alleles for HCRT receptors (HcrtR1 and 2) and selectively deleted these receptors in noradrenergic (NA) and dopaminergic cell groups. Deletion of HcrtR1 in NA cells revealed a bidirectional contribution to wakefulness quality according to the motivated behavior, as manifested under exposure to threatening vs rewarding stimuli. While stressors induced weakened EEG theta and fast-gamma waking responses in NA-specific *HcrtR1*-KO mice, a rewarding context had the opposite effect. Moreover, SWS after stress-associated arousal was deficient in slow-delta activity. Thus HCRT is a major determinant of brain states, which is critical to sustain electrocortical and behavioral arousal, and to mount a robust sleep homeostatic response.

Oral Presentations

OP.67

Regulation by hypocretin (orexin) of excitatory postsynaptic potentials in layer V pyramidal neurons of murine prefrontal cortex (PFC).

Colombo G[1], Coatti A[1], Vassalli A[2], Becchetti A[1]

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy; [2]Department of Physiology, University of Lausanne, Switzerland

Hypocretin (HCRT) 1 and 2 are neuropeptides synthesized by lateral hypothalamic neurons, most active during waking, and silent during sleep. By innervating subcortical arousal nuclei, HCRT cells set the level of cortical activation necessary for exploratory goal-oriented behaviors, in response to physiological or emotional drives. Moreover, by innervating PFC, HCRT neurons directly regulate cognitive tasks. HCRT actions are mediated by HCRT receptor 1 (HCRTR1) and 2 (HCRTR2), but little is known about how HCRTs regulate neocortical circuits. We studied the effect of HCRT1 on glutamate release in PFC frontal area 2 (Fr2), a premotor region implicated in goal-oriented behaviors. In slices from 4 to 9 week old mice, HCRT1 (500 nM) increased the frequency of spontaneous excitatory postsynaptic currents (EPSCs) on layer V pyramidal neurons, from 14 ± 5.5 to 19.2 ± 6.1 Hz ($p < 0.05$; paired t-test, n = 7), with no effect on EPSC amplitude. The effect was also produced by 100 nM HCRT1 and was abolished by 1 μM SB-334867 (an antagonist of HCRTR1). Next, HCRT1 was tested on a mouse model lacking HcrtR1 (Hcrtr1KO-Gfp/KO-Gfp mice; Vassalli et al. Sci Transl Med 7:314le2, 2015; Li et al. Sci Rep 8:1574, 2018). In these mice, HCRT1 failed to increase EPSC frequency, which was 2.9 ± 1.6 Hz in the absence of HCRT1, and 3.05 ± 1.9 Hz in the presence of HCRT1 (NS; paired t-test; n = 7). Our results support the notion that HCRT1 exerts its excitatory effect on layer V Py neurons through an HcrtR1-dependent mechanism, which could contribute to sustain neocortex activity during waking. Moreover, considering the implication of pyramidal neuron firing in θ rhythms, our data suggest how HCRT deficit could decrease EEG θ activity during wakefulness (Vassalli & Franken PNAS 114:E5464-73, 2017).

OP.68

Effects of very low calorie ketogenic diet upon orexinergic system, visceral adipose tissue and ROS production

Valenzano A[1], Polito R[1], Di Palma A[1], Aurora D[2], Di Maio G[3], Messina A[3], Monda V[3], Villano I[3], Monda M[3], Cibelli G[1], Messina G[1]

[1]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy; [2]Department of Biological and Pharmaceutical Environmental Sciences and Technologies, University of Campania "Luigi Vanvitelli", Caserta, Italy; [3]Department of Experimental Medicine, Section of Human Physiology and Unit of Dietetic and Sport Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy; *corresponding author: Giovanni.messina@unifg.it

Caloric restriction is a valid strategy to reduce visceral adipose tissue (VAT) content in obese. Orexin-A/Hypocretin1 is a neuropeptide synthesized in the lateral hypothalamus, that strongly modulates food intake, thus influencing adipose tissue accumulation. Therapeutic diets in obesity treatment may combine the advantages of caloric restriction and dietary ketosis. The current study aimed to evaluate the effect of a very low-calorie ketogenic diet (VLCKD) in a population of obese patients. Adiposity parameters and the Orexin-A serum profiling were quantified over a 8-week period. The effect of VLCKD on reactive oxygen species (ROS) production and cell viability was evaluated, in vivo, by culturing HepG2 cells in presence of VLCKD sera. Dietary intervention induced significant effects on body weight, adiposity and blood chemistry parameters. Moreover, a selective reduction in VAT was measured by dual-energy x-ray absorptiometry. Orexin-A levels significally increased after dietary treatment, as well. HepG2 cell viability was not affected after 24, 48 and 72 h incubation with patients' sera, before and after

VLCKD. Likewise, in the same model system, ROS production was not significantly influenced by dietary treatment. In conclusion, VLCKD exerts a positive effect on VAT decrease, ameliorating adiposity and blood chemistry parameters. Furthermore, short-term mild dietary ketosis does not appear to have a cytotoxic effect, nor does it represent a factor capable of increasing oxidative stress. Finally, to the best of our knowledge, this is the first study which shows an effect of VLCKD upon the orexinergic system, supporting the usefulness of such a therapeutic intervention in promoting reduction in the individual burden of disease.

Symposium 3

From whole-cell to single synapse engrams - Breaking the code for memory formation, storage and recall

Organizer: Marco Mainardi (Pisa, Italy)

Invited Oral Presentations

OP.69

Distinct granule cell populations are uniquely engaged in odor learning

Alonso M

Laboratory for Perception and Memory, Institut Pasteur and Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3571, France

Olfaction is an important sensory modality driving fundamental behaviors. To determine whether an odor is good or bad, the brain essentially need to attribute a significance to this sensory stimulus. The allocation of a positive significance to an odorant usually depends on its association during learning with a reward outcome. Moreover, multiple forms of plasticity are involved when such odor-reward associations are formed. In the adult olfactory bulb, the continual production of newborn interneurons contributes to the functional plasticity of the system. We demonstrate that adult-born neurons, but not preexisting ones, contain information about learned positive significance. We also found that adult-born neuron activation heightens olfactory learning and enhances the ability to update the odor significance. Moreover, we reveal that adult-born cells are massively connected by higher brain regions and these contacts might be sensitive to odor experiences. In summary, our results show a specific involvement of adult-born neurons in boosting odor-reward association that is linked with a distinct connectivity within the olfactory system. These data unveil the relevance of encoding odor significance at early stages of sensory processing.

OP.70

Two-photon calcium imaging of memory engrams throughout the hippocampal formation in behaving mice

Hainmueller T[1,2,3], **Bartos M**[1]

[1]Institute for Physiology I, Systemic and Cellular Neurophysiology, University of Freiburg, Germany; [2]Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany; [3]Faculty of Biology, University of Freiburg, Germany

Every day, we form memories about episodes of experiences that we make. The hippocampus is the cardinal brain structure for the formation of such episodic memories and cellular ensembles in this brain structure form representations or 'engrams' of each individual memory. To observe the formation and maintenance of such memory engrams, we repeatedly monitor the activity of thousands of hippocampal neurons using two-photon calcium imaging in head fixed mice. During our measurements, the mice perform memory tasks in a virtual environment, allowing us to monitor neuronal engram activity during encoding and recall of memories related to the virtual sceneries. Intriguingly, the coding properties of individual neurons differ vastly across the hippocampal formation. Pyramidal cells in the CA1 and CA3 areas show precise spatial tuning and remap rapidly between behavioural contexts, while granule cells of the dentate gyrus show a generalizing code. Furthermore, the firing fields of pyramidal cells are time-sensitive and remap as days pass, while those of granule cells are stable over many days. Our results suggest a multi-step process of memory assembly within the hippocampus with specific features being represented by the individual hippocampal subfields.

OP.71

Dendritic contributions to memory engrams: lessons from computational models

Poirazi P

Institute of Molecular Biology & Biotechnology (IMBB), FORTH, Greece

Associative memories are believed to be stored in distributed neuronal assemblies through synaptic and intrinsic plasticity. The long-term plasticity of synapses involves the long-term potentiation/depression of

synaptic responses, spine growth/elimination, protein synthesis and capture, homeostatic plasticity etc [1-2]. Based on experimental evidence, we developed a simplified computational model of plasticity that examines the role of dendrites and synaptic turnover dynamics during associative learning [3]. We use multi-scale modeling to model synaptic processes which span different temporal and spatial scales, such as calcium influx, protein synthesis and delivery, synaptic tagging and homeostasis to assess how memories are encoded in a population of neurons. Using the model, we show that memory storage increases the sparsity of population firing and that local protein synthesis promotes dendritic synapse clustering [3]. Moreover, our model suggests that memories learnt in close temporal proximity are stored in overlapping neuronal and dendritic populations. This overlap serves as the main mechanism for linking memories across time. These neuronal and dendritic overlaps underlie memory linking even in the absence of dendritic spikes, albeit at a very high cost of increased afferent connections, indicating that active dendrites serve as a means for resource savings. Finally, we propose that the same mechanisms can bind together sequential memories, creating memory episodes [3]. Our model also predicts that increased synaptic turnover facilitates the formation of synapse clusters within active dendrites, which in turn improves learning and maximizes the storage capacity of newly learnt memories [4]. References: [1] Sutton, M.A. & Schuman, E.M., 2006. Cell, 127,.49–58 [2]. Rogerson, T. et al., 2014. Nature Reviews Neuroscience, 15, 157–169. [3]. Kastellakis G., Silva, A.J., Poirazi, P. Cell Reports. 2016 Nov. [4]. Frank A.C., Huang S., Zhou M., Gdalyahu A., Kastellakis G., Silva T.K., Lu E., Wen X., Poirazi P., Trachtenberg J.T., Silva A.J. Nat Commun. 2018 January

Oral Presentations

OP.72

Responsibility of hippocampal asymmetry on the positive impact of predictable mild chronic stress on spatial memory in adolescent rats

Kaptan Z[1], Akgün Dar K[2], Kapucu A[2], Bulut H[3], Uzüm G[1]

[1]Department of Physiology, Istanbul University, Turkey; [2]Department of Biology, Istanbul University, Turkey; [3]Department of Biochemistry, Bezmialem University, Turkey

Stress exposed during adolescence structurally and functionally affect the hippocampus, which is the critical brain region for spatial memory. Studies show that the impact of stress on cognitive functions is related to its predictability. In addition, hippocampal lateralization is suggested to be associated with optimal function and developing in adolescence. Investigating the effect of predictable chronic stress (PCS) exposed in adolescence on the asymmetry of memory related molecules and the correlation between memory performance and asymmetry was the aim of this study. We applied mild chronic stress (as PCS) during adolescence. Male rats were divided in two groups: control and PCS; n=11 for each. 15 min/day immobilization stress for 4 weeks was used as a PCS model. After 4 weeks spatial memory was tested in Morris Water Maze. The left and right hippocampus and frontal cortices were separated. Brain derived neurotrophic factor (BDNF) and oxidative markers malondialdehyde (MDA), protein carbonyl (PCO) and antioxidant superoxide dismutase (SOD) were measured by ELISA. Spatial memory of PCS group was improved compared to the control group (0.05). Hippocampal BDNF of the control group had left>right asymmetry while PCS group had right>left asymmetry (0.001). In the hippocampus of PCS group, MDA had right>left asymmetry (0.001). There was no difference between the left and right sides in terms of oxidative markers in the hippocampus of control group and frontal cortex of both groups. Our original findings suggest that asymmetric expression of BDNF and MDA (recently, it is reported that MDA may be a signal molecule for plasticity) in the right hippocampus, may strengthen spatial memory.

OP.73

Oligomeric amyloid-beta at physiological concentrations rescues the impairment of hippocampal synaptic plasticity and memory in aged Amyloid Precursor Protein knockout mice

Tropea MR[1], Gulisano W[1], Teich A[2], Arancio O[2], Palmeri A[1], Puzzo D[1,3]

[1]Department of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Italy; [2]Department of Pathology and Cell Biology, Taub Institute, Columbia University, USA; 3Oasi Research Institute (IRCCS), Italy

Amyloid precursor protein (APP) is a widely expressed transmembrane protein that is cleaved to generate different fragments, among which Amyloid-β (Aβ) peptide, a key actor in Alzheimer's disease pathophysiology. Recent evidences indicate that the presence of APP is necessary for oligomeric Aβ and tau to exert a neurotoxic effect at the synapse, mediating their internalization into neurons. However, several studies have shown that APP and Aβ also play a role in physiological mechanisms underlying synaptic plasticity and memory. Here we have studied whether the genetic absence of APP affected different forms of synaptic plasticity and memory in APP knockout (APP

KO) mice. To this end, we performed in vitro electrophysiological recordings and behavioral studies to assess spatial learning, reference and fear memory in APP KO mice compared to WT animals. APP KO mice showed a significant impairment of CA3-CA1 hippocampal long-term potentiation (LTP) and different types of memory at 6 months of age, whereas an increase of paired-pulse facilitation (PPF), suggesting a decrease of neurotransmitter release, was found at 9 months of age. To study whether this impairment was due to the absence of endogenous Aβ, we treated APP KO mice with 200 pM oligomeric Aβ that was able to rescue the impairment of PPF, LTP and memory in APP KO animals. Taken together, these results show that APP is needed at the synapse and its absence determines an age-dependent impairment of synaptic function, which is rescued by low concentrations of oligomeric Aβ. This strengthens the importance of the physiological role of APP and Aβ in hippocampal plasticity and memory.

PHYSIOLOGY OF METABOLISM

Symposium 1

Gluscose metabolism in neurodegeneration

Organizer: Helena Chowdhury (Ljubljana, Slovenia)

Invited Oral Presentations

OP.74

Modulation of brain glucose metabolism by exercise and fasting

Jensen J, **Johansen E**

Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

Glucose is the main energy substrate for the brain under normal physiological conditions, and the brain consumes about 120 g of glucose daily. Humans have a carbohydrate intake 200-300 g (800-1200 kcal), and the brain oxidises a major part of the ingested glucose. Normally, blood glucose are relative stable (4-5 mM) but increases after carbohydrate rich meals. Type 2 diabetes and elevated blood glucose are associated with increased risk for cognitive impairments and increased risk for Alzheimer's disease. Regulation of glucose homeostasis involves several organs including liver and skeletal muscles. Skeletal muscles have very high capacity to oxidise glucose during exercise. In elite athletes, more than 4 g of glucose can metabolised each minute. During prolonged exercise, blood glucose can decrease below 3 mM and cause fatigue. Acute decline in blood glucose during exercise cause fatigue and impaired brain function. During prolonged fasting, blood glucose can also decline below 3 mM in many people, but cognitive function seems to remain normal. Importantly, blood ketone concentration increases and becomes the major substrate for the brain after 4-5 days of fasting. Interestingly, data indicate that ketone bodies improve brain function under some conditions, in particular in Alzheimer's disease. The talk will explain how exercise and fasting will influence accessibility of energy for the brain, and how this may influence physical performance and brain function.

OP.75

Energy Metabolism of Reactive Astrocyte during Brain injury

Morita M

Department of Biology, Kobe University, Japan

Impaired cerebral blood flow and extravagant increase of neuronal activities after brain injury deplete energy substrates in neurons and cause secondary neurodegeneration and poor network regeneration. Astrocytes are positioned between cerebral vasculature and neuronal network and play crucial roles in metabolism and delivery of energy substrates in the brain, however, astrocyte metabolic functions after brain injury are still largely elusive. Recent transcriptome analysis proposes multiple subpopulations of reactive astrocytes, thus astrocyte activation after brain injury likely impose complicated influences on energy metabolism. Accumulating publications suggest the upregulation of glycolysis in neurotoxic reactive astrocytes induced by pro-inflammatory cytokines, and the lactate produced by astrocytes may fuel neuron and/or accelerate inflammation. Meanwhile, neuroprotective scar-forming reactive astrocytes upregulate fatty acid oxidation, and likely produce ketone body for fueling neuron in addition to removing excessive fatty acid released from dead cells. These possibilities will be discussed by referring data we obtained in our originally-developed closed-head injury model, photo injury.

OP.76

Insulin and Adrenaline Modulate Cytoplasmic Glucose, Lactate and Glycogen Levels in Astrocytes

Kreft M[1,2,3], Fink K[1], Muhič M[1], Chowdhury H[1,2], Vardjan N[1,2], Zorec R[1,2]

[1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloska 4, Slovenia; [2]Celica Biomedical Center, Tehnoloski park 24, Ljubljana, Slovenia [3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Vecna pot 111, Slovenia

Astrocytes play a significant role in a number of processes, including the brain energy metabolism. Their anatomical position between blood vessels and neurons makes them an interface for effective glucose uptake from blood. Astrocytes contain glycogen, an energy buffer, which can bridge local short term energy requirements in the brain. Glycogen levels reflect a dynamic equilibrium between glycogen synthesis and glycogenolysis. Many factors that include hormones and neuropeptides, such as insulin and adrenaline likely modulate glycogen stores in astrocytes, but detailed mechanisms at the cellular level are sparse. We used a glucose nanosensor based on Förster resonance energy transfer to monitor cytosolic glucose and lactate concentration with high temporal resolution and a cytochemical approach to determine glycogen stores in single cells. We show that following the adrenaline or noradrenaline stimulation the availability of cytosolic glucose and lactate is increased promptly after stimulation. Insulin boosts the process of glycogen formation. Although astrocytes appear to express glucose transporter GLUT4, glucose entry across the astrocyte plasma membrane is not affected by insulin. Stimulation of cells with insulin decreased cytosolic glucose concentration, likely because of elevated glucose utilization for glycogen synthesis.

OP.77

ALS- and FTD-associated TDP-43 inclusions in astrocytes reduce β-adrenergic cAMP signalling and alter glucose and lipid metabolism

Velebit J[1], Horvat A[1,2], Prpar Mihevc S[3], Rogelj B[3,4,5], Zorec R[1,2], Vardjan N[1,2]

[1]LCI, Celica Biomedical, Ljubljana, Slovenia; [2]LN-MCP, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia; [3]Department of Biotechnology, Jožef Stefan Institute, Ljubljana, Slovenia; [4]Biomedical Research Institute BRIS, Ljubljana, Slovenia; [5]Faculty of Chemistry and Chemical Technology, University of Ljubljana, Ljubljana, Slovenia

Astrocytes are glial cells in the central nervous system with many homeostatic functions. They metabolically support neurons with nutrients, such as glucose, lactate and lipids. The neuronal energy provision during intense activity largely depends on the activation of noradrenergic system and release of noradrenaline (NA), which activates adrenergic receptors (ARs) on the surface of astrocytes. Activation of β-ARs stimulates cAMP signalling, augmenting glycolysis and the production of lactate, an important neuronal energy fuel. In most cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) neurons and also non-neuronal cells, including astrocytes, develop cytoplasmic inclusions of TAR DNA-binding protein 43 (TDP-43). Cytoplasmic TDP-43 inclusions in astrocytes alone can cause motor neuron death, however whether these alter astroglial metabolism and its noradrenergic regulation, which may affect astroglial metabolic support of neurons and contribute to TDP-43-mediated neurotoxicity, is unclear. To study the effect of astroglial TDP-43 inclusions on cell metabolism, isolated brain astrocytes were transfected with plasmids encoding the C-terminal fragment of TDP-43 (amino acids 208–414) to form cytoplasmic TDP-43 inclusions or wild type (WT) TDP-43 as control. Then the metabolism of lipids and glucose was studied in transfected cells using fluorescent dyes or genetically encoded nanosensors and fluorescence microscopy. Cytoplasmic TDP-43 inclusions reduced NA-induced cAMP signalling in astrocytes likely due to the downregulation of β2-ARs as determined immunocytochemically. This facilitated glycolysis and lipid metabolism, representing stress that likely impairs astroglial capacity to homeostatically support neurons in TDP-43-associated ALS and FTD.

Oral presentations

OP.78

3,5-diiodo-L-thyronine (T2) down-regulates miR-22-3p to modulate gluconeogenic pathway in high-fat diet rats

Senese R[1], Cioffi F[2], Petito G[1], Lange P[1], Russo A[1], Goglia F[2], Lanni A[1*], Potenza N[1]

[1]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "L. Vanvitelli", Caserta, Italy; [2]Department of Sciences and Technologies, University of Sannio, Benevento, Italy

Over 30 years of research has demonstrated that 3,5-diiodo-L-thyronine (T2), an endogenous metabolite of thyroid hormones, exhibits interesting metabolic activities. Particularly, its beneficial effects on glucose

metabolism is well established. However, little is known about its molecular mechanisms. Many reports have demonstrated the involvement of microRNAs in various diseases, including metabolic disorders such as obesity and type 2 diabetes (T2D), which are characterized by an impaired glucose homeostasis. Based on those considerations, the aim of this study was to examine the potential involvement of miRNAs in the mechanisms of action by which T2 affects glucose homeostasis in rats fed on a high-fat-diet, a condition known to impair glucose homeostasis. Three groups of rats were used: i) receiving a standard diet for four weeks (N); ii) receiving a high fat diet for four weeks (HFD),; iii) receiving a high fat diet for four weeks with a contemporary administration of T2 (25µg/100 BW) (HFD-T2). The results showed that T2- treated rats had a better tolerance to glucose load and a better performance at the insulin tolerance test in comparison to HFD animals. Interestingly, in the serum of the animals treated with T2 there was a general decrease of miRNAs with miR-22a-3p, miR-34c-5p and miR-33a-3p significantly downregulated. Furthermore, miR-22a-3p had the largest variation pointing toward its preeminent role in T2 metabolic effect. In fact, in liver there was an upregulation of its target (Transcription Factor 7) Tcf7, which had an important impact on gluconeogenesis. This study provide, for the first time, evidences that miRNAs are involved in the effects exerted by T2 on glucose homeostasis.

OP.79

Beneficial effects of Sicilian Black Bee Honey on the obesity-related glucose dismetabolism

Terzo S[1], Mulè F [2], Amato A[2]

[1]Department of Experimental Biomedicine and Clinical Neuroscience, University of Palermo, Italy; [2]Department of Biological- Chemical- Pharmaceutical Science and Technology, University of Palermo, Italy

Honey is known for the nutritive and therapeutic values. The health promoting characteristics are due to the presence of molecules with recognized antioxidants and antiinflammatory properties, such as vitamins, minerals, enzymes and flavonoids. The aim of the present study was to analyse the preventive effects of sicilian Black Bee Honey daily intake on dismetabolism and insulin resistance in mice fed high-fat diet (HFD), because so far data are few and controversial. Three groups of mice were fed with standard diet (STD), HFD or HFD supplemented with honey (HFD-H) for 16 weeks. Body weight, food intake, hyperglycaemia, glucose tolerance, insulin sensitivity, lipids, AST and ALT, central (brain gene expression of p-AKT, p-ERK and p-GSK3) and peripheral insulin resistance were analysed and compared between the different groups of animals. HFD mice showed increased body weight, food intake, triglyceride and cholesterol serum concentration, hepatic enzymes in comparison with STD group. No difference was observed in comparison with HFD-H. On the contrary, fasting glucose and insulin levels, glucose tolerance, insulin sensitivity were significantly ameliorated in HFD-H mice compared to HFD, although the values were different from STD mice. Honey intake significantly reduced the HOMA index, which indeed was increased in HFD mice, suggesting a beneficial effect on insulin resistance. Moreover, mRNA expression of p-AKT and p-GSK3, which was respectively decreased and increased in HFD, index of central insulin resistance, were similar to STD in HFD-H mice. Taken together, the results suggest that black bee honey consumption ameliorates glucose homeostasis as well as the peripheral and central insulin resistance.

Symposium 2

Brain regulation of metabolism: new insights in physiopathology

Organizer: Luc Penicaud (Paris, France)

Invited Oral presentations

OP.80

Brain Sugar Sensing

Fioramonti X

NutriNeuro, UMR, 1286 INRA- University of Bordeaux, France

Brain function and glucose metabolism are intimately linked. Indeed, glucose is the main, if not the only, energy substrate of this organ. This implies that blood glucose level must remain stable. Any decrease in blood glucose level would have immediate consequences on brain functions. Increased blood level will not have acute consequences but sustained hyperglycemia will be deleterious on the long term as seen in patients with uncontrolled Diabetes mellitus. The brain plays a critical role in the regulation of blood glucose level to ensure a glucose homeostasis well controlled. Thus, to be able to control the level of blood glucose, the brain must be able to sense any change. This function is ensured by selective glucose-sensing neurons which are able to adapt their electrical activity in response to physiological changes in brain level. The fact that these neurons sense physiological changes raises the question of the glucose level present in the brain. We will discuss here the mechanisms involved in brain glucose-sensing and the physiological functions

controlled by these neurons. Glucose is not the only sugar we consume. Indeed, fructose is more and more present in our alimentation due to the increase consumption of transformed food and sodas. The idea that fructose can impact brain cells will also be discussed in order to give a broad view of brain sugar-sensing.

OP.81

Emerging role of hypothalamic astrocytes in the metabolic control

García-Cáceres C

Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany.

The interplay between peripheral metabolic cues and neurotransmitters govern the regulation of feeding and energy expenditure, in which the hypothalamus plays a pivotal role in the transduction of peripheral afferents cues into satiety and feeding signals. Interestingly, we have recently shown that hypothalamic astrocytes respond to both circulating nutrients and hormones, in addition to enabling the transport of these molecules between the periphery and the brain. Interestingly, we also found that astroglia coordinate with neurons to efficiently regulate energy metabolism, and respond to hypercaloric diets prior to significant diet-induced body weight gain, suggesting the potentially functional role of astrocytes in the pathogenesis of obesity. Notably, our recent discoveries indicate that astrocytes regulate diet-induced vascular remodeling within the hypothalamus, a phenomenon that has been observed in both diabetic mice and humans. In addition to this, their hormone/nutrient signaling and cellular metabolism seem to be determinant of the manner in which astrocytes sense whole-body metabolic demands and consecutively cooperate with neurons in the control of feeding behavior, as well as systemic glucose and lipid metabolism. Overall, our recent findings suggest a novel model in which astrocytes are actively involved in sensing the whole-body metabolic status, and they may well be potential targets for enhanced pharmacological strategies against metabolic diseases.

OP.82

Reward System and Energy Metabolism

la Fleur SE

Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Netherlands and the Netherlands Institute for Neuroscience (NIN), Royal Dutch Academy of Arts and Sciences (KNAW), Amsterdam, Netherlands

Added sugar, often consumed in the form of sweetened beverages, is currently labeled as a big evil that increases our risk to develop obesity, cardiovascular diseases, and diabetes. Only a few decades ago, however, saturated fat received a similar negative label. Instead of singling out one of these factors we are interested to understand how fat and sugar intake interact, thus influencing brain function, metabolism and behavior. We recently showed that free choice during simultaneous fat and sugar consumption exponentially increases the risk of overeating, obesity development and occurrence of metabolic disturbances in rats. Moreover, the brain's response to this obesogenic choice diet does not promote counter regulatory mechanisms but reflects high energy demands (i.e. the brain shows a fasting-like response) pointing to aberrant nutrient signaling. During this presentation, the effects of the choice diet on the reward system will be reviewed which includes changes in neuropeptide signaling in striatum and neuronal responses to sugar drinking while eating a fatty diet or a healthier grain-based chow diet.

Oral Presentations

OP.83

Stress induces lipid droplet biogenesis in rat brain astrocytes

Smolič T[1], Tavčar P[1] , Horvat A[1,2], Zorec R[1,2], Vardjan N[1,2]

[1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, 1000 Ljubljana, Slovenia; [2]Laboratory of Cell Engineering, Celica Biomedical, 1000 Ljubljana, Slovenia

Astrocytes are an abundant subtype of neuroglia involved in maintaining homeostasis, synaptic transmission and metabolism of the central nervous system. Compared to glucose handling, that is in astrocytes highly regulated by noradrenergic system, the major brain stress response system, mechanisms underlying regulation of lipid metabolism, especially the (patho)physiological relevance of the biogenesis of lipid droplets (LDs), are still unknown. LDs are dynamic lipid storage organelles composed of a core of neutral lipids surrounded by a phospholipid monolayer. In non-adipose cells LDs presumably form in response to stress and may function as energy source for β-

oxidation and protection against lipotoxicity. The aim of the study was to determine whether extracellular stress stimuli and activation of adrenergic receptors induce the formation of LDs and whether stress affects the cytoplasmic mobility of LDs. We exposed astrocytes in culture and organotypic brain tissue slices for 24 h to nutrient deprivation, excess of fatty acids, lactate, hypoxia and various adrenergic agonists, labeled LDs with the fluorescent markers (Nile Red, BODIPY493/503) and monitored the mobility and biogenesis of LDs by confocal microscopy. Nutrient deprivation (replacement of growth medium with 10 mM glucose extracellular solution), exposure to excess oleic acid or lactate, hypoxia (induced by 1% O2 gas mixture) and stimulation with noradrenaline increased the amount of LDs in cultured and tissue astrocytes over 2-fold, indicating LD biogenesis. The mobility of LDs in astrocytes was reduced under nutrient deprivation. Our result show that LD biogenesis in astrocytes in vitro and in situ is regulated by extracellular stress-related stimuli, which may regulate LD formation also in vivo.

OP.84

The Neural Control for Thermoregulatory Inversion

Tupone D[1,2], Cano G[3], Conceição EPS[2], Chiavetta P[1], Morrison SF[2]

[1]Dept. of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2] Dept. of Neurological Surgery, Oregon Health & Science University, Portland, USA; [3]Dept. of Neuroscience, University of Pittsburgh, Pittsburgh, USA

To maintain body temperature under cold conditions, the CNS thermoregulatory networks normally respond to skin cooling by increasing brown adipose tissue (BAT) and shivering thermogenesis. In contrast, several behavioral states (torpor, REM sleep) are characterized by a thermoregulatory inversion (TI) in which cold exposure inhibits thermogenesis and skin warming activates it. We hypothesize that interruption of specific outputs from the preoptic area (POA) can initiate TI, and that TI is mediated by a previously unknown CNS thermoregulatory pathway. Pharmacological inhibition of POA neurons or pre-DMH transection was used to induce TI in anesthetized rats. Skin cooling and warming, plus pharmacological manipulation of dorsomedial hypothalamus (DMH) and parabrachial nucleus (PBN), were used to determine thermoregulatory pathways mediating TI. During TI, skin warming increased BAT thermogenesis and c-Fos expression in DMH neurons and in external lateral (el) PBN neurons projecting to DMH. Inhibition of neuronal activity in DMH or in elPBN blocked the warm-evoked activation of BAT thermogenesis, consistent with elPBN neurons relaying warm thermal information to DMH during TI. Skin cooling increased c-Fos expression in dorsolateral PBN, which contains dynorphinergic neurons that project to DMH. The skin cooling-evoked inhibition of BAT thermogenesis was prevented by injection of the k-receptor antagonist, NOR-BNI, in the DMH.Thus, TI is mediated by a novel pathway that relays skin thermal afferent signals from PBN directly to DMH, and a dynorphinergic input to DMH is required for the cold-evoked inhibition of BAT thermogenesis during TI. Understanding the CNS thermoregulatory mechanisms during TI could suggest novel mechanisms for induction of hypothermia.

RENAL PHYSIOLOGY

Symposium

Hot topics in chronic kidney disease

Organizer: Timo Rieg (Tampa, Florida, USA)

Invited Oral presentations

OP.85

***In vivo* Npt2a inhibition in CKD**

Thomas L[1], Xue J[1], Fenton RA[2], Dominguez Rieg JA[1], Rieg T[1]

[1]Molecular Pharmacology and Physiology, University of South Florida, USA; [2]Biomedicine, Aarhus University, Denmark.

Hyperphosphatemia is common in patients with chronic kidney disease and associated with increased mortality. In the kidney, the sodium-phosphate cotransporter Npt2a is responsible for bulk uptake of phosphate in the proximal tubule. Recently, an orally bioavailable selective Npt2a inhibitor (Npt2a-I, PF-06869206) has been described to reduce phosphate uptake in HEK cells transfected with mouse or rat Npt2a. So far, its physiological *in vivo* function has not been tested. We used C57BL/6J mice to study the effect of Npt2a-I (oral gavage, 1% of body weight) or vehicle. Npt2a-I dose-response (0.3- 300 mg*kg-1) relationships of phosphate excretion were assessed in metabolic cages for 3 hours. Npt2a inhibition caused a dose-dependent increase in urinary phosphate excretion (from 27±6 nmol*min-1 in response to vehicle to a maximum of 150±14 nmol*min-1 at 300 mg*kg-1, ED50 ~21 mg*kg-1). Plasma phosphate (Δ -0.5±0.1 mmol/L, $P<0.05$) and PTH (Δ -113±12 pg*mL, $P<0.05$) significantly decreased after 3 hours, with both

returning to near baseline levels after 24 hours. To study CKD we used the 5/6 nephrectomy model. Vehicle application showed a significantly higher phosphate excretion in 5/6 nephrectomy compared to Sham (60±7 vs 15±2 nmol*min-1, P<0.05). The maximal response of urinary phosphate excretion to Npt2a inhibition was ~10-fold in Sham and ~2-fold in 5/6 nephrectomy mice. Thus, inhibiting Npt2a might be a useful treatment strategy for hyperphosphatemia.

OP.86

Sympathetic regulation of renal function: physiological aspects and therapeutic implications

Milano S[1], Gerbino A[1], Carmosino M[1], Dal Monte M[2], Svelto M[1], Procino G[1]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy; [2]Department of Biology, University of Pisa, Italy

X-linked nephrogenic diabetes insipidus (X-NDI) is a rare disease caused by inactivating mutations of the vasopressin (AVP) type-2 receptor (V2R) gene. In X-NDI, AVP cannot activate the cAMP pathway regulating the transcription, activation and plasma membrane localization of solute and water transporters triggering antidiuresis. This causes severe polyuria and risk of dehydration in patients. The current therapeutic options are limited and only partially beneficial. Over the past years we explored the activation of adenylyl cyclase-coupled GPCRs expressed in the AVP-sensitive nephron segments as a strategy to bypass the V2R inactivation and restore proper hydro-saline homeostasis. We recently showed in mice that the β3-adrenoreceptor (β3AR) is localized in most of the nephron segments expressing the V2R and genetic inactivation of β3AR in mice is associated with significantly increased urine excretion of water and electrolytes. Strikingly, short-term β3AR agonism promoted a potent antidiuretic effect in X-NDI mice. We also demonstrated *in vitro* that β3AR is resistant to agonist-induced desensitization in renal cells, thus indicating β3AR as a potential pharmacological target to cure X-NDI. More recently we demonstrated that β3AR may be also be involved in regulating H+ excretion *via* V-ATPase in renal intercalated cells. Taken together, these data suggest that sympathetic stimulation of β3AR may regulate water, salt and H+ homeostasis in the kidney. Importantly, pharmacological stimulation of β3AR may either improve the impaired concentrating ability of X-NDI patients or increase the beneficial effects of the current therapy.

OP.87

Intracellular Calcium Signaling in Podocytes in Diabetic Nephropathy

Palygin O[1], Ilatovskaya D[1], Spires D[1], Khedr S[1], Shalygin A[1,2], Kaznacheyeva E[2], Staruschenko A[1]

[1]Department of Physiology, Medical College of Wisconsin, Milwaukee, WI, USA; [2]Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia

Diabetic nephropathy (DN) is the leading cause of chronic renal disease and, therefore, is the subject of major research efforts. The podocyte (glomerular epithelial cell) has become a central focus for novel interventions in CKD. Injury to podocytes is considered a major contributor to DKD: their loss causes proteinuria and progressive glomerulosclerosis. Elevation of intracellular calcium ([Ca2+]i) level due to a variety of stimuli leads to podocytes injury and following disturbances of glomerular permeability and the development of albuminuria. We have identified that agonists of protease-activated receptors (PARs) trigger a rapid elevation of [Ca2+]i in podocytes. Serine proteases promote activation of PARs-TRPC6 cascade in podocytes and these signaling pathways are highly upregulated in a rat model of type 2 Diabetic Nephropathy (T2DN rats), similar to clinical observations in human patients. Furthermore, the destroying of the actin cytoskeleton results in activation of TRPC6 channels, which might also contribute to podocyte injury. Scanning Ion Conductance Microscopy also demonstrated morphological changes in podocytes under DN conditions and following activation of PAR signaling. Moreover, we revealed that ATP-triggered calcium influx is enhanced in T2DN rats compared to control animals with even low concentrations of ATP causing activation of calcium flux and augmented [Ca2+]i concentration, correspondingly. All these mechanisms might be important determinants of podocyte injury occurring in DN and their study uncovers new therapeutic targets leading to a new treatment for DN. Inhibition of [Ca2+]i in podocytes could mitigate podocyte damage, and may be of therapeutic benefit in DN. Supported by NIH, VA and RSF №19-14-00114.

Oral Presentations

OP.88

Association of blood bicarbonate and pH with mineral metabolism disturbance and outcome after kidney transplantation

Bienaimé F, Brazier F, Jouffroy J, Martinez F, Anglicheau D, Legendre C, Neuraz A, Prié D

Hôpital Necker-Enfants Malades

In kidney transplant recipient (KTR), low blood bicarbonate level associates with reduced graft survival and mineral metabolism disorder. Yet, the relative association of blood pH and bicarbonate level with transplantation outcome or mineral metabolism disorders have not been assessed, nor the influence of blood collection site (arterial fistula *vs* peripheral vein) on bicarbonate level, which represent a specific concern in KTR. To investigate these questions we analysed blood gas parameters in a single center cohort of 1260 stable KTR, 3 months after transplantation. Inspection of PO2 distribution allowed the unambiguous identification of the arterial (i.e.: drawn from arterio-venous fistula; N=914) or venous (N=346) origin of the blood. The origin of the blood used for bicarbonate measurement was an independent predictor of bicarbonate level. In patients with arterial blood samples, 435 (46%) had bicarbonate below 22mmol/l. Among them, 196 (40%) were acidemic (blood pH < 7.38). IN multivariable analysis, acidemia associated with increased ionized calcium and phosphate level and reduced FGF23, but not with transplantation outcome. In contrast low bicarbonate levelpredicted allograft loss independently of mGFR and other potential confounders. In KTR, reduced arterial blood bicarbonate predicts outcome while acidemia is associated with altered mineral metabolism. Blood collection site should be taken into account when assessing acid-base status.

RESPIRATORY PHYSIOLOGY

Symposium

Breathing through the ages - Rhythm generation and modulatory mechanisms

Organizer: Donatella Mutolo (Firenze, Italy)

Invited Oral Presentations

OP.89

Glial and purinergic excitation of the preBötzinger complex shape the hypoxic ventilatory response

Funk GD

Department of Physiology, University of Alberta, Canada

The mammalian brain depends on a constant supply of oxygen to meet its energy needs. Failure of this supply for even a few minutes can result in permanent brain damage or death. A host of adaptive responses have evolved to protect brain oxygen. Prominent among these is the biphasic hypoxic ventilatory response in which an acute fall in oxygen levels (hypoxia) in the blood supplying the brain stimulates carotid body chemoreceptors, triggering a rapid (within 1 minute), Phase I increase in breathing. If oxygen levels are not restored immediately, breathing falls over the next 4-5 minutes to a lower steady state Phase II level (the secondary hypoxic respiratory depression). This secondary depression is most severe and life threatening in premature infants with apnea of prematurity (AOP). Dogma holds that the hypoxic ventilatory response results from two main processes, a peripheral chemoreceptor- mediated Phase I excitation followed by a centrally mediated depression to Phase II; i.e., the only contribution of the central nervous system to the hypoxic ventilatory response is inhibition. Recent data challenge this view with evidence that the preBötzinger Complex (preBötC), a brainstem region critical for inspiratory rhythm generation, mounts an excitatory response to hypoxia that attenuates the hypoxic respiratory depression. Specifically, astrocytes in the preBötC appear to sense hypoxia and release ATP, which in turn acts via P2Y1 receptors to excite inspiratory neurons and increase ventilation (Angelova et al. J. Neurosci. 35; 10460–73, 2015; Rajani et al. J. Physiol. online, 2017). Discussion will focus on the glial, purinergic and ionic mechanisms through which P2Y1 receptor activation excites preBötC neurons and increases ventilation in response to hypoxia. Research supported by Canadian Institutes of Health Research, Natural Science and Engineering Research Council, Canadian Foundation for Innovation and the Women and Children's Health Research Institute (University of Alberta).

OP.90

Evolutionary aspects of neural mechanisms underlying respiratory rhythm generation in vertebrates

Iovino L, Cinelli E, Bongianni F, Pantaleo T, Mutolo D

Department of Experimental and Clinical medicine, University of Florence, Italy

The lamprey, which diverged from the main vertebrate line around 560 million years ago, proved to be highly useful to identify neuronal circuits underlying rhythmic motor behaviours, such as locomotion and respiration. The isolated brainstem of the adult lamprey spontaneously generates rhythmic respiratory activity *in vitro.* The respiratory central pattern generator (CPG) is located in the paratrigeminal respiratory group (pTRG), a region rostral to the trigeminal motor

nucleus. The pTRG shows many similarities with the preBötzinger Complex (preBötC), the proposed mammalian inspiratory CPG. It is well known that ATP plays a role in the control of the preBötC and that astrocytes contribute to purinergic modulation. Recently, this issue has been investigated also in the lamprey. The results show for the first time that astrocytes strongly contribute to the maintenance of the activity of the pTRG via the glutamate-glutamine cycle. In addition, they are involved in the genesis of ATP-induced increases in respiratory frequency at the pTRG level. Acidification evokes ATP-independent increases in the respiratory motor output that requires astrocyte metabolic support. Another important neuromodulator of the preBötC is serotonin (5-HT). In the adult rabbit, 5-HT has been shown to play a pivotal role, especially through a 5-HT1A receptor-mediated inhibition of GABAergic inhibitory interneurons. The existence of a similar mechanism mediated by both GABAergic and glycinergic neurons has also been shown at the pTRG level. The results support the notion that some important features of the neural circuit underlying respiratory rhythm generation are highly conserved throughout phylogeny.

OP.91

Neuromodulation, reconfiguration and the neuronal control of breathing

Ramirez JM

Center for Integrative Brain Research Seattle Children's Research Institute, Departments of Neurological Surgery and Pediatrics, University of Washington School of Medicine, USA

All mammals developed effective strategies to cope with reduced oxygen availability or other metabolic, environmental and behavioral challenges. An important prerequisite for survival is the necessity to be flexible and adaptive, while maintaining functional integrity during times of extreme challenges. This is particularly important for the neuronal network that controls breathing. This network is amenable to a rigorous cellular and subcellular analysis. Using modern transgenic, optogenetic and molecular techniques we identify the critical microcircuits for breathing and demonstrate that neuromodulators imbue this network with the dynamic ability to reconfigure and alter the distribution of respiratory activity within the ventral respiratory column in the brainstem. This network reconfiguration involves the differential activation and inhibition of identified excitatory and inhibitory respiratory neurons as well as glia. We also show that breathing can occur as a 1-, 2-, or 3-phase rhythm, and that every breath is assembled stochastically, with each phase being generated independently by a dedicated excitatory microcircuit. The ability of these microcircuits to reconfigure may allow breathing to remain robust, yet plastic enough to adapt not only to metabolic challenges, but also to conform to non- ventilatory behaviors such as vocalization, swallowing and coughing. Lessons learned from the respiratory network may translate to other highly dynamic and integrated rhythmic systems.

Oral Presentations

OP.92

New data on hypnic and breathing phenotype of a mouse model of down syndrome

Bastianini S, Alvente S, Bartesaghi R, Bartolucci ML, Berteotti C, Guidi S, Lo Martire V, Silvani A, Stagni F, Zoccoli G

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

Down Syndrome (DS) patients commonly develop persistent sleep disorders and sleep apneas. Since the use of mouse models accelerates the understanding of DS pathophysiology, we analyzed the sleep and breathing phenotype in adult Ts65Dn mice (TS; a validated model of DS) and their controls (CTRL). Each mouse was implanted with electrodes to record electroencephalogram (EEG), neck electromyogram (nEMG) and diaphragmatic activity (DA). After 1 week, each mouse was placed in a whole-body plethysmographic (WBP) chamber for 8h during the resting (light) phase to simultaneously record breathing activity together with EEG, nEMG and DA. Based on the EEG and nEMG signals, we then discriminated wakefulness (W), non-rapid- eye-movement sleep (non-REMS) and REMS, while based on WBP signal, we checked for the occurrence of sleep apneas. The analysis of DA (still in progress) during apneic events will allow to discriminate between central and obstructive sleep apneas. Preliminary data (TS = 5, CTRL = 3) show that TS mice spent less time in W and more time both in non-REMS and REMS compared to CTRL. Moreover, TS mice showed fragmentation of W and non-REMS while, during REMS, they tended to have more sleep apneas than CRTL. These preliminary results corroborate previously published data on excessive sleep time in TS mice while reporting completely new findings on wake-sleep cycle fragmentation and increased sleep apnea episodes during REMS. These results lay the ground to understand the neural and molecular pathways of sleep and breathing alteration in DS.

OP.93

Respiratory and inflammatory alterations after therapy with surfactant based on recombinant sp-c analogue in rabbit model of acute respiratory distress syndrome

Mikolka P[1,2], Zebialowicz J[1], Massaro F[3], Perchiazzi G[3], Basabe-Burgos O[1], Curstedt T[4], Feinstein R[5], Larsson A[6], Johansson J[1], Rising A[1,7]

1Department of Neurobiology, Karolinska Institutet, Sweden; 2Biomedical Center Martin and Department of Physiology, , Comenius University, Slovakia; 3Anesthesia and Intesive Care, Villa Anthea Hospital, Italy; 4Department of Molecular Medicine and Surgery, Karolinska University Hospital, Sweden; 5Department of Pathology, The Swedish National Veterinary Institute, Sweden; 6Department of Surgical Sciences, Uppsala University, Sweden; 7Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Sweden

Exogenous surfactant could potentially be used to treat acute respiratory distress syndrome (ARDS). However clinical trials have so far been disappointing, possibly due due to too low doses given, surfactant inactivation caused by alveolar epithelium damage, leakage of plasma proteins and diffuse inflammation. In this study, synthetic surfactant based on a recombinant surfactant protein C analogue (rSP-C33Leu) was compared with natural derived surfactant (poractant alfa, provided by Chiesi Farmaceutici S.p.A., Parma, Italy) in an experimental model of ARDS. Adult New Zealand rabbits with respiratory failure (P/F ratio < 26.7 kPa) induced by lung lavages followed by injurious ventilation were treated with two intratracheal boluses of 2.5 mL/kg of 80 mg phospholipids/mL containing 2% rSP-C33Leu in DPPC/egg PC/POPG, 50:40:10, or with the same dose of poractant alfa, or with air as control. All animals were then conventionally ventilated for 3 h and cardio-respiratory parameters were recorded. Histological appearances of the lungs, oedema formation and levels of TNF IL-6 and IL-8 in lung homogenates were evaluated. Both surfactants improved lung function, reduced inflammation and atelectasis scores, and formation of lung oedema to similar degrees vs. the controls. Poractant alfa improved dynamic compliance at 1 h, P/F ratio and alveolar-arterial gradient at 1.5 h, and atelectasis to a larger extent compared to rSP-C33Leu surfactant. Treatment with synthetic surfactant based on rSP-C33Leu improves lung function and attenuates inflammation in experimental ARDS. Support: Swedish Research Council, The Swedish Heart Lung Foundation, FORMAS, CIMED, APVV-15-0075 and Chiesi Farmaceutici S.p.A

JOINT WORKSHOP AUSTRIA/AFRICA

Organizer: Nandu Goswami (Graz, Austria)

Oral presentations

OP.94

Exploring the Underlying Mechanisms Driving HIV-mediated Cardiovascular Diseases Onset – Focus on Immune-related Changes

Essop F

CMRG, Department of Physiological Sciences, Stellenbosch University, South Africa

With the successful roll-out of combination anti-retroviral therapy, HIV is now increasingly managed as a chronic condition. Thus HIV-infected persons are displaying longer lifespans together with a rise in cardiovascular diseases (CVD) onset. The World Health Organization warned that this phenomenon together with the robust increase in non-communicable diseases in non-infected populations constitutes a 'double burden of disease'. This projection holds serious consequences in terms of health care costs and economic growth for developing countries in particular. The development of CVD in HIV-infected individuals is a multi-factorial process where traditional risk factors (e.g. smoking, diet), side-effects of anti-retroviral drug cocktails and persistent immune activation are all implicated. Of note, although most treated HIV-infected patients attain viral suppression this does not always lead to a complete immunological recovery. The spotlight is therefore focused on the impact of immune-related perturbations on CVD onset in HIV-infected persons. Here increased immune activation and oxidative stress emerge as pivotal drivers of this process. In addition, metabolic re-programming of immune cells can contribute to coagulation pathway activation, providing a putative mechanistic link between HIV and CVD development. Future studies probing immune-related alterations and the nature of metabolic changes in the immune system should eventually help to lower the CVD risk in people living with HIV.

OP.95

Innovative Methods of Vascular Health Assessment in HIV

Goswami N

Department of Physiological Sciences, University of Stellenbosch, South Africa.

This paper discusses some innovative methods for vascular function assessment in HIV. These methods are currently being used in the ongoing EU Africa project. These assessments are particularly innovative in that they can be carried out non-invasively. These include assessments of endothelial health via flow mediated dilatation (FMD), assessment of arterial stiffness using pulse Wave velocity (PWV) and microvascular assessments by studying changes in the retinal microvasculature. The data were measured at two time points, separated by 18 months in three cohorts of persons: HIV negative (Controls); HIV positive plus on Antiretroviral therapy (ART/HAART); and HIV positive patients not yet on ART/HAART. The talk then discusses the methodology of each of these measurements and identifies the potential challenges that arise from the usage of these methods in the field. Quality assurance as well as the need for careful training of the operators are particularly important aspects that must be considered when planning such experiments.

OP.96

Comparability of Pulse Wave Velocity in Children and Adolescents living with HIV

Mellin J[1], Goswami N[2], Klein N[3]

[1]Institute of Physiology, Medical University of Graz, Austria; [2]Institute of Physiology, Medical University of Graz, Austria; [3]Institute of Child Health, University College of London, Great Britain;

HIV has become a manageable disease, bringing into focus the long-term effects such as an increased risk of cardiovascular disease. Many studies have shown on early onset of endothelial dysfunction and therefore atherosclerosis in adults in comparison to healthy controls. This brings into question whether these changes can already be seen and measured in children and adolescents. Plasma markers, flow mediated dilatation and intima media thickness are the most reported measurements in adults; invasiveness and long duration make these measurements impractical for the use in children and adolescents. Pulse wave velocity however is non-invasive and does not require the child or adolescent to lie still for a long period of time. Analyzing a study of HIV positive adolescents in Great Britain has brought several difficulties into perspective. Similar to flow mediated dilatation there is no standard operation procedure for obtaining pulse wave velocity measurements. The greatest problem is not the choice of recording device, but the technique of obtaining the distance between the carotid and femoral measurement site. There are several different methods that differ significantly. Since pulse wave velocity equals the distance of the pulse wave travelled over time, length is a defining measurement. In comparison to adult studies where absolute cut-off values have been put into place, pediatric reference values for age and sex are available for most variables such as blood pressure and biometric markers. Some pulse wave velocity reference values for children have evolved in the past couple of years. These are based on specific measurement techniques and relatively small sample sizes so far. We found that the abovementioned different measurement techniques make the comparability to reference values very difficult.

OP.97

Antiretroviral Drugs and the Human Gut Microbiome: Implications for the development of cardiovascular diseases in people living with HIV - An Austrian/South African Project

Musarurwa HT[1], Sewani-Rusike CR[1], Nkeh-Chungag BN[2], Goswami N[3]

[1]Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, Mthatha, South Africa; [2]Department of Environmental and Biological Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha, South Africa; [3]Otto Loewi Research Centre, Division of Physiology, Medical University of Graz, Graz, Austria

Sustained presence of HIV, together with protease inhibitors and nucleoside reverse transcriptase inhibitors used in HIV treatment alters gut microflora and are linked to changes in lipid profile and vascular function. This results in the development of a unique variant of metabolic syndrome, characterised by lipid imbalances, body fat redistribution, and insulin resistance making ART an interface of non-communicable and infectious diseases. Although the link between HIV infection, ART, metabolic diseases, and the enteric microbiome has been suggested, there is still need for exhaustive investigations to confirm the relationship. Thus, this study aims to simultaneously investigate changes in gut microbial dynamics, lipid profile, and endothelial function and in their relation to changes in viral load of HIV positive individuals on ART. This study presents a platform on which metagenomics, metabolomics and flow-mediated dilation ultrasound techniques are employed to characterise effects of sustained ARV use results in the observed diverse metabolic and physiological phenotypes observed in people living with HIV. The data produced will further clarify the HIV-microbiome-lipid complex.

OP.98

Obesity and related cardiometabolic risk factors in HIV-infected individuals

Strijdom H[1], Kamau F[1], Goswami N[2], Essop F[3]

[1]Division of Medical Physiology, University of Stellenbosch, South Africa; [2]Otto Loewi Research Centre for Vascular Biology, Immunology and Inflammation, Medical University of Graz, Austria; [3]Department of Physiological Sciences, University of Stellenbosch, South Africa.

There is growing evidence of an interplay between HIV-1-infection, anti-retroviral therapy (ART) and cardiovascular disease (CVD). Since the introduction of HAART, studies in Europe and North America have shown an increased incidence of coronary heart disease in HIV infected populations compared to the general population. Other known HIV-related cardiovascular comorbidities include obesity, diabetes, non-alcoholic fatty liver disease and overt metabolic derangements. Sub-Saharan Africa (SSA) is home to approximately 26-million (70%) of the world's HIV-infected population. Concurrent to the HIV/AIDS epidemic, many countries in SSA have observed a rapidly expanding non-communicable diseases epidemic, characterised by, among others, increases in CVD risk factors and disease. In view of this, it is surprising that the impact of high HIV-infection rates and HAART on the increasing prevalence of CVD in SSA has received relatively little research attention. An often overlooked and seemingly counterintuitive emerging risk factor in HIV-populations is that of obesity. Although the prevalence of obesity in HIV-infected populations has previously been described in developed countries, where it has been shown to rapidly approach that of the general population, data from the SSA region remain scarce. The cardiovascular risk posed by obesity in the general population is well described; however, the role of obesity and its metabolic effects in HIV-infected individuals remains unclear, particularly in view of the putative interaction with the cardiometabolic sequelae ascribed to the HI-virus and ART. In this lecture, the emerging risk posed by obesity in HIV-infected individuals will be discussed, supported by results from the EndoAfrica study currently conducted in South Africa.

Workshop

NUTRITION, GUT MICROBIOTA, AND HEALTH

Oral presentations

OP.99

Effects of western diet and short chain fatty acids on colonic inflammation in rats

Cantali Ozturk C[1], Sevim M[1], Ali Mergen M[2], Ceren Gullu E[2], Yaren Ayvaz E[2], Temel M[2], Oluc M[2], Sirvanci S[3], Abbak Ural M[4], Cevik O[4], Yegen BC[1], Yildirim A[1]

[1]Marmara University School of Medicine, Department of Physiology, Turkey; [2]Marmara University School of Medicine, Turkey; [3]Marmara University School of Medicine, Department of Histology & Embryology, Turkey; [4]Adnan Menderes University School of Medicine, Department of Biochemistry, Turkey.

The Western type diet (WD) worsens inflammatory events in the colon, while short chain fatty acids (SCFA) improve inflammation and other adverse effects of WD. In this study, preventive and/or therapeutic effects of SCFA were investigated by co- administration of SCFA and WD in rats with dextran sodium sulfate (DSS)-induced colonic inflammation. In normal diet–fed rats, DSS formed an inflammation in the colon, while feeding with WD and adding SCFA into drinking water for 10 days reduced the macroscopic score. However, 3-week SCFA administration enhanced the inflammation. Increased colonic lipid peroxidation levels in WD+DSS group was diminished with addition of SCFA. Despite that DSS in normal diet had no inflammatory effect on the liver, WD- induced hepatic inflammation was reduced by SCFA administration. Increased serum lipid (VLDL, triglyceride) levels with WD intake were reduced with administration of SCFA. In contrast, long-term SCFA consumption in WD-fed rats significantly increased the body and liver weights when compared to WD without SCFA. DSS did not reveal any adverse effects on renal functions but adding SCFA to normal diet elevated serum urea and BUN levels. Surprisingly WD reversed the effects of SCFA on renal functions. Taken together; short-term SCFA consumption has a beneficial effect against DSS- induced colonic inflammation and WD-induced hepatic inflammation. The beneficial effect of short-term SCFA treatment is consistent with the literature. Contrarily, long- term administration of SCFA has deleterious effects, while short or long-term SCFA administration disrupts renal function. Increased calorie intake along with SCFA may contribute to the loss of its favorable effect and exaggerate inflammation when accompanied by long-term administration of SCFA.

OP.100

Changes in tissue lipid mediators induced by severe negative energy balance in female rats

Carta G, Giunti E, Scherma M, Abolghasemi A, Murru E, Fadda P, Banni S

Department of Biomedical Science, University of Cagliari, Italy

The survival of any organism requires efficient energy substrate utilization. When energy intake decreases, it takes place an adaptive decrease in energy expenditure and increased motivation to eat to maintain a steady body fat composition. Changes of lipid deposition and distribution among tissues are partially mediated by specific lipid mediators such as N-acylethanolamides (NAEs) and 2-monoacylglycerols (2-MGs), which influence fatty acid oxidation and lipogenesis in peripheral tissues, and food intake and satiety in brain areas. The aim of this study was to evaluate whether in female rats dramatic changes of tissue lipid deposition, as it occurs during calorie restriction, following its replenishment during the recovery phase may influence tissue concentration of NAEs and 2-MGs which may mediate the physiological adaptation of the acute adipose tissue (AT) depletion/replenishment. Our results showed that strong negative energy unbalance resulted in a steep increase of NAEs in the two key tissues for lipid metabolism, liver and AT, that may be due to a relative depletion of fatty acids owed to their β-oxidation and exportation to relevant tissues for energy expenditure such as muscles, and/or an inhibitory effect, possibly mediated by PPAR , of degrading enzymes for NAEs and 2-MGs. Interestingly, the strong increase of some NAEs may further enhance PPAR activity which facilitates the use of fatty acid as a fuel to meet the energy needs during extreme food restriction. Remarkably, in the recovery phase, a prompt rescue of fat deposition is associated to normal levels of the lipid mediators. A nutritional approach targeting NAEs and 2-MGs biosynthesis modulated by dietary fatty acids, may regulate fat deposition and distribution during acute AT depletion/replenishment.

OP.101

Initial Brain Aging and High Fat-High Fructose Diet: Effect on Mitochondrial Bioenergetics, Oxidative Status and Cholesterol Homeostasis in Rat Brain

Crescenzo R[1], Spagnuolo MS[2], Iannotta L[1], Cancelliere R[1], Mazzoli A[1], Gatto C[1], Canè M[1], Nazzaro M[1], Iossa S[1], Cigliano L[1]

[1]Dept of Biology, University of Naples Federico II, Italy; [2]ISPAAM, CNR, Italy

Middle age is an early stage of the aging process, during which the consumption of diets rich in saturated fats and/or simple sugars might influence brain function, but only few data are available on this issue. Our aim was to investigate the impact of a diet rich in saturated fat and fructose (HFF) on mitochondrial physiology and cholesterol homeostasis in brain, where this lipid is involved in the maintenance of several neuronal processes. In particular we focused on critical areas for learning and memory, i.e. hippocampus and frontal cortex of middle-aged rats (11 months old), by including a group of adult rats (90 days) as negative control, lacking the putative effect of aging. Middle-aged rats were fed HFF or control diet for 4 weeks. Mitochondrial function was analyzed by high-resolution respirometry and by assessing respiratory complexes levels. A decrease in the activity of complex I was detected in both brain areas of middle-aged rats. In hippocampus, an age-decrease in mitochondrial respiratory capacity and complex IV content, partly reversed by HFF diet, was evident. Higher oxidative protein damage decreased antioxidant defenses, and increased UCP2 and PGC-1α were found in hippocampus of middle-aged rats. HFF feeding induced a significant reduction in the amount of UCP2, PGC-1α and PPARα, together with higher protein oxidative damage, in both brain areas. Notably HFF feeding also induced the alteration in key proteins of the regulatory network of brain cholesterol levels (LXR-β, HMGR, LDLr, Apolipoprotein E etc) that could predispose to neurodegenerative diseases. Overall, our results point to middle age as a condition of early brain aging for mitochondrial function, with hippocampus being an area more susceptible to metabolic impairment than frontal cortex.

OP.102

Long-term effect of a classical ketogenic diet on glucose metabolism: A 12-months longitudinal study

De Amicis R, Leone A, Foppiani A, Lessa C, Ravella S, Battezzati A, Bertoli S

International Center for the Assessment of Nutritional Status (ICANS), Department of Food Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

The classical ketogenic diet (cKD) is a normocaloric, high-fat, very low-carbohydrate diet that induces ketosis, mimicking starvation state. The ketone bodies are alternative body fuel and they pass into the brain, replacing glucose as an energy source; for these reasons, it's used as a recognised treatment for drug-resistant epilepsy (DRE), GLUT1 Deficiency Syndrome (GLUT1DS) and PDH deficiency, and it's currently assessed for obesity, metabolic syndrome and type 2 diabetes. Glucose profile on patients treated with a long- term cKD has poorly investigated, so we evaluated the effect of a 12-months cKD on glucose metabolism of 29 children affected by DRE and GLUT1DS (mean age: 8.0 y, range: 0.5- 16.6 y; 17 females; 22 GLUT1DS), to determine fasting HOmeostatic Model Assessment- Insulin Resistance

(HOMA-IR) and Quantitative Insulin Sensitivity Index (QUICKI). BMI- zscore (-0.22±1.87 vs -0.38±1.39, P=0.453) and percentage of body fat (22.7±7.8 vs 22.2±1.3, P=0.488) didn't change during the treatment. Children showed a not significant reduction in fasting blood glucose (84.9±1.6 mg/dl vs 80.8±1.6 mg/dl; P=0.085). However, fasting insulin significantly decreased (9.2±1.0 μU/mL vs 5.5±1.0 μU/mL; P=0.013), and both HOMA-IR and QUICKI indexes were significantly changed (2.0±0.2 vs 1.2±0.2; P=0.018; 0.51±0.00 vs 0.52±0.00; P=0.041, respectively). After 12 months of cKD, fasting blood glucose doesn't change, but a significant improvement is observed in HOMA-IR and QUICKI indexes, corroborating our previously published data of short-term effect of cKD on glucose metabolism. These results suggest potential interesting implications of the KD in insulin metabolism alterations; however, long-term studies on adult patients are needed to confirm these adaptive metabolic changes during cKD.

OP.103

Fumonisins induced toxic mechanisms on intestinal epithelial models

Garbetta A[1], Martino NA[2], Debellis L[3]

[1]Institute of Sciences of Food Production (ISPA), CNR, Italy; [2]Dept. of Veterinary Science, University of Turin, Italy; [3]Dept. of Bioscience, Biotechnologies and Biopharmaceutics, University of Bari, Italy

Fumonisins (FBs) are Fusarium mycotoxins, common contaminants in corn products, with toxic effects on animal and human health linked mainly to inhibition of ceramide synthase. The study aimed to assess, on *ex-vivo* human and rat intestine, the effects induced by FBs exposure on: a) epithelial transport index (short circuit current); b) lipid peroxidation (malondialdehyde levels, MDA). Moreover, in order to understand FBs toxicity mechanisms, several functional parameters, such as cell proliferation, oxidative status, immunomodulatory effect and changes in membrane microviscosity, assessed by fluorescence anisotropy, were tested on human intestinal cell line HT-29. Exposure to contaminated (FBs 0.7 to 90 μg/ml) corn chyme samples affected significantly (up to 30%) the electrogenic transports in both intestinal epithelia models, inducing also a significant FBs concentration-related lipid peroxidation (up to 200% MDA increase), probably due to interactions between mycotoxins and intestinal membranes. The experiments carried out on HT-29 line showed an early intracytoplasmatic FB1-FITC localization, confirmed the lipid peroxidation, followed by a significant decrease in IL-8 inflammatory response (up to 24%) and a significant reduction in membrane microviscosity. The lipid peroxidation, concomitant with modification in membrane fluidity, could explain the alterations in the physiological process of cell-mediated transport found in *ex vivo* intestinal tracts.

OP.104

A nutritional intervention based on egg white for phosphorus control in hemodialyis patients.

Di Maro M, Di Lauro T, Trio R, Salomone E, Di Martino R, Di Lauro M, Sacco E, Colantuoni A, Guida B

Department of Clinical Medicine and Surgery, Physiology Nutrition Unit, Federico II University of Naples, Italy

The aim of the present study was to evaluate a dietary intervention for hyperphosphatemia in dialysis patients based on the partial replacement of meat and fish with egg white, a virtually phosphorous-free protein source. 23 hyperphosphatemic patients on chronic standard 4 h, three times weekly, bicarbonate hemodialysis were enrolled in this open-label, randomized controlled trial. Patients in the intervention group were instructed to replace fish or meat with egg white in three meals a week for three months whereas diet was unchanged in the control group. At the end of the study, serum phosphate concentrations were significantly lower in the intervention group than in controls (4.9 ± 1.0 vs 6.6 ± 0.8; $p < 0.001$). Phosphate concentrations decreased more from baseline in the intervention than in the control group both after one (-1.2 ± 1.1 vs 05 ± 1.1; $p = 0.004$) and after three (-1.7 ± 1,1 vs -0.6 ± 1,1; $p < 0.001$) months of follow-up. No change either in body weight or in body composition assessed with bioelectrical impedance analysis or in serum albumin concentration was observed in either group. The partial replacement of meat and fish with egg white induces a significant decrease in serum phosphate without causing protein malnutrition and could represent a useful instrument to control serum phosphate levels in hemodialysis patients.

OP.105

Dietary supply of the antioxidant and prebiotic mix promotes muscle growth and improves disease resistance in cultivated fish

Lysenko L, Kantserova N, Parshukov A, Sukhovskaya I

Institute of Biology KarRC RAS, Petrozavodsk, Russia

In order to maintain welfare of reared fish and to improve performance natural antioxidants and

immunostimulants have been used as dietary supplements. Bioactive compounds of plant origin revealing various activities, such as antioxidant, anti-inflammatory, prebiotic, etc., could be promising in fish production improving individual weight gain, feed efficiency, and disease resistance of individuals. In rainbow trout juveniles, the effect of dihydroquercetin and arabinogalactan mix was shown. Key parameters of fish growth, such as growth rate, specific growth rate (SGR), and the molecular mechanisms of muscle growth, such as myofibrillar protein expression, pro-myogenic regulation, protease activities, net protein turnover, were studied in detail. In individuals differentiated by an infectious status, gut and stomach microbiome compositions by 16S rRNA gene sequencing and annotation was identified. Our results have shown that the dietary mix promotes feed conversion in fish and net protein metabolism in their skeletal muscle. Muscle growth responds to diet supplementation through enhanced anabolic and breakdown-associated cellular signaling and mRNA expression. Immunity of fish was also perturbed by supplements resulting in higher survival rate against sporadic bacterial infection and increased taxonomic variability of gut microbiome. Despite the differences observed in cumulative mortality against pathogens, just a little difference was found in SGR between groups fed by a standard or supplemented feed. Apparently, the bioactive mix occurs to improve some immune and growth-promoting mechanisms in rainbow trout opening the possibility to the use it in fish diets. The study was financially supported by the Russian Science Foundation, project no. 17-74-20098.

OP.106

Rifaximin-dependent modulation of gut commensal microbiota and host:bacterial interactions in a murine model of DSS-induced colitis

Ferrer M, Martínez V

Department of Cell Biology Physiology and Immunology, Universitat Autònoma de Barcelona, Spain

Rifaximin is a wide-spectrum antibiotic that improves clinical signs of Inflammatory Bowel Disease (IBD), although the mechanism of action is still unknown. Here, we characterized the effects of Rifaximin in host:bacterial interactions and gut commensal microbiota (GCM) in a murine model of colitis. Dextran sulfate sodium (DSS, 3%, 5 days) was added to the water to induce colitis in C57BL/6NCrl female mice. Animals were treated preventively with Rifaximin (50 or 150 mg/kg/day, 9 days, PO) or Doxycyclin (30 mg/kg/day, 9 days, PO). Daily clinical signs, histological colonic inflammation, GCM (shotgun metagenomic sequencing), local expression (qRT-PCR) of TLRs (2, 3, 4, 5 and 7), antimicrobial peptides (DEF 24, RELMβ and RegIIIγ) and inflammatory markers (IL-6, IFNγ, TNF , IL-1β, RANTES, IL-10) were assessed. Regardless the dose tested, Rifaximin did not affect the clinical course of colitis, while Doxycyclin attenuated clinical signs. In non-inflamed animals, Rifaximin did not alter total bacterial counts and had minor effects on GCM. DSS-induced colitis was associated to mild microbial changes that were only slightly altered after Rifaximin treatment. Bacterial diversity was not affected in any of the groups, except for a minor decrease due to Doxycyclin treatment in colitic mice. Colitis-associated up-regulation of inflammatory markers was attenuated by Doxycyclin but not modified by Rifaximin. Moreover, Rifaximin resulted in down-regulation of TLR3, 4 and 5 and up-regulation of TLR7 and RegIIIγ; whilst Doxycyclin was without effects. In the DSS-induced colitis model, Rifaximin does not show neither clear anti-inflammatory nor antimicrobial activities. The mechanisms mediating the beneficial effects of Rifaximin in IBD remain elusive.

Workshop

REPRODUCTIVE PHYSIOLOGY

Oral presentations

OP.107

Role of acupuncture in female infertility. A pilot study

Aloisi AM, Fiorenzani P, Ghiandai G, Martini N, Tian Fu Bao

Department of Medicine, Surgery and Neuroscience, University of Siena, Italy

In Traditional Chinese Medicine, reproductive capacity is under the control of the kidney because it serves the vital essence (Jing) of the egg and then the uterus in females and testis in men. Acupuncture can be used to treat infertility in cases of hormonal (high gonadotropins) or idiopathic causes. The aim of this study was to evaluate the acupuncture-induced effects on hormones and reproductive capacity in infertile women. The levels of FSH and other hormones were determined before and after a cycle of acupuncture treatment. Thirty infertile women (mean 38, range 29-49 years) with high FSH levels and no other pathology, who had tried the procedure for assisted reproduction at least once, were included in the study. Acupuncture consisted in 10 sessions (30 min each) carried out 2-3 times per week. The following acupoints were used:

CV-4 Guanyuan, ST-29 Guilai, CV-3 Zhongji, SP-9 Yinlingquan, SP-6 Sanyinjiao. The results showed a significant reduction in FSH levels: before 20,12 mUI/ml (± 5,57) vs. after 8,18 mUI/ml (± 2,61). After 3 months, 90% (27/30) of the patients were pregnant. In conclusion, it was confirmed that acupuncture is able to restore physiological FSH blood levels and to resolve infertility in women. New studies will be carried out to completely define the involved mechanisms.

OP.108

Placental adaptations to altered maternal glucose supply

Benincasa L, Ietta F, Manzan-Martins C, Passaponti S, Romagnoli R, Cresti L, Paulesu L.

Department of Life Sciences, University of Siena, Italy

Glucose represents the essential fuel for placental-fetal development since fetal gluconeogenesis has not been reported yet. The correct and physiological supply of glucose to the fetus almost totally depends on placental exchange. However, the mechanisms linking maternal under/over glucose supply to placental-fetal development are largely unknown. This study aimed to examine placental strategies of adaptation to perturbed glucose homeostasis. Placenta villi explants from elective termination of pregnancy and HTR8/SV-neo cells were exposed to under/over-glucose nourished media. Cultures were examined for glucose uptake and the presence of glucose transporters (GLUTs). The data showed that trophoblast glucose uptake is glucose supply-dependent. Moreover, under/over- glucose nourishment determined a reset of cells/tissues GLUTs expression and localization. The reset of glucose transporters might compromise glucose transfer across the placenta with potentially dangerous consequences on placental-fetal development.

OP.109

Does diet induce obesity changes the contribution of RhoA/Rho kinase pathway to myometrial contraction in pregnant rats?

Demeli M[1], Bayrak S[2], Korkmaz H[1,2], Pehlivanoğlu B[1]

[1]Department of Physiology, Hacettepe Universityì, Turkey; [2]Formerly Department of Physiology, Hacettepe University, Turkey; [3]Department of Physiology, Gazi University, Turkey

Obesity is an important public health problem with increasing prevalence and accompanying risk for various morbidity and mortality. It is well documented that obesity leads to maternal and perinatal fetal complications during pregnancy. The previous data also suggest reduced myometrial contractility, prolonged, dysfunctional labour in obese women leading to emergency caesarean section, which is supported by unstable asynchronous myometrial contractions in obese animals. Since RhoA/ROCK pathway is an effective intracellular mechanism in uterine smooth muscle, determining its share in contraction of pregnant myometrium in lean and obese individuals may present a new therapeutic window. On this background we investigated the effect of RhoA/ROCK pathway inhibition on spontaneous and oxytocin induced myometrial contractions in high fat diet-fed obese pregnant rats. Our results showed significant difference in contraction properties of lean and obese rats. Although the amplitude of spontaneous and oxytocin induced contractions were significantly lower in obese groups, the frequencies were similar in both groups. When agonist stimulated myometrial strips were treated with RhoA/ROCK inhibitor H1152 cumulatively (10-9-10-5) both frequency and amplitude of the contractions decreased. The impact of H1152 was significantly more prominent in control group. These results indicate a modulation in contractile process of myometrium in obese pregnant rats. Although the results should further be clarified by protein expression levels and functional studies, still they suggest a promising role for the RhoA/ROCK pathway.

OP.110

Pre-eclampsia and intrauterine growth restriction: manifestations of oxidative stress during pregnancy

Raina G[1], Farruggio S[1], Surico D[2], Bordino V[2], Cantaluppi V[3], Mary D[1], Melluzza C[2], Grossini E[1]

[1]Department of Translational Medicine, University East Piedmont, Italy; [2]Department of Translational Medicine, University East Piedmont and Azienda Ospedaliera Universitaria Maggiore della Carità, Novara, Italy; [3]Department of Translational Medicine, University East Piedmont and Azienda Ospedaliera Universitaria Maggiore della Carità, Italy

Oxidative stress is involved in the pathogenesis and maintenance of pregnancy-related conditions, such as intrauterine growth restriction (IUGR) and preeclampsia (PE). Human umbilical cord mesenchymal stem cells (hUMSCs) have been suggested as a possible therapeutic tool for the treatment of pregnancy-related disorders in view of their paracrine actions on trophoblast cells. To analyze the role of oxidative stress *in vivo* in patients affected by PE and IUGR and *in vitro* by using hUMSCs from physiological and pathological pregnancies and a

trophoblast cell line. In pathological and physiological pregnancies, the plasma markers of oxidative stress, arterial blood pressure, serum uric acid, 24h proteinuria, weight gain and body mass index (BMI) were examined. Furthermore, the pulsatility index (PI) of uterine arteries, umbilical arteries and fetal medium cerebral artery was measured. *In vitro*, the different responses of hUMSCs, taken from physiological and pathological pregnancies, and of trophoblast cells to pregnancy-related hormones in terms of viability and nitric oxide (NO) release were investigated. In some experiments, the above measurements were performed on co- cultures between trophoblast cells and hUMSCs. The results obtained have shown that in pathological pregnancies, clinical parameters were worse than those found in physiological ones and markers of oxidative stress were higher. Moreover, in PE and IUGR, a relation was observed between laboratory and clinical findings and the increased levels of oxidative stress. Trophoblast cells and hUMSCs showed worse viability and increased NO production when stressed with H_2O_2. Finally, trophoblast cells cultured in crosstalk with hUMSCs from pathological pregnancies showed worse response to hormones in terms of cell viability and NO release. Oxidative stress could play a role in the onset of IUGR and PE, by interfering with the crosstalk between hUMSCs and trophoblast cells.

OP.111

Glycodelin at the maternal-fetal interface

Ietta F[1], Benincasa L[1], Pavone V[1], Passaponti S[1], Ermini L[1], Luddi A[2], Piomboni P[2], Paulesu L[1]

[1]Department of Life Sciences, University of Siena, Italy; [2]Department of Molecular and Developmental Medicine, University of Siena, Italy.

Glycodelin (Gd) is a multifunctional glycoprotein that regulates critical physiological processes during reproduction, in particular Gd is an indispensable molecule for the establishment, maintenance and progression of pregnancy. The Gd is produced by the endometrium in the luteal phase of the menstrual cycle and during pregnancy. Furthermore, as a maternal product, the Gd has also been described in the placenta. In this study we performed experiments to characterize and evaluate the qualitative and quantitative expression of Gd in human placenta during the first trimester of pregnancy. By a proteomic approach, placenta tissues at different gestational ages were used to evaluate the presence and the glycosylation patterns of the protein in comparison to endometrial tissues. In vitro experiments by means of placenta explants cultures were also established to evaluate the production and release of Gd over time. The results obtained show that, in addition to share common Gd glycoforms with maternal endometrium, the placenta produces unique glycoforms of Gd. The data suggest the Gd of placenta origin as a new glycoprotein involved in the fetal- maternal cross-talk.

OP.112

Is alarin really a novel hormone for reproduction?

Solak Gormus ZI, Ozen Koca R, Solak H, Sahin Z, Kutlu S

Department of Physiology, University of Necmettin Erbakan, Meram Medical School, Turkey

Alarin is a new found peptide hormone which is a member of galanin family neuropeptides. It was found in gangliocytes of neuroblastic tumors of human first, and expressed in central nervous system/periphery tissue such as vascular smooth muscle cells and thymus. Its effect on reproductive system, especially on rat uterus is not investigated particularly experimentally. We aimed to investigate the effect of alarin on rat uterus. For this study we used 24 Wistar Albino rats in three groups: Control (Group 1, n=8), spontaneous contraction (Group 2, n=8), prostaglandin (PG) induced contraction (Group3, n=8). Uterus tissues were isolated quickly and sectioned into 2x12 mm strips. Uterus strips then placed in organ baths containing Krebs–Henseleit solution, which is thermoregulated at 37 C and aerated (95% O_2 and 5% CO_2). Changes in isometric tension of uterus strips were recorded using a four-channel force displacement transducer. In Group 1, only spontaneous contractions were observed. Group 2, when contractions were stable cumulative alarin doses (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) were given for spontaneous contractions. Group 3, strips contracted with PG (60 µl) and then cumulative alarin doses (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) were applied. Alarin treatments at doses 10^{-8}, 10^{-7}, 10^{-6} M had no significant changes on muscle contractility. But alarin inhibited the contraction at 10^{-5} M concentration dose following PE administration ($p<0.05$). This effect of alarin occured in a dose dependent manner. These findings have a potential to contribute to studies owing to effect of alarin on female reproductive system. Further studies are needed to clarify the mechanism(s) of alarin.

OP.113

Bisphenol A Compromises Uterine Arterial Remodeling and Function During Pregnancy and Reduces Placental Growth

Barberio L[1], Bertoli S[2], Colciago A[3], Pallottini V[4], Paulesu L[5], Rolfo A[6], Mandalà M[1]

[1]Department of Biology, Ecology & Earth Science, University of Calabria, Italy; [2]Department of Food, Environmental, & Nutritional Sciences, University of Milan, Italy; [3]Department of Pharmacological & Biomolecular Sciences, University Milan, Italy; [4]Department of Science, Biomedical Sciences & Technologies, University Roma Tre, Italy; [5]Department of Life Sciences, University Siena, Italy; [6]Department of Surgical Sciences, OIRM S. Anna Hospital, Italy

Bisphenol A (BPA), a widespread environmental contaminant, has been found in urine, blood and amniotic fluid. Studies in humans suggest that maternal BPA exposure is associated with alterations in pregnancy outcomes. Because adaptation of the maternal uterine circulation is essential for normal placental perfusion, we hypothesized that BPA compromises uterine artery remodeling and the development of the fetoplacental unit. To compare the effect of BPA in the reproductive vs systemic circulation, we examined the effects of BPA on uterine and mesenteric artery structure and function. Fetuses and placentas were weighed to provide an index of reproductive performance. Rats, divided into three groups of n =6 were treated for a month before pregnancy and during gestation with: BPA at 2.5 and 250 μg/kg or vehicle (ethanol, control). Arteries from pregnant rats were studied on day 20 of gestation (P20) by pressure myography. In uterine arteries, treatment with BPA reduced: a) lumen diameter, $p<0.01$ at 250 μg/kg BPA, b) distensibility, $p<0.05$ at both BPA concentrations, and c) vasodilation to acetylcholine, $p<0.05$ (2,5 μg/kg) and $p<0.01$ (250 μg/kg). Conversely, no changes were observed in mesenteric arteries. Although BPA did not influence fetal weights, placental weights were significantly ($p<0.05$) reduced at both BPA treatment levels. For the first time, our results suggest that treatment with BPA reduces arterial outward remodeling and induces endothelial dysfunction in uterine but not in systemic vessels. BPA may also hamper placental development and result in placental underperfusion. Interestingly, normal fetal weights suggest the existence of compensatory mechanisms that augment placental efficiency through mechanisms that are not yet known.

OP.114

Ω3 Fatty Acids Administration During Pregnancy and Lactation Induces a Long-lasting Modulation of Mevalonate Pathway in the rat brain

Tonini C[1], Schiavi S[1], Macca F[1], Segatto M[2], Trezza V[1], Pallottini V[1]

[1]Department of Science, University Roma Tre, Italy; [2]Department of Biosciences and Territory University of Molise, Italy.

Maternal nutrition during pregnancy and lactation is very important for the offspring health, not only for fetuses and newborns, but also for adults. The idea is that an under- or oversupply of some nutrients at a specific developmental stage may affect the function of organs or systems in the offspring. It has been established that a proper neurodevelopment is dependent on particular nutrients supplied by the mother from diet intake, including Omega-3 (3) fatty acids. Fatty acids intake during pregnancy could affect neurodevelopment also via mechanisms not directly related to themselves, since they could influence other metabolic processes important for brain development. Cholesterol biosynthetic pathway, also known as mevalonate (MVA) pathway, may represent one of these metabolic processes. 3 fatty acids are recommended to pregnant and lactating women to help offspring brain development and prevent alterations in adult life. To date, no information is available regarding the impact of this diet supplementation on MVA pathway in selected brain areas. Thus, we aimed at evaluating the long-lasting impact of perinatal exposition to 3 fatty acids on MVA pathway. The adult male offspring born from female rats fed with 3-enriched diet during pregnancy and lactation was used as experimental model. Cholesterol synthesis and downstream effects was then analyzed in six different brain areas. Our results demonstrate that perinatal supplementation of 3 induces a long-lasting modulation of mevalonate pathway in specific brain areas, without affecting liver metabolism. This 3-induced modulation is able to regulate neurotrophin content. Taken together our data show a new mechanism of action of 3-induced beneficial effects on brain.

Workshop

ANIMAL AND ENVIRONMENTAL PHYSIOLOGY

Oral presentations

OP.115

Estrogens in molluscs revisited: not hormones but powerful exogenous modulators of physiological functions?

Canesi L[1], Ciacci C[2], Balbi T[1]

[1]Department of Earth, Environment and Life sciences-DISTAV, University of Genoa, Italy; [2]Department of Biomolecular Sciences, DISB, University of Urbino "Carlo Bo", Italy

In the last decade, knowledge on endocrine physiology in mollusks has enormously increased, with the identification of receptors/signaling pathways/effectors involved in steroid signaling and metabolism. From these studies, considerable debate emerged on the role of natural steroids in mollusk physiology. This is a key point to understand both the basic endocrine mechanisms and the possible impact of estrogenic chemicals in this relevant invertebrate group. In this work, we will focus on available knowledge on occurrence, effects and mechanisms of action of estrogens in mollusks, reporting pros- and cons- on their role as 'hormones' in regulation of physiological processes. Most information comes from bivalves, widespread in different aquatic environments, and most likely affected by exposure to estrogenic compounds, and on the effects of 17B-estradiol-E2 in the marine bivalve Mytilus spp. The results obtained in vitro, in the immune cells, the hemocytes, and in vivo, in early embryo development, will be integrated with –omics data obtained in the tissues of adult mussels exposed to estrogenic compounds. The results, through independent lines of evidence, strongly support the rapid, non-genomic signaling pathways of estrogen action. In this light, regardless of whether bivalves synthesize estrogens de novo, or rely on uptake of exogenous estrogens from the environment, they are clearly powerful bioactive compounds that affect multiple physiological functions.

OP.116

Slc15a1 transporters in teleosts fish: PepT1a and PepT1b, comparative functional studies

Vacca F[1], Bossi E[1], Gomes AS[2], Cinquetti R[1], Barca A[3], Verri T[3], Murashita K[2,4], Rønnestad I[2]

[1]Department of Biotechnology and Life Sciences, University of Insubria, Italy. [2]Deptartment of Biological Sciences, University of Bergen, Norway. [3]Department of Biological and Environmental Sciences and Technologies, University of Salento, Italy. [4]National Research Institute of Aquaculture, and Fisheries Research and Education Agency, Japan

Di/tripeptides are key nutrients in animal diets, and fundamental for growth. They are transported across the membrane of enterocytes *via* the Slc15a1/PepT1 peptide transporter, that uses an inwardly-directed proton electrochemical gradient to drive the uptake. Due to a genome duplication event, PepT1a and PepT1b paralogues are found in teleost fish. Two PepT1a transporters, respectively cloned from the Atlantic salmon (*Salmo salar*) and zebrafish (*Danio rerio*), namely asPepT1a and zfPepT1a, have been characterized. For both orthologs, function was verified by heterologous expression in *Xenopus laevis* oocytes, highlighting electrogenic, Na^+-independent and pH-dependent transport similarly to the well-known PepT1b. The transient currents and the transport currents recorded from asPept1a and zfPepT1a indicate significant functional differences with respect to PepT1b. PepT1a can be described as a low-affinity/high-capacity system, but its substrates preference profile is peculiar in the species, particularly for charged dipeptides. Moreover, the pre-steady state (PSS) currents, that reflect the first steps of transport cycle display in the charge/voltage relationship differences with respect to Pept1b in the voltage dependence. Considering that the PSS are consequence of the rearrangement of the protein in the membrane electric field, PepT1a transporters interact with the substrate at physiological pH, differently from PepT1b. In addition, PepT1a and PepT1b have similar expression profile but different expression levels. These data together with a significant dissimilar substrate specificity, support the idea of distinct roles for these proteins in peptide recognition and transport.

OP.117

Cardiac adaptation to hypoxia: the role of beta3-adrenoceptors in the goldfish (*Carassius auratus*)

Imbrogno S., Filice M, Leo S, Mazza R, Gattuso A, Cerra MC

Deptartment of Biology, Ecology and Earth Sciences (B.E.ST), University of Calabria, Italy

Insufficient oxygen supply (hypoxia) occurs in natural environments and is experienced by mammalian and non-mammalian vertebrates under both healthy and diseased conditions. In mammals, environmental hypoxia, and the resulting inability to meet cellular energy demands, mainly leads to death within few minutes. Contrarily, several non-mammalian species tolerate prolonged period of reduced O_2 availability. Among teleosts, cyprinids of the genus *Carassius*, such as the goldfish *Carassius auratus*, survive hypoxia for days to months thanks to compensative mechanisms, still under investigation. By using an ex vivo preparation, we showed that, under hypoxia, the goldfish heart increases its performance and sensitivity to heterometric regulation, this representing a putative component of hypoxia tolerance. However, the mechanisms that in the goldfish sustain the hypoxia-dependent increase of cardiac contractility have not been yet elucidated. Since in teleosts hypoxia is often associated with an increased sympathetic tone, we aimed to evaluate the role of adrenergic receptors

(ARs) in the goldfish cardiac response to hypoxia. We first characterized the effects of β3-ARs stimulation in normoxic goldfish heart in terms of contractility and signal transduction. Then, we analysed their role in the hemodynamic response of the goldfish heart exposed to acute hypoxia. We found that goldfish cardiac β3-ARs expression is affected by hypoxia. Our data suggest that the activation of β3-ARs contributes to the increased contractility which characterizes the hypoxic goldfish heart.

OP.118

Sensitivity of carbonic anhydrase to metal exposure in the model organisms *Mytilus galloprovincialis*: *in vitro*, *in vivo* and in field approach

Caricato R[1], Salmina S[2], Giordano ME[1], Schettino T[1], Lionetto MG[1]

1Dept. of Biological and Environmental Sciences and Technologies, University of Salento, Italy; 2University of Ghent, Belgium

Carbonic anhydrase (CA) is a ubiquitous metalloenzyme involved in a number of physiological processes. Its sensitivity to chemical pollutants has been recently recognized. The work was aimed to study the CA sensitivity to metal exposure in the digestive gland of the model organism *Mytilus galloprovincialis* under *in vitro*, *in vivo* and in field conditions, with reference to the functional involvement of CA in the lysosomal response to pollutant exposure. The study was carried out by immunofluorescence confocal microscopy, electrometric assay of CA activity, spectrofluorimetric and confocal analysis of the lysosomal system. Under *in vitro* exposure to $CdCl_2$ or $CuCl_2$, CA activity was dose dependently inhibited with an IC50 of 8.7 10^{-5}M for copper and 1.1 10^{-3} M for cadmium. On the other hand, under *in vivo* chronic exposure to $CuCl_2$ (0.3 10^{-6} M) or $CdCl_2$ (0.54 10^{-6} M) for 14 days, CA showed a significant upregulation, paralleled by the increased fluorescence of LysoSensor green charged cells, indicative of lysosome proliferation/increase in size. The metal induced lysosomal activation was prevented by the *in vivo* exposure to the specific CA inhibitor acetazolamide, demonstrating a key role of CA in the pollutant induced lysosomal activation. The response of CA upregulation paralleled by lysosomal activation was validated in field by an active biomonitoring approach in coastal marine sites interested by metal contamination. In conclusion, data showed the complexity and multi-aspect nature of the CA sensitivity to metals, which can be CA inhibitors at higher concentrations and modulator of CA expression at lower concentrations typical of chronic exposure. In this condition CA upregulation can be functional to the prolonged increased requirement of H^+ under lysosomal activation.

OP.119

Adverse effects of sunscreen agents on a marine flatfish: oxidative stress and energetic profiles in response to titanium dioxide nanoparticles and oxybenzone

Pereira B[1], Carvalhais A[1], Sabato M[2], Dolbeth M[3], Marques A[1], Guilherme S[1], Pacheco M[1], Mieiro C[1]

[1]CESAM and Department of Biology, University of Aveiro, Portugal; [2]Università degli Studi di Messina, Italy; [3]CIIMAR, University of Porto, Portugal

The increasing awareness of the negative effects of exposure to solar radiation has contributed to the growing use of sunscreens worldwide. These commercial products have in their composition both organic and inorganic UV filters, which have the ability to endure protection against radiation. The presence of UV filter ingredients has been detected in marine waters with potential toxic consequences for the marine biota. Hence, the main goal of this study was to evaluate the oxidative stress and metabolic response profiles induced by the most commonly used UV filters (an inorganic: titanium dioxide nanoparticles - TiO_2 Np and an organic one: oxybenzone - BP-3), on the marine flatfish *Scophthalmus maximus*. Fish were intraperitoneally injected with 3.0 $\mu g.g^{-1}$ per body weight of each compound and their mixture, and analysed after 72 and 168 h. Liver, kidney and intestine were sampled to assess the antioxidant profiles (CAT, SOD, GPx, GR activities and GSHt content) and membrane damage (LPO). Potential alterations of the energetic processes were also evaluated by the activities of IDH (it can also provide information regarding oxidative stress defences) and LDH activities in the liver. The oxidative stress profile suggested that the individual effect of TiO_2 Np or BP-3 was changed when in mixture in most organs, but without enduring membrane damage. The intestine was the most susceptible organ to the effects of the UV filters while kidney seemed not to be a target for these compounds. The alteration of the metabolic responses was only observed after 168 h, suggesting that the mixture may impair the energetic processes of fish. Thus, the combined use of TiO_2 Np and BP-3 UV filters in sunscreens, and the subsequent co-occurrence in marine systems, might adversely affect fish physiology

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OP.120

Effects of long-term treatment of the green olive leaf extract (OLE) and functional responses in renal cells exposed to low doses of cadmium

Ranieri M[1], Di Mise A[1], Difonzo G[2], Centrone M[1], Venneri M[1], Pellegrino T[1], Russo A[1], Mastrodonato M[3], Caponio F[2], Valenti G[1,4,5], Tamma G[1,4]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, Italy; [2]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Italy; [3]Department of Biology, University of Bari Aldo Moro, Italy; [4]Istituto Nazionale di Biostrutture e Biosistemi (I.N.B.B.), Italy; [5]Center of Excellence in Comparative Genomics (CEGBA), University of Bari, Italy.

Cadmium (Cd) is a heavy and highly toxic metal that contaminates air, food, and water. Cd accumulates in several organs, such as liver and kidney, causing deep functionality deregulations. The kidney is the major organ at risk of damage from chronic exposure to Cd as a contaminant in food and water. This study aims to investigate the beneficial effects of OLE in renal collecting duct MCD4 cells exposed to low dose of Cd (1 μM). In MCD4 cells Cd caused an increase in ROS production, as well as the generation of lipid droplets and reduced cell viability. Moreover, Cd exposure led to a remarkable increase in the frequency of micronuclei and DNA double-strand breaks, assessed using the alkaline comet assay. Furthermore, Cd dramatically altered cell cytoskeleton architecture and caused the S-glutathionylation of actin. Of note, all Cd-induced cellular deregulations were impaired by co-treatment with OLE, possibly due to its antioxidant action and to the presence of bioactive phytocompounds. Indeed, OLE treatment attenuated Cd-induced actin S-glutathionylation, thereby stabilizing actin filaments. Taken together, these observations provide a novel insight into the biological action of OLE in renal cells and support the notion that OLE may serve as a potential adjuvant against Cd-induced nephrotoxicity.

OP.121

Diversity in proton movement and coupling to substrate in vertebrate PepT$_1$ proteins: filling the gaps through the 'phylogenetic' approach

Verri T[1], Bossi E[2], Barca A[1], Del Vecchio G[1,4], Mazzei A[1], Piccinno P[1,§], Vacca F[2], Cinquetti R[2], Murashita K[3,4], Gomes AS[4], Rønnestad I[4]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Italy; [2]Department of Biotechnology and Life Sciences, University of Insubria, Italy; [3]National Research Institute of Aquaculture and Fisheries Research and Education Agency, Japan; [4]Department of Biological Sciences, University of Bergen, Norway; §present affiliation: University of Trento, Italy.

POT transporters drive the concentrative uptake of their substrates by coupling to the transmembrane H^+ electrochemical gradient. POTs recognize highly diverse di/tripeptides. Substrate extent expands with mammalian PepT1/PepT2, which also transport β-lactam antibiotics and peptide-based prodrugs. While substrate recognition changes, protonation sites seem conserved among members. In POTs, transport is achieved through the movement of the gating helices around the central binding site. The extracellular (EC) gate, formed by TM1,2 and TM7,8, serves to control access to the binding site from the EC side of the membrane. The intracellular (IC) gate, formed by TM4,5 and TM10,11, controls the release of peptide and protons on the inside of the cell. Two salt bridges coordinate these helices and control protein conformation. Whereas the IC gate contains a conserved Lys-Glu pair, the EC gate salt bridge is less conserved. In most of the bacterial POTs, the EC gate salt bridge is an Arg-Glu pair, while in mammalian PepT1, a conserved His on TM2 combined with an Arg-Asp salt bridge on TM1 and TM7 is seen. TM2 His is also found in "mammalian-like" bacterial members. In zebrafish PepT1b, the only vertebrate PepT1 known to work at alkaline pH, Lys replaces Arg on TM1. Likewise, PepT1 equally diverging from the "mammalian-like" transporters were retrieved (GenBank) from teleost fish (Cypriniformes, Cyprinodontiformes, Gymnotiformes, Gadiformes), birds (Apodiformes, Trochiliformes, Passeriformes, Piciformes), and even mammals (Chiroptera, Macroscelidea, Insectivora, Primates). Our findings extend the number of PepT1 prone to structure-function analyses and open to understand how their molecular diversity meets the physiology of the species and/or the environment where the species lives.

Workshop

INTEGRATIVE NEUROPHYSIOLOGY

Oral Presentations

OP.122

The vision-for-action network in monkey brain

Gamberini M, Passarelli L, Impieri D, Fattori P, Galletti C

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

The dorsal visual stream, the cortical circuit in the primate brain mainly dedicated to the visual control of actions, has been split into two routes, a lateral and a medial one, both involved in coding different aspects of sensorimotor control. The lateral route, more recently named "lateral grasping network" is mainly involved in the control of grasping under the perceptual and cognitive aspects. The medial route, originally described as involved in the control of arm transport in reaching movements, has been recently reported to be involved in the control of the full sequence of the prehension action, and has been named “reach-to-grasp network”. In macaque monkeys, this network specifically involves several areas of the superior parietal lobule (SPL), areas that show a clear caudo-rostral trend in their visuosomatic properties, and that are directly connected with the dorsal pre-motor cortex. The visual input to the network comes from area V6, a pure retinotopically-organized visual area located in the caudalmost portion of SPL. More dorso-rostrally, the associative areas V6A and PEc combine visual, somatosensory, and motor signals, useful to control limb movements, specifically the arms for V6A and both arms and legs for area PEc. The most anteriorly located area PE is a pure somatosensory region with a rough map of the body, which emphasizes limb representations. Neuronal retrograde tracers injected in areas V6, V6A, PEc, and PE, show in detail the reach-to-grasp network, supporting the functional features of these regions as well as their role in the control of prehension when limbs interact with structured and dynamic environments.

OP.123

Voluntary action modulates visually evoked cortical responses in primary visual cortex: an integrated ultra-high field fMRI and EEG study

Benedetto A[1], Binda P[1], Ho T[1], Costagli M[2], Tosetti M[2], Morrone MC[1,2]

[1]Department of Translational Research on New Technologies in Medicines and Surgery, University of Pisa, Pisa, Italy. [2]IRCCS Stella Maris, Calambrone, Pisa, Italy

To interact effectively with the external world, motor and visual processing need to be tightly synchronized in time. One possible synchronization mechanism may be synchronous phase resetting of endogenous rhythms of motor and visual cortex. In previous studies we have shown that voluntary actions can induce long-lasting theta behavioral oscillations. These behavioral oscillations have been observed for several visual functions, including temporal order judgments, orientation discrimination and contrast sensitivity. To study how behavioral oscillations are related to endogenous neuronal oscillations, we investigated the spatial and temporal characteristics of the visual response around the time of a voluntary action in an ultra-high field (7T) fMRI experiment, and in an event-related EEG experiment (experiment 1 and 2, respectively). Participants (N=13 and 18 for experiment 1 and 2) discriminated the spatial frequency of two very brief gratings, presented randomly in the upper or lower visual field after a free self-initiated button press. The stimulus was displayed randomly with either 70 ms or 150 ms delay from button-press, corresponding to the first minimum and maximum of the oscillation in visual sensitivity. Stimuli presented with a stimulus onset asynchrony of 150 ms evoked a stronger V1 BOLD response than stimuli presented at 70 ms (i.e., the predicted peak\through of the excitability cycle, respectively). Consistently, the occipital VEP to the 150 ms stimulus revealed a higher amplitude respect to the 70 ms stimulus. These results suggest an early visuo-motor interaction, at the level of V1. The rhythmic modulation points to a synchronization between vision and action, shaping vision by alternatively suppressing and enhancing visual processing. This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No 801715 - PUPILTRAITS) and from the ERC-FP7 ECSPLAIN (grant no. 338866).

OP.124

Topographic organization of the “third tier” dorsomedial visual cortex in the macaque

Chadjidimitrakis K[1,2,3], Bakola S [2,3], Chaplin TA[2,3], Yu HH[2,3], Alanazi O[2], Chan JM[2], Worthy KH[2], Rosa MGP[2,3,4]

[1]Department of Biomedical and Visuomotor Sciences, University of Bologna, Piazza di Porta San Donato, Bologna, Italy; [2]Biomedicine Discovery Institute and Department of Physiology, Monash University, Clayton, Australia; [3]Australian Research Council, Centre of Excellence for Integrative Brain Function, Monash University Node, Clayton, Australia; [4] School of Biomedical Sciences, The University of Queensland, Brisbane, Australia

The boundaries of the visual areas located anterior to secondary visual area (V2) in the dorsomedial region of the macaque cortex remain contentious. This region is usually conceptualized as including two functional subdivisions: the dorsal component of area V3 (V3d), laterally, and another area medially, named the parietooccipital area (PO) or V6. However, the nature of the putative border between V3d and PO/V6 has remained undefined. We recorded the receptive fields of multiunit activity in adult male macaques, and

reconstructed the locations of recording sites using histological sections and flat cortical maps. Immediately adjacent to dorsomedial V2 we found a representation of the lower contralateral quadrant, which represented the vertical meridian at its rostral border. This region, corresponding to V3d of previous studies, formed a simple eccentricity gradient, from approximately <5˚ in the annectant gyrus, to >60 in the parietooccipital sulcus. However, there was no topographic reversal as it would be expected to be at the border between V3d and PO/V6. Instead, near the midline, this lower quadrant map continued directly into a representation of the peripheral upper visual field, without an intervening lower quadrant representation that could be unambiguously assigned to PO/V6. Thus, V3d and PO/V6 form a continuous visuotopic map, which includes parts of both quadrants. Together with previous observations that V3d and PO/V6 are both densely myelinated relative to adjacent cortex, and share similar input from V1, these results suggest that they are parts of a single area, which is distinct from the one forming the ventral component of the third tier complex.

OP.125

Microsaccades could indicate the locus of attention during self-motion perception

Piras A, **Trofè A**, **Perazzolo M**, **Raffi M**

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2]Department for Life Quality Studies University of Bologna, Italy

The visual perception of self-motion is mainly due to the optic flow fields. In daily life, during locomotion, the eyes scan the environment, so the gaze is not always directed to the focus of expansion of the optic flow. A particular kind of small eye movements, called microsaccades, are produced during fixations. Such eye movements change the retinal position of the focus of expansion with respect to the fovea. We sought to investigate whether the microsaccadic activity was modulated by eye positions during the view of radial optic flow stimuli. We manipulated the spatial distribution of dot speed and the fixation point position to simulate specific heading directions combined with different gaze positions. Random dot motion and fixation in the dark were used as control stimuli. The experiments were performed on 19 healthy volunteers, eye positions were recorded binocularly using the EyeLink II tracking system (Sr-Research, Canada). The analysis showed a linear relationship between microsaccades amplitude and peak velocity. We found that the microsaccade rates increased in each condition from the first to the last trial, indicating a possible increment in the attentional function. The analysis of the microsaccade directionsshowed that the different combinations of optic flow and eye positions evoked non-uniform directions of microsaccades in all stimulations with mean vectors oriented toward the lowerleft quadrant of the visual field. The control stimuli also evoked non-uniform directions, with the mean vectors in the lower visual field. These results show that, during self-motion perception, attention could be oriented toward the lower visual field; such attentional focus might monitor the floor to avoid obstacles and stabilise posture.

OP.126

Motor adaptation to a virtual perturbation incompatible with muscle synergies across multiple days

Borzelli D[1], **Gurgone S**[2], **de Pasquale P**[1,3], **Berger D**[3], **Acri G**[1], **d'Avella A**[1,3]

[1]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy; [2]Department of Mathematical and Computer Sciences, Physical Sciences, and Earth Sciences, University of Messina, Messina, Italy; [3]Laboratory of Neuromotor Physiology, IRCCS Santa Lucia Foundation, Rome, Italy

Myoelectric control of a virtual end-effector to perform a reaching task, during the generation of multi-directional isometric forces, allows to investigate motor adaptation and its relationship with the muscle synergies underlying force generation at the hand. After a simulated perturbation of muscle pulling directions (virtual surgery) that is constructed to be incompatible with the muscle synergies, i.e. such that only recruiting the synergies a subject cannot reach all the targets, adaptation requires exploration of new motor strategies and possibly learning of new synergies. Previous work demonstrated that subjects could not adapt to an incompatible perturbation in a single experimental session, possibly because of limited practice time. In this study, we investigated the effect of longer practice. Eight subjects tried to adapt to a virtual incompatible surgery while attempting to reach eight targets with a cursor controlled by myoelectric signals recorded from 15 arm muscles during three sessions performed in consecutive days. Only four subjects were able to adapt and to increase the number of successful trials across sessions. Moreover, only for two of these four subjects performance improvement was associated to a decrease of the error in the initial movement direction, suggesting that they had learned to recruit new synergies in addition to more effective online corrections. These results indicate that there are remarkable inter-individual differences in the process underlying the adaptation to incompatible virtual surgeries and in its dependence on improvements of

feedback or feedforward control. Further analyses will address the possible sources of these differences.

OP.127

Effects of the visual context on ocular and interceptive responses to partially occluded ballistic trajectories with different laws of motion

Delle Monache S[1,2,3], Lacquaniti F [1,2,3], Bosco G [1,2,3]

1Department of Systems Medicine, Neuroscience Section, and 2Center of Space Biomedicine, University of Rome Tor Vergata, Rome, Italy; 3Laboratory of Neuromotor Physiology, Santa Lucia Foundation, Rome, Italy.

Accurate tracking of a moving target is essential to acquire detailed visual information for perception as well as for complex motor interactions like manual interceptions. In addition, visual context cues may help interpreting the causal nature of the target motion, especially when visual motion information is noisy or missing. To gain further insight, we studied the influence of visual context cues on eye tracking movements and manual interceptions of computer-generated ballistic trajectories that accounted for Earth's gravity effects in the ascending portion, while being either perturbed or not with altered gravity effects (0g | 2g) in the remaining part. Shortly after the perturbation, targets were occluded for either 450 or 650 ms and then were visible again until landing. Trajectories were shown either in a realistic pictorial scenario or in a dark grey background, with a counterbalanced design across subjects. In Experiment 1, subjects were asked only to track the target throughout its trajectory. In Experiment 2 they tracked the target and provided manual reaction time responses to random color changes of the target. In Experiment 3 subjects tracked thetarget and intercept it at its landing location by means of a mouse cursor. We analysed saccadic and smooth pursuit parameters extracted from the eye movement recordings and the interceptive timing and location derived from mouse cursor recordings in Experiment 3. Ocular tracking parameters indicated higher anticipation of the target trajectory in the realistic scenario, and the same tendency was observed also for interceptive responses in Experiment 3. The present results suggest the existence of common predictive mechanisms for the control of eye tracking movements and manual interceptions.

OP.128

Evidence for object-mirroring mechanism specificity in monkey's mirror neuron network

Lanzilotto M[1], Livi A[2], Maranesi M[1], Ferroni CG[1], Bonini L[1]

[1] Department of Medicine and Surgery, University of Parma, Parma, Italy; [2] Department of Neuroscience, Washington University in St. Louis, St. Louis, MO, USA.

Several human and monkey brain regions are recruited for planning action as well as for predicting those of others, and the discovery of mirror neurons constituted a major advance in this field. Nevertheless, how our brain uses contextual-object information for predicting others' actions has been unclear for a long time. A recent study from our group sheds light on this issue by demonstrating the presence of a novel mechanism in the monkey pre-supplementary motor area (F6), through which observers could predict other's impending action by recruiting the same motor representation they would activate if they were to act upon the same object in the same context (object-mirroring mechanism). Here we looked for evidence of this mechanism in another node of the mirror neuron network, the anterior intraparietal area (AIP). To this purpose, we employed the same paradigm used for studying area F6, recording single-neuron activity from area AIP of two monkeys while they performed a visuomotor Go/No-Go reaching-grasping task and while they observed the same task performed by an experimenter in the same context. In agreement with previous studies, we found distinct sets of action-related neurons encoding selectively monkey's and/or other's action. Most interestingly, we found that neurons responding during the object presentation (object-related neurons) were markedly related to the processing of the object visual features rather than to the predictive representation of others' action. Population decoding approaches showed that, in contrast to F6, AIP seems to be mostly involved in the processing of general object affordance rather than object mirroring, suggesting that motor prediction may be a hallmark of motor rather than visual brain areas.

OP.129

Neuronal dynamics of signal selective motor plan cancellation in macaque dorsal premotor cortex

Giarrocco F[1,2], Giamundo M[1], Fabbrini F[1], Brunamonti E[1], Pani P[1], Ferraina S[1]

[1]Department of Physiology and Pharmacology, Sapienza University, Rome, Italy; [2]Behavioural Neuroscience PhD program, Sapienza University, Rome, Italy

The ability to selectively inhibit actions in response to the environment changes is extremely relevant for both cognitive and executive functions and it is a prerequisite for adaptive behavior. Despite its

relevance, no studies have investigated the neuronal dynamics underpinning this behavior. For this purpose, we recorded multi-unit activity from Utah array in the dorsal premotor cortex (PMd) of two rhesus monkeys trained in a modified version of the Stop Task. In this version, most of trials required to perform a movement after a go signal presentation. In a minority of trials, one of two signals could follow the go signal: the Stop or the Ignore signals, with only the Stop signal requiring to inhibit the movement. We found that monkeys adopted two behavioural strategies: they could opt for a discrimination of the signal followed by the inhibition only after the Stop signal, or they could choose to inhibit whatever the signal that appears, then discriminate it and generate the movement once the Ignore signal has been identified. Through a state-space approach, we found that movements are generated if the neuronal dynamics showed trajectories towards a sub-space that allows their execution. Along this trajectories, when movement inhibition occurred the neuronal evolution is reversed towards the initial sub-space, which does not allow movement generation. Following the Ignore signal, a momentarly inversion of the evolution is observed only when a specific behavioural strategy is adopted, suggesting a temporary activation of the inhibitory process. Our data show that PMd neuronal dynamics signal movement inhibition, whenever the behavioral strategy require it, even only temporarily. Thus, PMd is confirmed as a key structure for movement inhibition.

Workshop

CELLULAR PHYSIOLOGY AND NEUROPHYSIOLOGY

Oral presentations

OP.130

Alphavbeta3 integrin/Rac1 pathway as a possible target in retinitis pigmentosa

Dal Monte M[1], Cammalleri M[1], Locri F[1], Pecci V[1], De Rosa M[2], Pavone V[3], Bagnoli P[1]

[1]Department of Biology, University of Pisa, Italy; [2]Department of Experimental Medicine, Second University of Napoli, Italy; [3]Department Department of Chemical Sciences, University of Napoli Federico II, Napoli, Italy

Retinitis pigmentosa (RP) is a disease characterized by progressive loss of vision due to photoreceptor degeneration leading to secondary inflammation. In the rd10 mouse model of RP, we recently demonstrated that UPARANT, a peptide inhibiting the interactions between urokinase-type plasminogen activator (uPA) receptor (uPAR) and its membrane partners, including αvβ3 integrin, reduced upregulated levels of inflammation. When analyzing molecular targets underlying ameliorative effects of UPARANT, we found that uPA and uPAR were only minimally expressed in the rd10 retina because of the low HIF-1 levels in the hyperoxic environment characterizing the RP model. We demonstrated that uPA/uPAR expression was regulated by HIF-1 as its retinal levels were restored by stabilization of HIF-1α, the oxygen-sensitive subunit of HIF-1. Among the lateral partners of uPAR, αvβ3 integrin and its downstream effector Rac1 were upregulated in the rd10 retina indicating their involvement in RP. UPARANT would act downstream uPAR by inhibiting the overactivated αvβ3 integrin/Rac1 pathway and the downstream inflammatory cascade coupled to RP-associated damages. At the functional level, UPARANT improved photopic phototransduction in rd10 mice as determined by the photopic bwave analysis and cone immunohistochemistry in retinal whole mounts. In contrast, kinetic analysis of the a-wave and rod markers determination seem to exclude ameliorative effects on scotopic phototransduction. Overall the present results indicate that in the retina the expression of uPA/uPAR genes is regulated by HIF-1 and that the αvβ3 integrin/Rac1 pathway is a promising target for the development of novel therapeutic approaches to treat RP-associated damages. Funded by Kaleyde Pharmaceuticals AG

OP.131

Photoswitchable lipid membrane-spanning molecules for light-dependent modulation of neuronal activity

DiFrancesco ML[1,2§], Lodola F[3§], Colombo E[1,2§], Maragliano L[1,2], Paternò GM[3], Bramini M[1,2], Cimò S[3,4], Colella L[4], Fazzi D[5], Eleftheriou CG[1,2], Maya-Vetencourt JF[1,2], Bertarelli C[3,4§], Lanzani G[3§], Benfenati F[1,2§].

[§]equal contribution [1]Center for Synaptic Neuroscience, Istituto Italiano di Tecnologia, Genova, Italy; [2]IRCCS Ospedale Policlinico San Martino, Genova, Italy; [3]Center for Nano Science and Technology, Istituto Italiano di Tecnologia, Milano, Italy; [4]Dipartimento di Chimica, Materiali e Ingegneria Chimica "Giulio Natta", Politecnico di Milano, Milano, Italy; [5]Department of Chemistry, Institut für Physikalische Chemie, University of Cologne, Köln, Germany

The possibility to modulate neuronal activity in a light-dependent manner is becoming increasingly important in neuroscience and biomedicine, and the interest in more potent, precise and efficient optical technologies is growing. We present in this study photoswitchable molecules able to insert in lipid membranes, where they induce a thinning of the bilayer caused by trans-dimerization. Light-stimulation in the Cyan region of the

spectrum promotes the rearrangement of their molecular structure, resulting in the loss of trans-dimerization and a consequent bilayer relaxation, as suggested by Molecular Dynamics Simulations. We performed Confocal Microscopy, Patch-Clamp and MultiElectrode Array analysis in order to describe the physiological outcome resulting from lightstimulation of HEK-293 cells and primary hippocampal neurons incubated with photochromic molecules. We found that about the 60% of neuronal plasma membrane was covered by photoswitchable molecules, colocalizing preferentially with lipid drafts. Under light-stimulation, we measured a transient drop in membrane capacitance, corresponding to membrane hyperpolarization at the light onset, followed by a late depolarization peaking after the light offset. In hippocampal neurons, such a membrane hyperpolarizationdepolarization stably and reproducibly triggered action potentials at several light-stimulus frequencies. Firing modulation was described i) under physiological conditions, ii) with synaptically isolated neurons, and iii) 7 days after molecule incubation. A modulation of neuronal activity was measured also in vivo from the somatosensory cortex of injected mice. In the future, we aim to engineer Graphene flakes with photoswitchable molecules for a cellspecific targeting. Funded by the European Graphene Flagship.

OP.132

Moderate concentration of ketone body β-hydroxybutirate inhibits endocytosis and exocytosis in rat brain synaptosomes.

Fedorovich S[1, 2], **Hrynevich S**[1]

[1]Institute of Biophysics and Cell Engineering NASB, Minsk, Belarus; [2]Department of Biochemistry, Belarusian State University, Minsk, Belarus

The ketogenic diet is used as a prophylactic treatment for different types of brain diseases, such as epilepsy or Alzheimer's disease. In such a diet, carbohydrates are replaced by fats in everyday food, resulting in an elevation of blood-borne ketone bodies levels. Despite clinical applications of this treatment, the molecular mechanisms by which the ketogenic diet exerts its beneficial effects are still uncertain. In this study, we investigated the effect of replacing glucose by the ketone body DLβ-hydroxybutyrate in therapeutic concentration 8 mM as the main energy substrate on synaptic vesicle recycling in rat brain synaptosomes. First, we observed that exposing presynaptic terminals to nonglycolytic energy substrates instead of glucose did not alter the plasma membrane potential. Next, we found that synaptosomes were able to maintain the synaptic vesicle cycle monitored with the fluorescent dye acridine orange when glucose was replaced by β-hydroxybutyrate. However, in presence of β-hydroxybutyrate, synaptic vesicle recycling was modified with reduced endocytosis. Replacing glucose by pyruvate also led to a reduced endocytosis. Addition of β-hydroxybutyrate to glucose-containing incubation medium was without effect. Reduced endocytosis in presence of βhydroxybutyrate as sole energy substrate was confirmed using the fluorescent dye FM2-10. In conclusion, the nonglycolytic energy substrates β-hydroxybutyrate and pyruvate are able to support synaptic vesicle recycling. However, they both reduce endocytosis. Reduction of both endocytosis and exocytosis together with misbalance between endocytosis and exocytosis could be involved in the anticonvulsant activity of the ketogenic diet.

OP.133

Bi-directional Cross-talk between cells and microenvironment

Fiocchetti M, **Fernandez VS**, **Montalesi E**, **Marino M**

Department of Science, Roma Tre University, Viale Guglielmo Marconi 446, I-00146 Rome, Italy

In physiological condition, cells react to environmental changes to maintain the homeostatic balance of whole organism. Cells must adapt continuously to fluctuations in extracellular microenvironment and, in turn, they remodel and sculpt the external surrounding. Cell "secretome", which refers to a collection of proteins (growth factors, hormones, cytokines and molecular mediators) released by cells, reflect intracellular response to external stimuli. The secretome, in turn, influences cell functions including growth, differentiation, migration and survival. In this context, the present work aims to define the functional cellular response and related molecular mechanisms in cells in response to cultured medium pre-conditioned by hormonal treatment and stress conditions. Cancer cells, the prototype of cross talk between cells and their environment have been used as experimental model. In particular, breast cancer cells MCF-7 were cultured in conditioned medium generated by cells treated for 48h with vehicle, 17β-Estradiol (E2,10 nM) or a sub-toxic dose of hydrogen peroxide (H2O2, 200 μM). The influence of conditioned medium on cell proliferation, migration/invasion and survival/apoptosis balance was analyzed. Obtained results reinforce the idea that external stimuli compel cells to modify their functions and functional responses as well as their microenvironment extending the response to the surrounding area and indirectly affecting/matching the function of neighboring cancer cells that will cope stresses sustaining cancer progression. Such results change the dogma stimuli/response pathway highlighting the existence of a bidirectional cross talk between cells and their microenvironment that could

generate a loop of stimuli/response/stimuli to maintain the homeostasis.

OP.134

Copper Dyshomeostasis In Neurodegenerative Diseases

Maffia M[1,2], Greco M[1], Rizzo F[1], Garzarelli V[1], Intini V[1], Maffia MC[3], Danieli A[1], Vergara D[1,2], De Riccardis L[1,2]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [2]Laboratory of Clinical Proteomic, ASL-Lecce, Italy; 3San Raffaele-Telethon Institute for Gene Therapy, Vita Salute San Raffaele University, Milan, Italy

Copper (Cu) homeostasis is required for a number of processes included brain development. Cu dyshomeostasis and oxidative stress play a pivotal role in several neuropathologies such as Parkinson disease (PD). In such diseases, metal accumulation in the central nervous system could result in loss-of-function of proteins involved in copper metabolism and in a copper redox cycling that generates reactive oxygen species. Moreover, neurodegenerative disorders imply the presence of an excess of misfolded proteins known to lead to neuronal damage: in PD, copper accumulates in the brain, binding alpha-synuclein and initiating its aggregation. In this work, we assessed the correlation between neuronal differentiation and copper homeostasis regulation, in both physiological and pathological conditions. At this purpose, we used SHSY5Y neuroblastoma cell line, treated with retinoic acid and brain derived growth factor (BDNF) in order to induce neuronal differentiation, and rotenone, able to cause neuron degeneration. Upon Cu treatment, we analyzed transcriptional and metabolic levels of proteins directly or indirectly involved within copper homeostasis such as Cu transporters and chaperones, together with α-synuclein and the prion protein (PrP). Aberrant conformations of these soluble proteins facilitate their precipitation and the tendency to form insoluble and toxic deposits: identifying Cu dependent alterations in the pathways responsible for the protein-misfolding may potentially offer new opportunities for clinical intervention.

OP.135

The Effect of Growth Hormone and/or Swimming Exercise on PI3K/AKT/mTOR Signaling Pathway and Bone Mineral Density in Rats Skeletal Muscle

Palabiyik O[1], Tayfur P[2], Tastekin E[3]

[1]Department of Medical Services and Techniques, Trakya University Health Services Vocational College, Turkey; [2]Department of Physiology, Trakya University, Turkey; 3Department of Pathology, Trakya University, Turkey

Athletes misuse recombinant human growth hormone (r-hGH) to enhance their performance. The aim of this study was to investigate possible effects of r-hGH use and/or exercise on phosphatidylinositol 3-kinase(PI3K)/protein kinase B(Akt)/Mammalian target of rapamycin(mTOR) signaling pathway and bone mineral density in rats skeletal muscle. 36 Sprague-Dawley male rats were divided into control (C,n=9), swimming exercise (E,n=8), r-hGH (GH,n=10) and swimming exercise+r-hGH (E+GH,n=9) groups. Exercise groups completed a 1-h swimming exercise 5 times a week for 8 weeks. Subcutaneous r-hGH was administered as 0.3 mg/kg/day. Protein expression of PI3K, AKT1, mTOR were assessed by immunohistochemistry. Total body bone mineral content(BMC), bone mineral density(BMD), lean mass(LM) and fat(%fat) were performed using Dual-energy X-ray absorptiometry. One-way ANOVA and Tukey post-hoc test were used for statistical comparisons. PI3K, AKT1 and mTOR protein expression were higher in the GH, E and E+GH groups compared with in C group ($p<0.05$, <0.05 and <0.005, respectively). Average values of BMC, GH and GH+exercise groups were found to be lower than those of only exercise groups (p:0.001). Growth hormone administered coupled with swimming exercise appeared to affect the PI3K/AKT/mTOR signaling pathway. On the contrary to the common opinion, GH didn't increase lean mass but caused only a partial decrease in adipose tissue.

OP.136

Mechanotransductive signaling pathway in human islets of Langerhans: implications for β-cell survival and function.

Galli A[1], Marku A[1], Castagna M[1], Maffioli E[2], Tedeschi G[2], Milani P[3], Lenardi C[3], Perego C[1]

[1]DISFEB Dept of Excellence Pharmacological and Biomolecular Sciences, [2]Dipartimento di Medicina Veterinaria. [3]CIMAINA and Dipartimento di Fisica, University of Milano, Italy

Pancreatic β-cells are constantly exposed to mechanical stimuli arising from the surrounding extracellular matrix (ECM), neighboring cells and blood flow, but whether and how these stimuli contribute to β-cell differentiation and function is largely unknown. By exploiting cluster-assembled zirconia substrates with tailored roughness to mimic the nanotopography and stiffness of the ECM, we investigate the effect of mechanical forces on human islet of Langerhans

survival and function. Human β-cells viability and function are improved on nanostructured substrates: β-cells contain several dispersed insulin granules and show increased glucose-sensitive calcium currents and insulin secretion. Quantitative immunofluorescence analysis reveals reorganization of the cell-substrate adhesion complexes, the actin cytoskeleton and the nuclear architecture. Proteomic analysis demonstrate protein changes that are congruent with the functional and morphological results and shows that β-cells respond to mechanical forces through the activation of a certain number of mechanosensors, including mechanosensitive ion channels and integrins (Gene Ontology GO terms: 0005925). Their activation causes remodeling of the actomyosin cytoskeleton (GO: 0005856) and nuclear architecture (GO: 0031891) and is conveyed to the nucleus where it modulates gene expression. The characterization of the mechanotransduction signaling pathway may offer a unique possibility to understand how beta cells work and can lead to the identification of new targets of pharmacological intervention in diabetes mellitus.

OP.137

Hyper-excitability and hyper-plasticity disrupt cerebellar signal transfer in the IB2 KO mouse model of autism

Prestori F

Department of Brain and Behavioral Sciences, University of Pavia, Italy

Autism spectrum disorders (ASD) are pervasive neurodevelopmental conditions that often involve mutations affecting synaptic mechanisms. Recently, the involvement of cerebellum in ASD has been suggested but the underlying functional alterations remained obscure. Thus, we exploited a combination of whole-cell patch-clamp recordings with voltage sensitive dye imaging (VSDi) in acute cerebellar slices in WT and IB2 KO mice to investigate single-neuron and microcircuit properties. The IB2 gene (chr22q13.3 terminal region) deletion occurs in virtually all cases of Phelan–McDermid syndrome, causing autistic symptoms and a severe delay in motor skill acquisition. The granular layer of these mice revealed severe alterations in synaptic transmission, neuronal excitation and long-term synaptic plasticity. A 2.5-times larger NMDA receptor-mediated current in IB2 KO granule cells enhanced synaptic plasticity (WT = 20.4±4.2%, n=12 vs. IB2 KO = 107.7±44.4%, n=9; p<0.05) along with the excitatory/inhibitory (E/I) balance (WT = 0.98±0.27, n=6 vs. IB2 KO = 2.78±0.32, n=7; p<0.01). At the same time, the spatial organization of granular layer responses to mossy fiber inputs shifted from a "Mexican hat" to a "stovepipe hat" profile, with stronger excitation in the core (WT = 12.9±1.7 μm vs. IB2 KO = 29.5±4.9 μm, n=5 for both; p<0.01) and limited inhibition in the surround (WT/KO ratio IWT/KO = 2.83±0.17, n=5). The IB2 KO mouse model therefore configures a complex cerebellar synaptopathy centered on NMDA receptor gain of function, that in several respects resembles alterations also observed in cortical minicolumns. The profound changes of signal processing at the cerebellar input stage unveil a possible new mechanism contributing to the pathogenesis

Workshop

EXERCISE AND CARDIOVASCULAR PHYSIOLOGY

Oral presentations

OP.138

Cardiovascular kinetics during moderate3intensity arm and leg exercise: a preliminary report.

Bruseghini P[1], Vinetti G[1], Taboni A[2], Fagoni N[1], Ferretti G[1,2]

[1]Department of Molecular and Translational Medicine, University of Brescia [2]Departments APSI and NEUFO, University of Geneva

The phase I cardiovascular response to exercise implies an instantaneous cardiac output ($\dot{Q}$) increase, due to the effect of sudden vagal withdrawal on heart rate (fH) and of sudden venous return increase, due to muscle pump action, on stroke volume (SV). If the latter is the case, we would expect that, when exercise is performed with small active muscle mass, the cardiovascular responses at exercise are depressed. On 8 healthy young subjects, we measured beat3by3beat fH, SV and $\dot{Q}$ during arm ergometer and cycle ergometer exercise transitions, from rest to 50W. A double exponential model was applied to the transient phase, and we computed amplitudes and time constants of phase I (A1 and T1). For arm cranking, steady state fH was 65.2±7, and 102.3±7.8 bpm, at rest and 50 W exercise, respectively V corresponding SV was 106.1±16.5, and 112.9±13.4 mL, so that $\dot{Q}$ was 6.6±0.8, and 11.8±1,4 L/min. For leg cycling, fH was 68.4±7.8, and 92.7±6 bpm, SV was 101.8±14.4, and 117.1±16 mL, and $\dot{Q}$ was 6.9±0.6, and 10.8±1.2 L/min, at rest and exercise, respectively. For fH, A1 and T1, for arm exercise (18.4±8.1 bpm and 7.5±5 s, respectively) were greater (p<.05) than the corresponding values for leg exercise (9.1±2.2 bpm and 3.2±2 s, respectively). No significant differences appeared in A1 and T1 for SV and $\dot{Q}$ between the two

exercise types. Exercises with different muscle masses acted on the kinetics of fH, but not on that of SV, and thus essentially on the vagal withdrawal mechanism.

OP.139

Effects of hormone replacement therapy in combination with swimming exercise and/or melatonin on oxidative tissue damage in postmenopausal rats

Tamer SA[1], Altınoluk T[1], Emran M[2], Korkmaz S[2], Yüksel RG[2], Baykal Z[2], Dur ZS[2], Levent HN[3], Ural MA[4], Yüksel M[5], Çevik O[4], Ercan F[3], Yıldırım A[1], Yeğen BÇ[1]

[1]Marmara University, Department of Physiology, Istanbul, Turkey; [2]Marmara University, Istanbul, Turkey; [3]Marmara University, Department of Histology & Embryology, Istanbul, Turkey; [4]Adnan Menderes University, Department of Biochemistry, Aydin, Turkey; [5]Marmara University, Department of Medical Laboratory, Istanbul, Turkey

Hormone replacement therapy (HRT) or exercise (E) ameliorates postmenopausal symptoms and protects against oxidative tissue damage. In order to compare putative ameliorative effects of HRT, E or their combination on oxidative damage and to further evaluate the impact of adding melatonin (M) to HRT, E or both, half of Sprague Dawley rats with bilateral ovariectomy had swimming exercise (30 min/5 days/week; E, E+HRT, E+M, E+HRT+M; n=32), while other half was sedentary (S, S+HRT, S+M, S+HRT+M) for 8 weeks during which HRT (estradiol; 1 mg/kg/day) or M (4 mg/kg/day) was given in drinking water. Memory performance was not different among groups. Weight gain was lower in all HRT groups. Rats were decapitated at postsurgical 70th day, and heart, aorta, brain, liver and kidney tissues were obtained for biochemical and histological analyses. Compared to nontreated sedentary group, cerebral malondialdehyde levels were elevated, while antioxidant glutathione levels of cardiac and hepatic tissues were decreased in both HRT groups (S+HRT and E+HRT). On the other hand, addition of M treatment to S+HRT or E+HRT groups reversed these oxidative injury parameters back to levels of non-treated sedentary group. Addition of E to HRT or E+HRT+M combination decreased myeloperoxidase activity in brain, liver and kidney. Histological analysis revealed diminished aortic wall thickness, endothelial detachment and an irregular organization of cardiomyocyte fibers in sedentary groups, while addition of exercise or M to HRT resulted in normal aortic wall and cardiomyocyte organization. HRT either alone or combined with exercise impaired the oxidant/antioxidant balance, but addition of melatonin provided a protection against HRT induced oxidative stress, advocating postmenopausal use of melatonin.

OP.140

Non-invasive assessment of the vascular baroreflex arm

Javorka M[1], Krohova J[1], Czippelova B[1], Turianikova Z[1], Mazgutova N[1], Wiszt R[1], Lazarova Z[1], Faes L[2,3]

[1]Department of Physiology and Biomedical Centre Martin (BioMed Martin), Jessenius, Comenius University, Martin, Slovakia; [2]Department of Energy, Information engineering and Mathematical models (DEIM), University of Palermo, Italy; [3]BIOtech Center of the Department of Industrial Engineering, University of Trento, Italy

Baroreflex response is composed of cardiac chronotropic (effect on heart rate), cardiac inotropic (effect on contractility) and vascular (effect on vascular resistance) arms. Because of its measurement simplicity, cardiac chronotropic arm is the most often analysed baroreflex component. The aim of study was to introduce a method to analyse vascular baroreflex arm. Healthy volunteers (N = 78, median age: 18.6 yrs.) participated in this study. We recorded continuous systolic and mean blood pressure (SBP and MBP) by volume-clamp method (Finometer Pro, FMS), and R-R interval (RR) by ECG (CardioFax ECG-9620, NihonKohden). Cardiac output (CO) was recorded using impedance cardiography (CardioScreen® 2000, Medis). Then, we calculated the peripheral vascular resistance (PVR) as a ratio of MBP and CO. The directional spectral coupling and gain of cardiac chronotropic (SBP to RR) and vascular arms (SBP to PVR) were calculated in low frequency band (LF, 0.04 – 0.15 Hz). We analysed baroreflex vascular component characteristics during various physiological conditions (supine, head-up tilt (HUT), supine recovery, mental arithmetics (MA)). The coupling from SBP to PVR was significantly higher than the coupling from SBP to RR during whole protocol ($P < 0.0001$). The coupling in both assessed directions was significantly higher during HUT compared to supine rest ($P < 0.0001$ and $P = 0.0138$), but no differences were found during MA in comparison with the preceding supine recovery. No significant changes in the spectral gain across all phases were found ($0.1494 \leq P \leq 0.9053$). We conclude that changes in PVR are tightly coupled with the SBP oscillations via baroreflex with a stable gain. Analysis of the vascular baroreflex arm could reveal another aspect of blood pressure dysregulation. Grants: VEGA 1/0117/17 and VEGA 1/0200/19

OP.141

Heart Rate Kinetics and Sympathovagal Balance Accompanying a Maximal Sprint Test

Storniolo J, Esposti R, Cavallari P

Human Physiology Section of the De.P.T., Università degli Studi di Milano, Italy

During a sprint test, Heart Rate (HR) quickly increases, keeps high for some seconds and returns to basal value with a roughly exponential decay. While the decay and its time constant (off) have been widely studied, much less attention has been devoted to the time delay (tdelay) between the sprint end and the HR decay onset. Given the proven correlation between the sympathovagal balance and the exercise performance, as well as the frequent heart failures in cardiopath patients during the post-exercise phase, we evaluated the sympathovagal balance before and after the sprint, trying to correlate it with both tdelay and off. R-R intervals, recorded in 24 healthy adults for 5 min before, during and for 5 min after a 60-m sprint test (from Storniolo JL, Pavei G, Minetti AE. 2017 Front Physiol 8:868, with kind permission of all Authors), were re-processed by autoregressive method, so as to extract the HR variability power (LF and HF) in the low and high frequency ranges, respectively. The sympathovagal balance, estimated as the ratio LF/HF before (bef) and after (aft) exercise, was correlated with tdelay and off. Both (LF/HF)bef and (LF/HF)aft had a skewed distribution. Rank correlation ($p<0.05$) was found for (LF/HF)bef vs. off ($r=0.42$) and for (LF/HF)aft vs. both off ($r=0.41$) and tdelay ($r=0.43$). The difference (LF/HF)bef-aft had a normal distribution and a strong partial correlation with tdelay ($r=0.61$) but not with off. Thus, a long tdelay well fits with a high sympathetic activity after exercise, while an already high sympathetic activity before sprint leads to a slow recovery (high off), which should accompany a poor performance. These results confirm that the autonomic modulation plays an important role in both parameters depicting HR kinetics after a sprint test.

OP.142

Light Sensitive conjugated polymers optically tune the fate of Endothelial Progenitor Cells

Lodola F[1], Tullii G[1], Desii A[1], Tapella L[2], Catarsi P[3], Rosti V[3], Lim D[2], Moccia F[4], Antognazza MR[1]

[1]Center for Nano Science and Technology, IIT@PoliMi, Milano, Italy; [2]Department of Pharmaceutical Sciences, University of Eastern Piedmont "Amedeo Avogadro", Novara, Italy; [3]Center for the Study of Myelofibrosis, Research, Foundation IRCCS Policlinico San Matteo, Pavia, Italy; [4]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Italy.

Therapeutic angiogenesis of injured myocardium after an ischemic insult is hampered by the limited control of stem and progenitor cells which does not allow to fully prime the re-activation of the injured myocardium and vasculature. Here we propose a novel strategy to gain in vitro optical control of Endothelial Colony Forming Cells fate, which represent the only known truly endothelial precursor showing robust in vitro proliferation and overwhelming vessel formation in vivo. Our strategy is based on the combination of light sensitive conjugated polymers, used as photo-actuators, with the advantages offered by optical stimulation over current approaches, mainly based on electromechanical and chemical stimulation. Light modulation provides unprecedented spatial and temporal resolution, permitting at the same time lower invasiveness and higher selectivity. We demonstrate that polymer-mediated optical excitation induces a robust enhancement of proliferation and lumen formation in vitro. We identify the underlying biophysical pathway as due to light-induced activation of TRPV1 channel. Altogether our results appear as a novel effective application of semiconducting polymer-based optical modulation to induce angiogenesis in vitro, which represents the proof-of-principle to improve the outcome of autologous cell-based therapy in vivo.

OP.143

The Acute Effect of High-Intensity Interval Training on Energetic Substrate Oxidation Rate During Exercise and 1-Hour Post-Exercise Period in Young, Normal-Weight Women

Tiltina K, Ozolina-Moll L

Department of Human and Animal Physiology, University of Latvia, Latvia

Over the last decade, the high-intensity interval training (HIIT), which has gained great popularity, is used both to improve aerobic performance and body composition, including the reductions of fat mass. Despite the great popularity of HIIT, the acute physiological effects of HIIT and their mechanisms have not been studied. The aim of the study was to find out the acute effect of HIIT on the lipolysis and glycolysis during HIIT and 1-hour post-exercise period.The study involved 11 young (24□5 years old), healthy normal-weight women. The persons performed HIIT on a cycle ergometer. HIIT consisted from 7 cycles: 2 min high intensity exercise (80% of the maximum workout) and 1 min moderate load (45% of the maximum workout). VO2 and VCO2 were monitored using indirect calorimetry to determine metabolic rate and energetic substrate oxidation rate during rest conditions, exercise and for 1-hour post-exercise period. Changes in plasma triglycerides (TG) and glucose concentration were also controlled during the same conditions. Our results show that plasma glucose level does not significantly change during HIIT and post-exercise period, while plasma TG level increases by almost 50% during HIIT, but rapidly decreases during post-exercise period. The level of TG remains decreased for at least one hour after HIIT. This

indicates an increased use of TG in tissues during the post exercise period. The indirect calorimetry measurements also confirm these results. The respiratory quotient has a rapid and sustained decrease in the post-exercise period, which indicates the increased use of lipids during post-exercise period. The obtained results suggest that HIIT stimulates effective and sustained increase in lipid oxidation rate during post-exercise period in young women and can be used to efficient weight and fat mass management.

OP.144

Advanced Morpho-Functional Analysis on Ventricular and Atrial Tissue Reveals Cross-Bridge Kinetics Alterations and Sarcomere Energetic Impairment in HCM Patients

Poggesi C

University of Florence, Italy

Mutations in cardiac myosin-binding protein-C (cMyBP-C), are the most common cause of Hypertrophic CardioMyopathy (HCM). The E258K-cMyBP-C is a penetrant missense mutation with poorly understood molecular mechanisms. Mechanics and kinetics of contraction were investigated in left ventricular (LV) and atrial myofibrils from E258K and donor hearts while ATPase and isometric tension were simultaneously measured in permeabilized LV and atrial strips from the same hearts. The rate of tension generation following maximal Ca2+-activation was faster in both LV and atrial E258K myofibrils compared to donors. The rate of isometric relaxation was also faster in E258K myofibrils, suggesting faster cross-bridge detachment and increased energy cost of tension generation. Direct measurements in skinned LV and atrial strips confirmed that tension cost was higher in E258K vs controls. To check whether cardiomyocyte disarray, typical of HCM hearts, may have contributed to artificially increase the tension cost of the HCM preparations, the strips were clarified, immunostained and imaged at mesoscale level. An advanced tissue clearing method in combination with two-photon microscopy was employed to reconstruct the 3D image of the strips at sub-micrometer spatial resolution. A 3D cytoarchitecture analysis tool based on 3D Fourier Transform was used to determine cardiomyocyte orientation across and along the strips. Both global and local statistics of spatial disarray were derived and correlated to mechanical and energetic data. The results did not highlight structural differences between donor and HCM strips strengthening the conclusion that the E258K mutation primarily alters apparent cross-bridge kinetics and impairs sarcomere energetics. SILICOFCM grant agreement 777204 is acknowledged.

OP.145

The effect of gender differences on cardiac ischemic preconditioning in chronic kidney disease

Sárközy M[1*], Márványkövi F[1*], Szűcs G[1], Gáspár R[1], Dajka D[1], Siska A[2], Földesi I[2], Csont T[1] (*these authors are equally contributed)

[1]MEDICS Group, Department of Biochemistry, University of Szeged, Szeged, Hungary; [2]Department of Laboratory Medicine, University of Szeged, Szeged, Hungary

Cardiovascular complications including heart failure and acute myocardial infarction are the leading causes of death in CKD. About 60% of patients are women in the early stages of chronic kidney disease (CKD). We have previously shown that the infarct size (IS)-limiting effect of ischemic preconditioning (IPRE) is preserved in CKD in male rats. We aimed to investigate if gender difference could influence the ISlimiting effect of IPRE in CKD. CKD was induced by 5/6 nephrectomy in 9 weeks old male and female Wistar rats. Nine weeks later, serum urea and creatinine levels were measured to verify the development of CKD. Transthoracic echocardiography was performed to monitor the cardiac morphology and function. At week 9, hearts of both the 5/6-nephrectomized and sham-operated rats were isolated and subjected to 45 min aerobic perfusion, and 35 min global ischemia followed by 120 min reperfusion with or without preceding preconditioning induced by 3 intermittent cycles of 5 min ischemia and 5 min reperfusion. Both in males and females, serum urea and creatinine levels were significantly elevated, left ventricular hypertrophy developed with diastolic dysfunction and preserved systolic function in CKD. In male rats, the IPRE significantly decreased the IS both in the sham-operated and CKD groups. In female rats, the IPRE decreased the IS in the sham-operated group tendentiously (33,9±2,5 vs. 40,4±3,6%, p=0,15) and in the CKD group significantly (28,2±2,3 vs. 34,7±2,3%, p<0,05). Uremic cardiomyopathy was developed, and the IS-limiting effect of IPRE was preserved in both genders in CKD. However, the protective effect of IPRE seems to be lost in sham-operated female rats suggesting that IPRE and female sex did not confer additive cardioprotection on each other.

Poster Session I (1/2)

Neurophysiology - Integrative Neurophysiology

PP.1

Kir4.1 gain-of-function and gut dysbiosis appear as risk factors for autism-epilepsy phenotype in a new mouse model of autism

Coretti L[1], Ambrosini E[2], Cenciarini M[1], Sforna L[1], Belia S[3], Harold-Barry E[4], Hasan S[5], Lanciotti A[2], Brignone MS[2], Sicca F[6], Santorelli F[6], Chiarotti L[7], Lembo F[8], Pessia M[1,4], D'Adamo MC[4]

[1]Department of Experimental Medicine, University of Perugia, Italy; [2]Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy; [3]Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy; [4]Department of Physiology and Biochemistry, University of Malta, Msida, Malta; [5]Faculty of Medicine, Kuwait University, Kuwait; [6]Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Pisa, Italy; [7]Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II; Naples, Italy; [8]Department of Pharmacy, University of Naples Federico II; Naples, Italy

Despite numerous investigations, the pathophysiology of autism spectrum disorder (ASD) remains unresolved. Amongst the genes associated with ASD, KCNJ10 that codes inwardly-rectifying K+ channels has recently been attributed to the disorder. In a cohort of autistic patients with intellectual disability and epilepsy our collaborative network uncovered, for the time first time, gain-of-function mutations in the KCNJ10 gene. To assess the pathophysiological relevance of these mutations, we generated a mutant rodent model of the disease, using CRISPR-Cas9 technology (Kir4.1-R18Q). Patch-clamp recordings, immunofluorescence and biochemical analyses of cortical astrocytes cultured from Kir4.1-R18Q mice confirmed that the mutation resulted in a gain of channel function. Several behavioural tests were performed from young and old, male and female, animals which displayed increased anxiety and obsessive-compulsive behaviour compared to WT littermates. Altered gut microbiota (GM) composition has been linked to the pathogenesis of ASD. Here we show that the GM of Kir4.1-R18Q mice is characterized by marked alterations in the abundance of distinct bacterial species, including *Odoribacter, Parabacteroidetes, Ruminococcus, Oscillospira* and U.g. of *Desulfovibrionaceae*. Similar GM abnormalities have been described in autistic patients and other mouse models of ASD. Overall, our evidence indicates that genetically-induced enhancement of Kir4.1 expression and gut dysbiosis result in an autistic-like phenotype. Moreover, our newly established mouse model of autism could represent a powerful tool for uncovering new disease mechanisms associated with ASD and for the development of precision medicine being tailored to selected autistic patients.

PP.2

Object observation activates neurons in the ventrolateral prefrontal cortex of the macaque

Fogassi L

University of Parma, Italy

The ventrolateral prefrontal cortex (VLPF) is connected with dorsal and ventral visual stream areas and is involved in object categorization and visual working memory. Electrophysiological studies mainly focused on the role of restricted VLPF sectors in face and object coding/categorization, in this latter case using a limited number of stimuli. The first aim of our study was to assess the response of neurons of a wide region of VLPF to a large set of visual stimuli. We recorded single neurons from VLPF of two macaque monkeys (Macaca mulatta) presented with a set of 12 images. We found 863, visually responding neurons. Most of them (89.2%) did not show stimulus preference, while 10.8% selectively coded one (n=58) or a group of presented stimuli (n=35). In both monkeys, selective neurons tended to group in three clusters, two located in the central sector of the recorded region (areas c46VR and 12r) and one in the posterior sector (46VC/8). The second aim of the study was to assess whether VLPF neurons responding to images are also involved in action planning, by comparing the responses to objects in the visual task with those recorded from the same neurons in a Go/NoGo task, in which the monkeys had to observe or observe-and-grasp three of the objects also presented in the visual task. At the population level, the neural response recorded in the Go condition was the highest, followed by that observed in the NoGo condition, while that recorded in the purely visual task evoked the weakest discharge. Altogether, these data indicate that VLPF neurons, beyond participating to visual coding of objects, are strongly modulated by a visuomotor task, suggesting their involvement in the selection of actions to be performed on graspable objects.

PP.3

The splanchnic anti-inflammatory pathway can be exogenously activated to inhibit inflammation

Martelli D[1,2], McKinley MJ[2], McAllen RM[2]

[1]Department of Biomedical and Neuromotor Science, University of Bologna, Italy; [2]. Florey Institute of Neuroscience and Mental Health, Parkville, Australia

In response to a systemic immune challenge, the body reacts endogenously activating a neural reflex, termed the inflammatory reflex, to inhibit the ensuing inflammation. The efferent arm of the inflammatory reflex, the splanchnic anti-inflammatory pathway, travels in the greater splanchnic sympathetic nerves.

Exogenous electrical stimulation of peripheral autonomic nerves also inhibits inflammation. Vagus nerve stimulation (VNS) is considered one of the most promising electroceutical strategy to treat inflammatory diseases that are not responsive to classic pharmaceutical treatments. Recently, we showed that afferent VNS suppresses systemic inflammation via the splanchnic anti-inflammatory pathway, enhancing the endogenous reflex action of sympathetic splanchnic nerves. Therefore, we hypothesized that the direct exogenous electrical stimulation of the splanchnic nerves (SpNS) inhibits inflammation in an animal model of endotoxemia. We set out to prove our hypothesis in anaesthetized rats implanted with a bipolar cuff electrode around the left greater splanchnic nerve and challenged with i.v. injection of lipopolysaccharide (LPS, 60 μg/kg). One group of animals received SpNS, 10 minutes before and 10 minutes after LPS injection, at 20V, 0.5ms, 2Hz. Another group was sham stimulated. 90 minutes after LPS injection, blood was collected, and the plasma concentration of tumor necrosis factor (TNF), sufficient and necessary mediator of inflammation, assayed. The results showed that SpNS strongly reduces the TNF response to LPS. We concluded that SpNS activates the splanchnic anti-inflammatory pathway to inhibit inflammation. SpnS is therefore a valid alternative to VNS as new electroceutical strategy to treat inflammatory conditions.

PP.4

Study of reaching trajectories in physiological and pathological conditions: a decoding approach.

Bosco A[1], Bertini C[2], Ladavas E[2], Fattori P[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy;[2]Department of Psychology, University of Bologna, Italy.

One of the basic aspects of human cognitive abilities is represented by the reaching behavior that is important for the interaction with the environment. The knowledge of the physiology of Posterior Parietal Cortex (PPC) is relevant for understanding the control of reaching movements towards visual targets. Patients with lesions of PPC typically misreach when guiding a limb in peripheral space towards targets that are not foveated and most often do errors in the direction of their gaze. These effects are typically observed in the Optic Ataxia deficit. In the present study, we analysed the reaching performance of a female left-handed patient with right PPC damage and that of 5 left-handed healthy controls during visually-guided reaching movements in foveal and peripheral viewing conditions. We measured the trajectories and the reach errors in different eye/hand configurations varying in depth and direction. To compare the trajectories performed by the patient with those of the controls, we calculated the horizontal deviation between an ideal trajectory connecting the start position and the target position and the real trajectory and tested for significance by a linear discriminant classifier. We found that patient and controls shared the same trajectory strategy at the beginning of movement and then significantly diverged from 30% of movement in all eye/hand configurations tested. The patient showed higher reaching errors with respect to controls both in depth and direction in the peripheral viewing conditions. By application of a novel decoding approach, these results demonstrate how patient and controls shared the same motor plan but significantly differed in the on-line control of the movement. This allows to shed new light on the physiology of the reaching circuit.

PP.5

Activation of dopamine D_1 receptor signaling in the dentate gyrus is required for antidepressant action of SSRI in a mouse model of depression.

Kuroiwa M, Shuto T, Sotogaku N, Nishi A

Department of Pharmacology, Kurume University School of Medicine, Japan

Dopamine D1 receptor signaling plays an important role in the regulation of reward systems and cognitive function. Hippocampal dentate gyrus (DG) is one of target brain regions for antidepressants. Recently, chronic treatment with a selective serotonin reuptake inhibitor, fluoxetine, is reported to induce functional changes of mature granule cells in the DG, in addition to the facilitation of adult neurogenesis. In this study, we investigated the expression profile and function of dopamine D1 receptors in the DG after chronic antidepressant treatment. Treatment of mice with fluoxetine (15 mg/kg/day) for 14 days increased the expression of D1 receptors in mRNA and protein levels only in the DG, but not of other subtypes of dopamine receptor. The ability of a D1 receptor agonist, SKF81297, to phosphorylate DARPP-32 at Thr34 (PKA-site) in DG slices was enhanced in fluoxetine-treated mice. Mice subjected to severe restraint stress

(4 hr/day, 28 days), chronic treatment with fluoxetine failed to reduce the stress-induced increase in feeding latency in the novelty-suppressed feeding test (NSFT) and immobility time in the tail suspension test. However, chronic co-administration of a dopamine D1 receptor agonist, R(+)-SKF81297 (1.5 mg/kg/day, i.p. for 5 days), with fluoxetine reversed the depression-like behaviors. These results suggest that activation of dopamine D1 receptor signaling in the DG is required for therapeutic actions of SSRI. D1 receptors may be a therapeutic target in combination with SSRI antidepressants under SSRI-resistant stress conditions.

PP.6

Analysis of microsaccade direction during learning of an attentional task in the macaque monkey

Raffi M, **Meoni A**, **Piras A**

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy.

Microsaccades are small eye movements produced during fixations. The spatial location indicated by an attentional visual cue can bias microsaccade directions towards or away from the cue. Aim of this work was to evaluate the characteristics of saccades and microsaccades during the monkey's training, investigating the relationship between attentional orientation and learning. The experiments were performed in one macaque monkey (*M. fascicularis*) trained to fixate a target in a reaction time (RT) task. At the target onset, the monkey pressed a lever and an expanding optic flow stimulus appeared to the right of the target. After a variable delay of 750, 1000, 1250 or 1500 ms, an attentional cue appeared within the optic flow stimulus and the monkey had to release the lever in a maximum RT of 700 ms. In the control task no visual cue appeared within the optic flow stimulus and the monkey had to attend a change in the target color. Eye movements were recorded by the EyeLink II (Sr-Research, Canada). Data were recorded in 9 months. Results showed that RTs at the control task were always shorter that those at the attentional task. RTs decreased across times in all four attentional conditions, although were higher in the 750 ms condition. Both microsaccade and saccade directions were significantly clustered toward the attentional cue ($p<0.001$) (Oriana, Kovac computing). The microsaccade main sequence showed that the monkey made very fast microsaccades with large amplitude corresponding to the stimulus eccentricity. The number of microsaccades did not decrease significantly across time. These results, although preliminary, show that microsaccade directions could indicate the position of the attentional focus without significant variations during the learning process.

PP.7

Processing of depth and direction signals in the medial posterior parietal cortex of the macaque.

Breveglieri R[1], **De Vitis M**[1,2,3], **Hadjidimitrakis K**[1,4,5], **Galletti C**[1], **Fattori P**[1]

[1]Department of Biomedical and Visuomotor Sciences, University of Bologna, Italy; [2]Laboratory of Neuro- and Psychophysiology, Leuven, Belgium; [3]Leuven Brain Institute, Leuven, Belgium; [4]Biomedicine Discovery Institute and Department of Physiology, Monash University, Clayton, Australia; [5]Australian Research Council, Centre of Excellence for Integrative Brain Function, Monash University Node, Clayton, Australia

During reaching in 3D space, several areas of the posterior parietal cortex (PPC) combine visual and somatosensory inputs with motor commands to guide the arm at appropriate directions and depths. Reach depth and direction are thought to be processed in distinct circuits, but in literature there is little neurophysiological support. Here, we investigated the neural correlates of reach depth and direction in three medial PPC areas: V6A, located in the anterior bank of the parieto-occipital sulcus, PEc located just rostral to V6A, and PE, located more rostrally in the PPC. We characterized the temporal evolution of depth and direction processing during a fixation-to-reach task in darkness towards targets placed at different locations in 3D space. Single neuron activity was recorded extracellularly in four *Macaca fascicularis* monkeys and was quantified in several task phases: target fixation, reaching preparation, execution, and target holding. We found that the processing of depth information occurred mainly during and after movement execution in both PE and PEc, whereas in V6A it was evident during all task phases. Differently, in all areas, the number of cells coding for direction during the initial target fixation period was higher compared to the subsequent phases. In addition to the temporal segregation of depth and direction signals, we found that the number of single neurons that processed simultaneously both depth and direction decreased significantly going from V6A to PEc and then from PEc to PE. These trends draw parallels to well-established gradients in sensory processing observed along the caudorostral axis of medial PPC, i.e. the increase of somatosensory and the simultaneous decrease of visual processing and support several lines of behavioral evidence.

PP.8

Neurons modulated by action execution and observation in area V6A of the macaque.

Vaccari, FE[1], Tabanelli M[1], Breveglieri R[1], Bosco A[1], Gamberini M[1], Fattori P[1], Galletti C[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

In several cortical areas of the monkey, mirror neurons are modulated during the execution of an action and during the observation of the same action performed by another agent. Visuomotor area V6A in medial parietal cortex contains neurons modulated by the execution of grasping movements, but evidence about the presence of mirror neurons in V6A is still lacking. The aim of this study is to assess whether V6A cells are modulated by the execution of a grasping action and by the observation the same action performed by another agent. Single cell recordings were performed in area V6A of 2 Macaca fascicularis trained to perform a grasping task, an object observation task, and to observe grasping actions performed by an experimenter. We have found that the majority of cells (63%) was modulated only by reach-to-grasp execution, a few cells (2%) only by grasping observation, but, interestingly, a group of cells (18%) was modulated by both the execution and observation of grasping (putative mirror neurons). In these putative mirror neurons, neural representations during grasping execution and observation were not congruent, both at the single cell and at the population level. For this reason, we suggest that V6A putative mirror neurons are not involved in representing the specific type of grip that is observed. The majority of V6A mirror neurons responded also to the object observation, but the strength of the object-related response was maximal before own grasp execution whereas it was weaker when the object was the target of another agent's subsequent action or when no grasping was required. We thus suggest that V6A neurons encode the relevance, for the observer, of the target object.

PP.9

Postural Control during Gait Initiation in children with Cerebellar Ataxia

Farinelli V[1], Marchese SM[1], Strano C[1], D'Arrigo S[2], Ardissone A[2], Nardocci N[2], Bolzoni F[1], Esposti R[1], Cavallari P[1]

[1]Human Physiology section of the De.P.T., University of Milan, Italy; [2]Developmental Neurology Unit, IRCCS C. Besta Neurological Institute, Milan, Italy

Cerebellar Ataxia (CA) is a heterogeneous group of rare neurological diseases with often a genetic aetiology. The cerebellum plays a crucial role in both balance and locomotion, where the feed-forward control of dynamic phenomena prior to stepping is essential for walking effectively. Aim of this work was to evaluate how CA affects the postural control during gait initiation. Eight pathological and nine age-matched healthy children were asked to stand on a dynamometric platform for 30 seconds and then start walking spontaneously, self-selecting the stepping limb. Body kinematics were also recorded, by a 6-TVC optoelectronic system. During stance, the Centre of Pressure (CoP) covered a larger area (95% confidence ellipse) in CA patients than in healthy children, especially in the medial-lateral direction ($p<0.05$). With regard to the unloading and imbalance phases of the first step, only the anterior-posterior CoP displacement during imbalance was significantly larger in CA patients than in healthy children ($p<0.01$), while all other parameters (duration, length of CoP trajectory, medial-lateral CoP displacement & mean velocity) were comparable for both phases. However, the length and speed of the first step were lower in CA patients ($p<0.05$). The significant changes in CoP area during stance confirm in children that CA is associated to an impaired control of balance, underlining their difficulty in maintaining the upright posture. The fact that the imbalance and unloading phases were in most part comparable between the two groups indicates that CA patients are still able to output an operative motor program, like healthy individuals, but the slower and smaller first step may actually be considered a compensatory strategy for an impaired control of dynamic posture.

PP.10

Sleep loss affects membrane physical state of myelin sheaths

Fiorini R

Università politecnica delle Marche, Italy

Using electron and confocal microscopy we recently showed that prolonged sleep restriction (~5 days) reduced myelin thickness and increased node of Ranvier length without affecting internodal length, thus suggesting that sleep loss can lead to plastic remodeling of myelin. Since optimal plasma membrane fluidity is an essential requirement for cellular plasticity, we hypothesized that the fluidity of membranes forming myelin could be affected by sleep loss. Membrane fluidity of myelin enriched brain samples from sleeping (S, n=6, killed after 6 hrs of sleep during the light cycle) and sleep deprived c57bl/6 mice (SD, n=6, killed after 4 hrs of enforced wake during the light cycle) was

assessed by steady-state fluorescence spectroscopy. Two fluorophores were used, Laurdan, located at hydrophobic-hydrophilic interface of the membrane, and 1,6-diphenyl-1,3,5-hexatriene (DPH), incorporated in the hydrophobic lipid region. Moreover, in mouse forebrain samples, genome-wide analysis of messenger RNA profiling of oligodendrocyte lineage was conducted as a function of S and SD, to detect differentially expressed genes. A significant increase in membrane fluidity was found in myelin membrane core in SD relative to S (DPH anisotropy: S [0.231±0.008]; SD [0.222±0.005], $p<0.00019$), while no differences have been detected at the polar headgroups level. In addition, analysis of the oligodendroglia transcriptome revealed the up-regulation of Fad3 in SD (+176%; $p=0.004$). Fad3 codes for a fatty acid desaturase that regulates unsaturation of fatty acids and influences membrane fluidity. Thus, increased fluidity of the inner myelin membrane region could contribute to morphological modifications of myelin induced by sleep loss.

PP.11

Postural orientation contributes to modeling the effects of gravity for target interception in humans

La Scaleia B[1], **Lacquaniti F**[1,2,3], **Zago M**[1,3,4]

[1]Laboratory of Neuromotor Physiology, IRCCS Fondazione Santa Lucia, Rome, Italy; [2]Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy; [3]Centre of Space Bio-medicine, University of Rome Tor Vergata, Rome, Italy; [4]Department of Civil Engineering and Computer Science Engineering, University of Rome Tor Vergata, Rome, Italy

Interception of moving targets relies on visual signals and internal models. Less is known about the additional contribution of nonvisual cues about head and body orientation relative to gravity. We took advantage of Galileo's law of motion along an inclined plane to demonstrate the effects of vestibular and somatosensory cues about body posture on interception timing. We presented a virtual scene without any visual information about gravity direction in a head-mounted display. Participants were asked to hit a ball rolling in a gutter towards the eyes, resulting in image expansion. Participants were tilted backwards in the sagittal plane by 20 or 60 , while ball acceleration was compatible with rolling down a slope of 20 or 60 . At the beginning of the experiment, the timing errors were large and independent of the coherence between acceleration and pitch angle. This is what one would expect if the responses were based exclusively on visual information, since the visual stimuli were identical at both subject tilts. At the end of the experiment, however, the timing errors were systematically smaller in the coherent conditions than the incoherent ones. Moreover, the responses were significantly earlier when participants were pitched by 60 than when they were pitched by 20 .Therefore, practice with the task led to incorporation of postural information about head and body tilt relative to gravity for response timing. Instead, posture did not affect response timing in a control experiment in which participants hit a static target in synchrony with the last of a predictable series of stationary audio-visual stimuli.

PP.12

Exercise affects skeletal-muscle reinnervation after nerve crush via TrkB-A_2A receptor crosstalk

Sartini S[1], **Di Palma M**[1], **Ambrogini P**[1], **Lattanzi D**[1], **Pellegrino MA**[2], **Bottinelli R**[2], **Cuppini R**[1]

[1]Dept. of Biomolecular Sciences, University of Urbino Carlo Bo, Italy; [2]Dept. of Molecular Medicine, University of Pavia, Italy

The mechanisms behind the recovery of skeletal muscle innervation after peripheral nerve injury remain poorly understood. We previously showed that an intermittent, mid-intensity treadmill activity induces axonal sprouting and faster muscle re-innervation, but these effects were only partially explained by TrkB activation by muscle-derived BDNF. In the present work no significant difference in NT4, IGF1, CNTF, and GDNF expression was found between sedentary and running rats. Muscle activity increases adenosine release, and, moreover, a pivotal role of adenosine in TrkB activation has been recently demonstrated in brain. Therefore, we evaluated the role of A2A-TrkB crosstalk in muscle re-innervation by intracellular electrophysiological recordings. Ten days after nerve crush, the percentage of multiply innervated muscle cells (an index of nerve sprouting) was about 10 % in sedentary rats. This percentage increased to about 30% in runners, but not if they were treated with TrkB or A2A specific receptor antagonists. Moreover, sedentary controls administered with specific TrkB or A2A agonists showed an increase similar to that obtained under running conditions. These findings show a primary role of adenosine in intramuscular motor nerve sprouting and strongly suggest that the adenosine action through A2A receptors by gating the TrkB signaling might be the underlying mechanism.

PP.13

How the brain holds its horses: a new model of inhibitory control implementationg

Mirabella G[1,2]

[1]IRCCS Neuromed, Pozzilli (IS), Italy; [2]Department of Anatomy, Histology, Forensic Medicine & Orthopedics, Sapienza University, Rome, Italy

Inhibitory control is a key executive function as it allows for the implementation of adaptive and flexible behavioral strategies. This is because in the real world the course of events cannot be fully predicted.There are thus instances in which the value of a planned action might suddenly change and the pending action must be suppressed to avoid awful consequences, such as being struck by a truck suddenly appeared on the road when we are about to cross it.Given the importance ofaction countermandingit is not surprising that a wide number of cortical and subcortical brain regionsare involved[1]. However,the neural underpinnings of inhibitory controlare still largelydebated and controversial. I will propose a novel model of the inhibitory network, whose way of working conceptually resembles the idea of the race model exploited to interpret the behavioral outcome of the stop-signal task[2]. In this model, the two input components of the basal ganglia loop, the striatum, and the subthalamic nuclei (STN) play a key role. Thanks to their wide connectivity, they are capable of evaluating the pros and cons of the ongoing motor plan delivered by the motor cortices, against the wide array of context-dependent,emotional,and attentional signals coming from the prefrontal, parietal, and limbic cortices. The competition between the opposite signals coming from the striatum and the STN will determine the state of activation of the output nuclei of the basal ganglia. i.e. the globus pallidus internal segment (GPi),as far as arm movement is concerned,and the substantia nigra pars reticulata, as far as eye movement is concerned. Both these regions tonically inhibit targets in the thalamus and brainstem, blocking the excitatory activity that precedes movement onset, and thus leading to the suppression of the pending action. If the striatum wins the race than the movement is performed,whereas if the STN prevails the movement issuppressed. This circuit would allow for quick braking of pending actions when these are evaluated as no longer suitable to achieve the previously selected goal and would provide an explanation of why many different brain regions can be involved in action inhibition. [1] Mirabella (2014). Should I stay or should I go? Conceptual underpinnings of goal-directed actions. Front Syst Neurosci 8, 206. [2] Logan et al (1984). On the ability to inhibit simple and choice reaction time responses: a model and a method. J Exp Psychol Hum Percept Perform 10, 276-291

PP.14

Effects of occlusal rebalancing on cognitive performance

Tramonti Fantozzi MP[1], De Cicco V[1], Diciotti S[2], Tessa C[3], Vignali C[3], Barresi M[4], Cataldo E[5], Bruschini L[6], Faraguna U[1,7], Manzoni D[1]

[1]Department of Translational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa, Italy; [2] Department of Electrical, Electronic, and Information Engineering "Guglielmo Marconi", University of Bologna, Cesena, Italy; [3]Department of Radiology, Versilia Hospital, Azienda USL Toscana Nord Ovest, Lido di Camaiore, Italy; [4]Institut des Maladies Neurodégénératives, University of Bordeaux, Bordeaux, France; [5]Department of Physics, University of Pisa, Pisa, Italy; [6]Department of Surgical, Medical, Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy; [7]Department of Developmental Neuroscience, IRCCS Foundation Stella Maris, Pisa, Italy.

Trigeminal input to the ascending reticular activating system is important for the maintenance of arousal and may affect the discharge of the Locus Coeruleus (LC) noradrenergic neurons, whose activity influences both vigilance state and pupil size, inducing mydriasis. Pupil size evaluation is now considered as a proxy of LC activity. Trigeminal imbalance due to malocclusion may lead to imbalance in LC activity and, as a consequence, in the brain excitability. Such an imbalance might, in turn, lead to functional impairment of cognitive and motor functions. In healthy subjects, showing asymmetry in the electromyography activity of masseter muscles during clenching (> 24%), we evaluated whether malocclusion, modifying the symmetry of the trigeminal sensorimotor activity 1) induces changes in the cognitive performance, in the task-related mydriasis and in the pupil asymmetry; 2) modifies the brain activation during a pure motor task as evaluated by fMRI, in order to disentangle the possible role of motor behaviour in the performance of the cognitive task which consist of both cognitive and motor components. It was found that occlusal correction (through the wearing of an appropriate orthotic interposed between the dental arches) modifies the trigeminal input asymmetry and positively affects cognitive performance by changing the task-induced LC activation and its resting imbalance. Moreover, occlusal correction leads to a reduction in the brain activation during a pure motor task, suggesting the motor contribution into the cognitive performance improvement. So, trigeminal unbalance associated to malocclusion leads to an asymmetry in pupil size, while occlusal correction reduces both trigeminal and pupil size asymmetry and boost performance in a cognitive sensorimotor task.

PP.15

Influence of different facial expressions on face and hand primary motor cortices

Ginatempo F[1], Manzo N[2], Ibanez-Pereda J[3], Rocchi L[3], Rothwell JC[3], Deriu F[1]

[1]Department of Biomedical Sciences, University of Sassari, Sassari; [2]IRCCS NEUROMED, Pozzilli, IS, Italy; [3] Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, England.

In humans the ability to rapidly recognize and react to the view of facial expressions is crucial for social communication. This work investigated the effects of emotional stimuli, represented by different facial expressions, on the face primary motor area (M1) using TMS protocols in healthy subjects. Moreover, a comparison with data obtained from the hand M1 was performed. Thirty healthy subjects were randomly assigned to two groups undergoing TMS of face M1 or of hand M1. In both groups, short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF) were tested in depressor anguli oris (DAO) and first dorsal interroseus (FDI) muscles after 300 ms from the presentation of images reporting happy, sad, and neutral faces. Statistical analysis was performed through within- and between-subjects ANOVA. Statistical analysis of SICI effects comparing muscles, showed a non-significant effect of MUSCLE GROUP ($F_{1,28}$= 1.903, p= 0.179) but a significant effect of visual stimuli presented before TMS-protocols ($F_{2,56}$=6.860, p=0.004) and interaction among the factors ($F_{2,56}$=5.072, p=0.015). A significant reduction of SICInwas detected after presentation of sad and happy expressions compared with the neutral one (sad p=0.009; happy p=0.001). A clear difference was detected between FDI and DAO muscles only for happy expression (FDI vs DAO p= 0.026) with the DAO showing a larger reduction of SICI than FDI. No clear differences were detected in ICF protocol. Results showed that emotional stimuli influence significantly M1 with a stronger effect of pleasant stimulus (happy faces) on facial than hand muscles. Data suggest the different responses of face and hand M1s to different emotional stimuli may be due to the different functional role of these muscles.

PP.16

Thalamo-cortical projections to the macaque precuneate cortex

Passarelli L[1], Gamberini M[1], Impieri D[1], Fattori P[1], Galletti G[1], Rosa M[2,3], Bakola S[2,3]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2]Department of Physiology and Biomedicine Discovery Institute, Monash University,Clayton, Victoria, Australia; [3]Australian Research Council, Centre of Excellence for Integrative Brain Function, Monash University Node, Clayton, Victoria, , Australia

The precuneate cortex, located in the caudal medial surface of the parietal lobe, hosts areas PGm and 31. The aim of the present study was to characterize the thalamic afferents to the precuneate cortex in the macaque, based on a set of retrograde tracer injections in areas PGm, 31, and in nearby area PEci. These areas receive strong input from the posterior thalamus, the lateral posterior and the pulvinar complex, and moderate input from the medial, lateral, and intralaminar thalamic regions. We found that PGm has strong connectivity with the associative medial and visual lateral divisions of the pulvinar, whereas areas 31 and PEci receive exclusive afferents from the oral division of the pulvinar. Areas PGm, 31, and PEci receive input from the 'motor' division of lateral thalamus, in particular from the VL nucleus, while area PEci receives input from both the 'sensory' (VPL) and 'motor' domains of the lateral thalamus. Consistent thalamic input to all three areas arrived from the MD nucleus, while the LD nucleus, in the superior part of the thalamus, is uniquely connected with area PGm. These results indicate that macaque area PGm integrates information from a set of association, motor, and limbic regions of the thalamus. The connectivity pattern emphasizes links with visual and multimodal thalamic regions. The lack of input from the thalamic nuclei with somatosensory properties (VPL for areas PE, PEc, PEci, and oral pulvinar for area 31) differentiates PGm from adjacent superior parietal areas, mainly involved in sensorimotor analyses related to the use of limbs. For area PGm, we suggest a role in the processing of oculomotor, spatial, and attentional information useful to scan scenes for spatial navigation.

PP.17

The cognitive component of reaction time tasks in Parkinson's disease patients is improved after the program of "dry" immersion

Meigal A[1,3], Tretjakova O[1], Gerasimova-Meigal L[1], Sayenko I[2]

[1]Department of Human and Animal Physiology, Pathophysiology, Petrozavodsk State University, Russia; [2]Institute of Biomedical Problems, Russia; [3]Laboratory of Novel Methods in Physiology, Petrozavodsk State University, Russia

The course of analogue microgravity induced by the condition of "dry" immersion (DI) was reported to reduce motor deficits in patients with Parkinson's disease (PD) (Meigal et al., 2018; Miroshnishenko et al., 2018). In the present study, we instrumentally assessed the effect of the DI program (7 DI sessions, 45 min each, MEDSIM device, IMBP, Moscow) on reaction time in several tasks with different cognitive

demanding - the simple (SRT), choice (CRT), reaction time tasks, SRT with distraction of attention (SRT-DA), prediction motion task (PMT), and tapping test (TT). The study was performed with a help of PC-based tester (Neirosoft Ltd., Ivanovo, Russia). A total of 11 PD patients (Hoehn and Yahr staged 1-3) was studied. Data was collected in 4 study points (before, right after the program of DI, 2 weeks and 2 months after the program). In 6 PD patients of the reference group (with no DI) the tasks have not modified across the study time. In the group with DI, SRT has not changed, SRT-DA has decreased from 344 ms (302-353) before the DI program to 314 ms (296-322) 2 weeks after it ($p=0.029$). CRT has decreased from 443 ms (385-589) before the DI program to 402 ms (359-454) 2 weeks after it ($p=0.06$). At the point of 2 months all parameters tended to restore their values. In the PMT, precise hits constituted 50-57% and did not change over the study. The count of hand taps during TT task centered around 200 per 30 s, and it stood unchanged by DI in both groups. These results indicate that the tasks with stronger cognitive component (SRT-DA, CRT) are more eagerly modified by the microgravity condition than those with stronger motor component (SRT, PMT, TT). Thus, the mechanism of neuroplasticity and improved brain connectivity are supposingly involved by the DI program.

PP.18

Catodal or Anodal tDCS on Parietal Operculum do not affect Intra-limb Anticipatory Postural Adjustments

Marchese SM, **Esposti R**, **Bolzoni F**, **Cavallari P**

Human Physiology Section of the De.P.T., Università degli Studi di Milano, Italy

Recent data (Sepulcre 2014, Neuroscientist 20:499-508) suggests that the parietal operculum (PO) acts as an integration centre within a multimodal network, originating from different primary sensory cortices and projecting to frontal, parietal and temporal cortical hubs that govern cognitive and motor functions. Thus, PO might also play a crucial role in the control of voluntary movement and posture. In order to test this hypothesis, the Anticipatory Postural Adjustments (APAs) stabilizing the arm when the index-finger is briskly flexed (Cavallari et al 2016, Front Hum Neurosci 10:525) were recorded in three groups of 10 healthy subjects, before, during and after cathodal or anodal transcranial direct current stimulation (tDCS, 20 min at 2 mA) applied over PO. Results were compared to those obtained in a sham group. In agreement with literature data, in the sham group the activation of the prime mover Flexor Digitorum Superficialis was preceded by an inhibitory APA in Biceps Brachii and Anterior Deltoid, and almost simultaneous to an excitatory APA in Triceps Brachii. The same pattern was observed in both the cathodal and anodal groups, with no significant tDCS effects on APAs amplitude or timing. Index-finger kinematics were also unchanged. These negative results suggest that PO should be excluded from the key network governing intra-limb APAs, indirectly confirming, as indicated in the literature, that such structure is mainly committed to cognitive functions (learning and memory) and in defining the aim of the motor action.

PP.19

Neural States in V6A during reaching task.

Diomedi S, **Filippini M**, **Breveglieri R**, **Fattori P**

DIBINEM, Università of Bologna, Italy.

For a long time, it has been thought that single neurons are the functional and structural unities of the nervous system and that these could be classified in relation to a specific function. From this viewpoint, functionality of an area is defined in relation to the type of its neurons. Recently, new techniques gave us the possibility to study information coded in a set of neurons. This new approach allows us to discover the presence of various networks, composed by more neurons, throughout the brain. These networks encode various functional properties as pattern of neuron activations. A specific activation pattern is a cognitive neural state of the network, these states drive task execution. Now the common thought is that these networks are functional unities of the nervous system. Here, we applied a statistical model named Hidden Markov Model to the activity of a population of V6A neurons. V6A is part of the dorsomedial visual stream and is known to encode different aspects of prehension. We wanted to detect V6A's neural states during a reaching task. We trained macaques to perform a delayed reaching task with different targets located in different locations. We tried to detect different numbers of neural states consistent with real event timings, that we called hidden states. Results showed that it was possible to detect hidden states corresponding to several activation patterns, for the main behavioural epochs: free activity baseline, gaze fixation period, reaching planning and reaching movement. The natural presence of these hidden states proves the presence of different networks in V6A and highlights the complexity of the modulations of its neurons that can belong to different patterns of activation during different behavioural epochs with different modulations.

PP.20

Role of visual motion information and internalized gravity in motor and perceptual predictions

Bosco G[1,2,3], Delle Monache S[1,2,3], De Sá Teixeira N[1,2,3], Lacquaniti F[1,2,3]

[1]Department of Systems Medicine and [2]Center of Space Biomedicine, University of Rome Tor Vergata, Rome (Italy); [3]Laboratory of Neuromotor Physiology, Santa Lucia

Foundation, Rome (Italy)

Predictive processes are essential for both motor behavior and perception. Here, we examined the contribution of visual motion processing and internalized knowledge about physical invariants (i.e. gravity) in predicting natural object motion. In two experiments, we interfered, by using transcranial magnetic stimulation (TMS), with the activity of visual motion area hMT/V5+ and of the temporoparietal junction (TPJ), an area related to an internal representation of gravity. In one experiment, we disrupted TPJ and hMT/V5+ while subjects intercepted projectile trajectories perturbed with either hypo- or hypergravity effects. Trajectories were entirely visible or occluded for variable intervals before landing. Three TMS pulses at 10 Hz were timed either at the motion perturbation or at the occlusion. hMT/V5+ stimulation affected responses to all motion types, whereas TPJ affected preferentially responses to 1g motion, in line with their role in the processing of visual motion and internalized gravity information, respectively. In the second experiment, subjects reported the perceived vanishing location of moving targets. In this task, the reported location is systematically displaced forward, in the direction of motion (Representational Momentum), and downward, in the direction of gravity (Representational Gravity). Blocks of trials were presented prior to and following disruption of either hMT/V5+ or TPJ with continuous theta-burst stimulation. Representational Gravity increased following disruption of hMT/V5+, whereas Representational Momentum was enhanced by TPJ disruption, suggesting a reciprocal balance between perceived kinematics and anticipated dynamics. Thus, overall, our results indicated that motor and perceptual predictions may engage different neural mechanisms.

PP.21

A kinematic study of skilled reaching movement in Rat

Parmiani P[1,2], Bonifazzi C[1], Lucchetti C[3], Franchi G[1]

[1]Department of Biomedical and Specialty Surgical Sciences, Section of Human Physiology, University of Ferrara, Ferrara, Italy; [2]Center for Translational Neurophysiology, Istituto Italiano di Tecnologia, Ferrara, Italy; [3]Department of Biomedical, Metabolic and Neural Sciences, Section of Physiology and Neuroscience, University of Modena and Reggio Emilia, Modena, Italy

Skilled reaching, the act of reaching to grasp an object as occurs in a reach-to eat task, is an important model for investigating many topics in neurosciences research. It is composed of orienting (OR), reaching (RC), Grasping (GR) and Retract (RT) movements. We present a new method for quantitative assessment of skilled reaching in trained rats. An infrared 3D motion tracking system using three high speed digital cameras to capture at 100 Hz the motion of passive markers attached to the rat's body (Qualisys Motion Capture System, USA). Custom made software tools perform basic motion calculations markers movement kinematics. We present here preliminary results of OR movement from five rats: 1- The OR trajectory is smooth and continuous with a length and shape variability due to the position of starting point relative to target. 2- By means of kinematic parameters we were able to distinguish between two OR patterns characterized by a different numbers of sub-phases (2 vs 3). 3- The two OR patterns present different speed profiles and durations. Our data suggest that the NOSE works as a pointing system for wrist/paw positioning.

PP.22

Suppression history of visual locations shapes spatial priority maps sustaining transient ongoing control of saccadic behavior

Di Caro V[1], Della Libera C[1,2]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy; [2]National Institute of Neuroscience, Verona, Italy

The onset of a new stimulus in the visual field triggers an automatic orienting response, including a reflexive saccade towards its location, even when such stimulus is irrelevant and should be ignored. When irrelevant onsets accompany task relevant stimuli, they act as powerful distractors and lead to performance costs. Efficient behaviour thus depends on being able to filter out these distractors, by engaging inhibitory mechanisms which suppress their processing. In a previous study we showed that onset-driven reflexive saccades are affected by the suppression history associated with stimulus location, being reduced for onsets at locations that had been previously more often associated with distraction. By testing the permanence of these effects at different delays, we show that suppression history shapes reflexive orienting

saccades for a considerable time even after all unbalances in the spatial probability of the onset are removed. This phenomenon however is only found early on after the learning phase with biased spatial probabilities (Exp. 1), and no effects emerge on the next day (Exp. 2). Following unbalances in the spatial probability of irrelevant onsets, we propose that within neural priority maps of the visual space, which assist planning and implementation of saccades, the locations that have been more frequently suppressed accumulate stronger inhibitory signals which alter their representation and render them less capable of triggering gaze shifts. Our data suggest that such functional plasticity is transient in nature, and involves spatial representations which adaptively sustain ongoing oculomotor control.

PP.23

High Fat Diet Induces Neuroinflammation and Brain Oxidative Stress Affecting Cerebral and

Synaptic Mitochondria Function and Efficiency

Trinchese G[1], Cavaliere G[1], Penna E[1], Cimmino F[1], Catapano A[1], Pirozzi C[2], Lama A[2], Annunziata C[2], Piscopo O[1], Zammit C[3], Crispino M[1], Mollica MP[1]

[1]Department of Biology, University of Naples Federico II, Italy; [2]Department of Pharmacy, University of Naples Federico II, Italy; [3]Department of Anatomy, University of Malta, Malta

High fat diet (HFD) consumption induces obesity-related metabolic disorders characterized by low-grade inflammation. Mitochondria, the primary cellular energy-generating system, produce key factors during inflammation and constitute the main source of reactive oxygen species (ROS). In the brain, neuroinflammation is an important risk factor for neurodegenerative disorders. The brain requires high amounts of energy for numerous processes, thus subtle changes in mitochondrial energy production has a strong impact on the brain. Mitochondria specifically located at synapses (synaptosomal mitochondria) provide energy to support synaptic functions and plasticity, and the impairment of their function may lead to synaptic failure, which is a common hallmark of neurodegenerative disease. Therefore, to investigate the molecular mechanisms underlying the effects of HFD in cerebral region, we analysed the mitochondrial functions of brain cortex and synaptic in a mouse model of diet induced obesity. MaleC57Bl/6 mice were divided into two groups fed a standard diet or HFD for 18 weeks. Our data demonstrated that HFD, compared to CD, induced inflammation and oxidative stress in the brain cortex, that is even more pronounced in synaptic regions. These alterations are linked to alteration of BDNF pathway and mitochondrial dysfunctions. Indeed, brain cortex mitochondria show a decreased oxidative capacity, and synaptic mitochondria is characterized by a reduced basal and maximal respiration, and a decrease in ATP production. Analyzing the dynamics of synaptic regions of the brain open a new perspective in the investigation of the molecular mechanisms underlying responses of the nervous system to the HFD.

PP.24

Evaluation of the mechanisms involved in behavioral effects of obestatin

Szakács J[1], Csabafi K[1], Kovács D[1], Pataki I[1], Szabó G[1]

[1]University of Szeged, Faculty of Medicine, Department of Pathophysiology, Hungary

Obestatin is a preproghrelin gene-derived peptide, which was originally identified as a ghrelin antagonist, reducing food intake and body weight gain. Since its discovery, multiple crucial functions of this peptide were described, involving the glucose and lipid metabolism, the cardiovascular system, among others. However, in contrast to ghrelin, much less is known about the behavioral effects of obestatin. The aim of this study was to investigate the effects of obestatin using the forced swimming test (FST), an experimental model of depression. Male mice were treated acutely with different doses of intracerebroventricular obestatin (0.5–1-1.5 μg/2 μl aCSF). Other group of animals received pretreatment with ghrelin receptor antagonist [D-Lys3]-growth hormone releasing peptide-6 ([D-Lys3]GHRP-6 1 μg/2 μl aCSF), the CRF type 1 receptor antagonist antalarmin (0.1 μg/2 μl aCSF), or intraperitoneal injection of nelivaptan (1 mg/kg), a selective vasopressin V1B receptor antagonist. All animals were tested in the FST and the immobility time was registered. Obestatin treatment alone increased the immobility time indicating a depression-like behavior. Pretreatment with different antagonists however, reversed the depression-like effect of obestatin in the FST paradigm. The presented results of obestatin on depression-like behavior, correlate with our previous studies indicating an anxiogenic-like effect of this peptide. Both effects might involve multiple pathways participating in the mechanism of stress-related illnesses such as anxiety and depression. Similarly to ghrelin, obestatin is a pleiotropic brain-gut peptide, having both central and metabolic effects, which merits further thorough investigations. Grants: EFOP-3.6.2-16-2017-000006

PP.25

Visuospatial attention and saccadic inhibitory control in children with cerebral palsy

Maioli C[1*], Falciati L[1], Galli J[1,2], Micheletti S[2], Turetti L[1], Balconi M[3], Fazzi E[1,2]

[1]Department of Clinical and Experimental Sciences, University of Brescia, Italy; [2]Unit of Child Neurology and Psychiatry, ASST Spedali Civili of Brescia, Italy; [3]Department of Psychology, Catholic University of Milan, Italy

Cerebral palsy (CP) is a non-progressive syndrome due to an early brain injury, which frequently involves an impairment of non-motor abilities. The aim of this paper was to examine visuospatial attention and inhibitory control of prepotent motor responses in CP children with normal IQ or mild cognitive impairment, measuring their performance in purely oculomotor tasks. Ten children (9-16 year-old) with spastic CP and 13 age-matched, typically developing children participated in the study. Subjects performed a simple visually-guided saccade task and a *cue-target* task, in which they performed a saccade towards a peripheral target, after a non-informative visual cue was flashed 150 ms before the imperative target, either at the same (*valid*) or at a different (*invalid*) spatial position. CP children showed severe executive deficits in maintaining sustained attention and complying with task instructions. Moreover, saccadic inhibitory control appeared to be significantly impaired in presence of both stimulus-driven and goal-directed captures of attention. In fact, patients showed great difficulties in suppressing saccades not only to the cue stimuli, but also to the always-present target placeholders, which represented powerful attentional attractors that had to be covertly attended throughout the task execution. Moreover, impairment did not affect in equal manner the whole visual field, but showed a marked spatial selectivity in each subject. Saccade latencies in the *cue-target* task were faster in the *valid* than in the *invalid* condition in both child groups, indicating the preservation of low-level visuospatial attentive capabilities. These impairments of executive skills and in inhibitory control manifest in childhood but recover to virtually normal level during adolescence.

PP.26

T-Patterns in the study of movement disorders

Casarrubea M, Aiello S, Crescimanno G

Laboratory of Behavioural Physiology, Dept. of Biomedicine, Neurosciences and Advanced Diagnostic (Bi.N.D.), Human Physiology Section "Giuseppe Pagano", University of Palermo, Italy

An intrinsic feature of behaviour orbits around its temporal dimension consisting of patterns in time: investigations on behaviour necessarily deal with sequences often impossible to be perceived by the human eye [Eibl-Eibesfeldt I. Ethology: the biology of behavior. New York: Holt, Rinehart and Winston. 1970]. Thus, the study of behavioural sequencing in terms of higher order arrangements, how the sequences are structured and, importantly, their analysis in normal subjects and in subjects affected by specific movement/behavioural disorders may represent a stimulating but challenging task. By means of T-Pattern analysis (TPA), it is possible to study these hidden features of behaviour and, specifically, its sequential organization [Casarrubea M et al. J Neurosci Methods. 2015;239:34-46]. This is particularly interesting when such a multivariate technique is used to study animal models of neurological illnesses characterized by movement disorders such as Parkinson's disease, Tourette's syndrome or histamine-depleted conditions. Here we illustrate our recent findings, obtained by means of TPA, in different rodent models presenting movement disorders [Casarrubea M et al. Behav Brain Res 2019;362:28-35; Santangelo A et al. CNS Neurosci Ther 2018;24:703-711; Santangelo A et al. Neuropharmacology. 2017;113:533-542]. The results may represent a stimulating topic of discussion on what, from a translational perspective, each model may suggest in terms of human behavioural abnormalities. We propose TPA as a suitable tool to describe the architecture of a behaviour both in animal models of neurological disorders and human patients as well.

Poster Session I (2/2)

Neurophysiology - Miscellaneous

PP.27

Functional cerebellar organoid formation on peptide-functionalised polyethylene glycol hydrogels

Balion Z[1], Cėpla V[2], Svirskienė N[3], Valiokas R[2], Jekabsone A[1,3]

[1]Institute of Pharmaceutical Technologies, University of Health Sciences, Lithuania; [2]Ferentis UAB, Lithuania; [3]Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania

Although organ-on-a-chip constructs meet the demand of in vivo relevance by recreating organotypic chemical, mechanical, spatial cues and flow of body fluids, the technical complexity makes the systems expensive,

difficult to repeat in terms of cell interactions, and requires well-trained staff to operate them. Therefore, such models are not suitable for bigger scale production and application for high throughput screening. Aiming to meet the demand of simpler organotypic models, we have applied collagen mimetic peptide-polyethylene glycol (CMP-PEG) based hydrogels as multiwell plate inserts for cerebellar neuronal-glial cell culture. The hydrogels promoted development of cerebellar explants to form complex spheroidal bodies resembling cerebellar granule layer organization with incorporated astrocytes and mobile, predominantly rod-shaped microglia. Contrary to cultures on hydrogels, monolayer cultures grown on poly-L-lysine coated plastic had evenly distributed sporadic networks of neurons and astrocytes with relatively small numbers of round microglia on top. Number of astrocytes and microglia was higher in hydrogel cultures making glia to neuron ratio similar to that in developing cerebellum. Neuronal functionality in the cultures was confirmed by measuring spontaneous network activity. Neurons in the spheroids on hydrogels started to reveal functional activity after 4 days in culture, and formed functional networks after 7 days. In contrast, no or very little functional activity was observed in cultures on CMP-PEG coated glass coverslips or monolayer cultures on poly-L-lysine coated plastic and glass after the same time in culture. In conclusion, CMP-PEG hydrogel inserts provide a new platform for fast and easy repeatable functional neuronal-glial cell culture.

PP.28

Decoding Modality-invariant Spatial Targets from Planning-related Activity in Early Visual Areas

Turella L[1], **Malfatti G**[1], **Monaco S**[1], **Culham J**[2,3], **Cattaneo L**[1,4]

[1]Center for Mind/Brain Sciences (CIMeC), University of Trento, Italy; [2].Department of Psychology, University of Western Ontario, Canada; [3]Brain and Mind Institute, University of Western Ontario, Canada; [4]Department of Neuroscience, Biomedicine and Movement, University of Verona, Italy

Daily interactions with objects require knowledge of their spatial location in the environment. Spatial information may be obtained through different sensory modalities, such as hearing and vision. Nevertheless, spatially-oriented behavior is performed successfully regardless of the sensory modality signaling the target position. Does the brain represent spatial targets invariantly from their sensory quality during action planning? And, if so, which brain areas provide spatial maps invariant of sensory modality? fMRI investigations showed that, during action planning, it is possible to decode upcoming movements not only from motor networks, but also from V1. V1 might serve as an "active blackboard" where task-relevant information, such as target position, is maintained. We tested this possibility with fMRI. Human subjects performed a motor task, reaching one of two spatial targets following either a visual or auditory cue. The targets were positioned on the left and right side of a central LED. While in the MR, subjects were in complete darkness and fixated the central LED. We adopted a 2x2 factorial design with factors: cue modality (visual vs auditory) and target position (left vs right). We performed multivariate analysis on the planning phase within V1 and tested if this region contained information about upcoming actions towards specific spatial targets: a) following visual cue, b) auditory cue, or c) irrespective of cue modality (cross-decoding). Overall, we showed that: 1) planning-related activity in V1 represented actions towards targets signaled by specific cue modality; 2) cross-decoding confirmed that V1 represented the same information in both tasks. Our study adds to the increasing evidence reporting the involvement of V1 in spatial working memory.

PP.29

Laminar organization of the corticostriatal projections in the macaque

Borra E[1], **Gerbella M**[1], **Rizzo M**[1], **Rozzi S**[1], **Luppino G**[1]

[1]Department of Medicine and Surgery, University of Parma, Parma, Italy

Recently, we provided evidence for converging projections from parietal, premotor, and prefrontal cortical areas involved in controlling purposeful hand actions to two different sectors of the putamen, designated as rostral and caudal hand-related input channels (IC), respectively (Gerbella et al., CerebCortex 2016). These putaminal sectors are distinct from the sector receiving projections from the primary motor cortex. To obtain more detailed information on the contribution of each area to a given striatal IC, retrograde tracer injections were placed in two macaque monkeys in the putamen, at about the level corresponding to the location of the rostral hand-related IC. The laminar distribution of the labeled corticostriatal neurons was analyzed in cortical columns 250μm wide. The results showed differences in the laminar origin of the corticostriatal projections from different frontal and parietal sectors. In the parietal cortex, the labeled cells were by far predominantly located in layer V (>80%), whereas in the frontal cortex, the laminar pattern varied among the various labeled areas and also within each labeled area. In some frontal cortical sectors, labeled cells were predominant in layer V (50-80%), but also in layer III and in layer VI

with proportions varying from one zone to another. Furthermore, in other frontal cortical sectors labeled cells tended to be more evenly distributed across layers III, V, and VI. These data provide preliminary evidence for a further degree of complexity of the architecture of corticostriatal projections, suggesting differential contributions from the various cortical sectors projecting to a specific striatal IC.

PP.30

Endfoot targeting of AQP4ex is the determining factor of the anchoring of AQP4 water channel molecules at the brain–blood interface

Palazzo C[1,3], Buccoliero C[1], Mola MG[3], Abbrescia P[1], Nicchia GP[2,3], Trojano M[1], Frigeri A[1,2]

[1]Department of Basic Medical Sciences, Neurosciences and Sense Organs, School of Medicine, University of Bari Aldo Moro, Bari, Italy; [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine,Kennedy Center, Bronx, NY, USA; [3]Department of Bioscience, Biotechnologies and Biopharmaceutic, University of Bari "Aldo Moro", Bari, Italy

AQP plays a central role in the preservation of the CNS water homeostasis, essential for the maintenance of osmotic composition and volume within the glial and neuronal compartments. The recent discovery of the extended isoform of AQP4 (AQP4ex), generated by translational readthrough, revealed a potential new mechanism of water transport regulation and polarization at the brain-blood interface. We employed CRISPR/Cas9 technology to generate an AQP4ex-KO mouse model and evaluate the effect on the overall AQP4 expression, polarization, supramolecular organization in orthogonal arrays of particles (OAPs) and neuromyelitis optica (NMO-IgG) autoantibodies binding. In WT mouse, AQP4ex, representing about 10% of all AQP4 isoforms, showed a polarized distribution in the cerebrum mostly confined to the pericapillary astrocyte endfeet. AQP4ex removal completely suppressed the specific location of AQP4 at the astrocyte endfeet and was compensated by an increased expression of the canonical isoforms (M1 and M23) indicating that the KI stop codons tightly work. Without AQP4ex, AQP4 was mislocalized in the brain parenchima, and -syntrophin expression, the selective partner for AQP4 localization, was partially altered. The supramolecular organization of AQP4 in OAPs was subtly altered. Indeed, the absence of AQP4ex slightly reduced the size of AQP4-OAPs but the number of AQP4-OAPs pools remained largely the same. The absence of AQP4 at the perivascular pole completely abolished the binding of pathogenic human neuromyelitis optica autoantibodies to the brain. This study provides the first direct evidence in vivo on the specific role of AQP4ex in AQP4 perivascular OAP assembly and confinement, as well as its involvement as a structural component of the glial endfoot membrane protein functional unit.

PP.31

Inter-individual variability and predictability of throwing actions

Maselli A[1], Russo M[2], Lacquaniti F[1,2], d'Avella A[1,3]

[1]Laboratory of Neuromotor Physiology, IRCCS Fondazione Santa Lucia, Rome, Italy; [2]Centre of Space Bio-medicine and the Department of Systems Medicine, University of Rome Tor Vergata, Italy; [3]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy

Overarm throwing is a fundamental human motor skill, involving complex whole-body motions. Skills as throwing involving many degrees-of-freedom may allow for multiple solutions and different individual strategies which may differ in their predictability. We aimed at characterizing how the predictability of a throwing action is related to the individual strategy and whether individual style affects catching performance. For this, we recorded whole-body kinematics from twenty non-trained participants performing unconstrained overarm throws at four targets. The throwing predictability was characterized at the individual level performing linear discriminant analysis on different body markers at different time intervals. Spatiotemporal maps were extracted to quantify how accurately the outgoing ball direction could be correctly predicted from the kinematics of specific body parts at different times across the action course. Accurate predictions (above 80%) could be typically made as early as 400-500 ms before ball release. The spatiotemporal structure of throw direction predictability, however, differed across individuals. In parallel, we inspected individual and gender differences in the whole-body motor behavior adopted during a throwing task. We introduced a compact description of the throwing kinematics, based on spatiotemporal PCA, which supports identity and gender identification from single throws. Furthermore, the all recorded throws naturally cluster into four throwing styles, which present analogies with the main stages of throwing skill acquisition during development. The relation between throwing styles, spatiotemporal structures of predictability maps, and associated modulations of interceptive performances is currently under investigation.

PP.32

Sonic hedgehog signalling pathway on neural stem cells during regenerative processes in a mouse model of motoneuronal loss

Vicario N[1], Bernstock JD[2], Spitale FM[1], Giunta MAS[1], Spoto G[1], Giallongo C[3], Costantino A[1], Li Volti G[3], Tibullo D[3], Gulisano M[4], Leanza G[4], Gulino R[1], Parenti R[1]

[1]Dept. of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Italy; [2]Medical Scientist Training Program, The University of Alabama at Birmingham, AL, USA; [3]Dept. of Biomedical and Biotechnological Sciences, Section of Biochemistry, University of Catania, Italy; [4]Dept. of Drug Sciences, University of Catania, Catania, Italy

Neuronal loss represents the consequence of direct or indirect insults to neurons and the major factors mediating persistent disability. Sonic hedgehog (Shh) signalling plays a key role in developing and maintenance of the central nervous system (CNS), thus holding great potential in prompting CNS repair and regeneration. Herein we aimed to study the Shh pathway activation on neural stem cells (NSCs) both in vitro and in a mouse model of spinal motoneuronal depletion induced by Cholera toxin-B conjugated to saporin (CTB-Sap). The effects of Shh signalling modulation were evaluated in vitro on NSCs finding a significant increase of the growth rate (3.0±0.6 vs. 5.3±0.6, $p<0.05$) and neurospheres diameters (109.9±2.4μm vs. 129.6±3.7μm, $p<0.01$). Moreover, we found that Shh signalling stimulation with a known steroid (i.e. clobetasol) acting on smoothened was able to stimulate NSCs proliferation and clonal expansion by inducing canonical Gli1-dependent Shh signalling. Finally, we analysed the Shh pathway in vivo, upon CTB-Sap-induced selective ablation of lumbar spinal cord motoneurons. We found that Shh stimulation induced restorative effects on locomotor impairment at 6 weeks post-lesion (8.2±1.1 vs. 5.0±1.0 footfalls over meter, $p<0.05$) and increased the mean myofiber area as compared to lesioned controls (1.4±0.2 regenerative processes and represents an exploitable pathway in tissue repair and regeneration including recovery following spinal motoneuronal degeneration.

PP.33

Glial phagocytic clearance in health and disease

Giusti V[1], Gallasch L[2], Sandre M[1], Plotegher N[1], Bubacco L[1], Greggio E[1], Erlandsson A[2], Civiero L[1]

[1]Department of Biology, University of Padova, Italy; [2]Department of Public Health and Caring Sciences, Geriatrics, Uppsala University, Sweden

An emerging picture suggests that reactive gliosis and loss of glial cells functions can contribute or trigger neurodegenerative conditions. Among glial cells, microglia and astrocytes have been shown to play phagocytic roles by engulfing synapses, apoptotic cells, cell debris, and released toxic proteins. As pathogenic protein accumulation is a key feature in Parkinson's disease (PD), compromised phagocytic clearance might participate in PD pathogenesis. On the other hand, enhanced, uncontrolled and potentially toxic glial clearance capacity could contribute to synaptic degeneration. My current research is focused in the understanding of the molecular mechanisms underlying astroglial phagocytosis, focusing on the possible implication of phagocytic dysfunction in neuronal degeneration. Several endolysosomal proteins encoded by genes implicated in genetic PD are highly expressed by microglia and astrocytes. Here, I provide some preliminary evidence that lysosomal and/or membrane trafficking defects can affect phagocytic clearance and I discuss whether the restoring or the enhancement of lysosomal function might be therapeutically relevant in PD.

PP.34

Blunting neuroinflammation with resolvin D_1 prevents early signs of Parkinson's disease in a rat model

Krashia P[1], Cordella A[1], Nobili A[1,2], La Barbera L[1,3], Federici M[1], Pisani A[1,3], Calabresi P[1,4], Viscomi MT[5], Chiurchiù V[1,2], D'Amelio M[1,2], Mercuri NB[1,3]

[1]Department of Experimental Neurosciences, IRCCS Santa Lucia Foundation, Rome, Italy; [2]Department of Medicine, University Campus Bio-medico, Rome, Italy; [3]Department of Systems Medicine, University of Rome 'Tor Vergata', Rome, Italy; [4]Department of Philosophy, Human, Social and Educational Sciences, University of Perugia, Italy; [5]Institute of Histology and Embryology, Università Cattolica del Sacro Cuore, Rome, Italy

Chronic inflammation and neuroinflammation are well-established features of Parkinson's disease (PD), seemingly contributing to midbrain dopamine (DA) neuron degeneration. Recent studies link chronic inflammation with failure to resolve early inflammation, a process operated by specialized pro-resolving mediators, including resolvins. However, the effects of stimulating the resolution of inflammation in PD – with the aim of modulating the progression of the disease – still remain unexplored.Here we show that rats overexpressing the human -synuclein (Syn) display altered substantia nigra DA neuron properties, reduced striatal DA release and motor deficits. All these defects occur prior to DA neuron degeneration. Interestingly, these early alterations are coupled with microglia activation and perturbations of inflammatory and pro-

resolving mediators, namely IFN- and resolvin D1 (RvD1). We also demonstrate that chronic administration of RvD1 in Syn rats prevents central and peripheral inflammation as well as neuronal and motor deficits. Finally, we provide unprecedented evidence that RvD1-based impairments are also present in PD patients. Our results demonstrate an imbalance between neuroinflammatory and pro-resolving processes in PD, suggesting that the early use of pro-resolving mediators could be exploited therapeutically.

PP.35

The Effects of Melanocortin 4 Receptor Agonist RM-493 on the Behavioral outcomes of Western-Type Diet

Aslan B[1], Karakaya B[2], Şirvancı S[2], Yıldırım A[1]

[1]Department of Physiology, Marmara University School of Medicine, Turkey; [2]Department of Histology, Marmara University School of Medicine, Turkey

Western diet consumption alters the metabolic functions and has a negative effect on cognitive functions. The central melanocortin system is critical for controlling body energy balance, energy expenditure and food intake. The purpose of this study was to investigate the effects of melanocortin 4 receptor (MC4R) agonist RM-493 on metabolic and cognitive functions and mediating mechanisms. Compared to the normal diet, the western type diet increased blood lipids (cholesterol, HDL, LDL), and subcutaneous injection of RM-493 reduced these values. On the other hand, in the samples collected from the hippocampus, proopiomelanocortin (POMC), MC4R and brain-derived neurotrophic factor (BDNF) were increased in western-fed groups, and RM-493 treatment further increased these values. According to the results of the elevated plusmaze test used to evaluate cognition, the administration of RM-493 to the regular diet group prolonged the time spent in the open-arm. Treatment with RM-493 resulted in a rise in BDNF expression due to the increase in MC4R expression which plays a crucial role in brain plasticity. Histological analysis of the hippocampus revealed degeneration of stratum lucidum layer and pyramidal neurons in the western type diet group and these were normalized by injection of RM-493. In conclusion, an increase in body fat caused by western type of nutrition activates the leptin-mediated pathway leading from adipose tissue, and the RM-493, with its beneficial effect against brain damage-inducing western-type feeding can improve cognitive functions.

PP.36

Evaluation of the Effects of Recurrent Dexmedetomidine on Cognitive Functions and Brain Tissue in Streptozotocin-Induced Rats with Alzheimer's Disease

Arslan M[1], Küçük A[2], Meryem Kıran M[3], Dilmen Z[1], Kavutçu M[4], Kurtipek Ö[1]

[1]Gazi University, Faculty of Medicine, Department of Anesthesiology of Reanimation, Ankara, Turkey; [2]Kütahya Health Sciences University, Faculty of Medicine, Department of Physiology, Kütahya, Turkey; [3]Yıldırım Beyazit University, Faculty of Medicine, Department of Medical Pathology, Ankara, Turkey; [4]Gazi University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

The aim of this study was to evaluate the effects of recurrent dexmedetomidine on cognitive functions and brain histopathology in the elderly rat model which has Alzheimer disease created with streptozotocin (STZ). Totally 24 aged Wistar Albino rats were divided into 4 equal groups; control (Group C), sham (Group S), Alzheimer (Group A) and Alzheimer + dexmedetomidine (Group AD). All rats in Group A and Group AD received stereotaxic injection under ketamine (100 mg / kg, i.p.) anesthesia. Midline burr hole was entered under the dura. Group A and Group AD 3 mg/kg (10 ml) were induced by administering STZ experimental Alzheimer intracerebroventricularly. Four weeks after the surgery, Group S and Group AD received 100 μg/kg (i.p) dexmedetomidine for 3 consecutive days. Each group were tested with RAM test. After 24 hours, all rats were euthanized under anesthesia and brain tissue was taken. Biochemical and hippocampus tissues were evaluated histopathologically. At the beginning, the number of RAM input-output is similar in all groups, but 3 weeks after the Alzheimer's formation RAM input-output decreased significantly. In Group AD, the number of RAM input-outputs increased significantly compared to Group A after 2nd and 3rd anesthesia applications. Glial fibrillary acidic protein levels were significantly higher in Group A compared to K and S groups in hippocampus tissue. In Group AD, it was found to be significantly lower than Group A. In group A, catalase, TBARS and PON-1 activities of brain tissue were found higher than Group K and S. TBARS activity of Group AD brain tissue was significantly lower than Group A. We concluded that recurrent dexmedetomidine administration in rats treated with STZ positively affects cognitive functions evaluated with RAM in the presence of recurrent dexmedetomidine. Also histopathological and biochemical markers supported these findings. We concluded that observational studies should be performed in large series to correlate these results with clinical applications.

PP.37

Cord blood serum (CBS) based eye drops mitigate light-induced retinal neurodegeneration

Di Marco S[1,2], Riccitelli S[1], Di Paolo M[1], Buzzi M[3], Ciavarella C[4], Versura P[4], Campos E[4], Bisti S[1,2]

[1] Department of Applied Clinical Science and Biotechnology, University of L'Aquila, L'Aquila, Italy; [2]Istituto Nazionale di Biostrutture e Biosistemi (INBB), Roma, Italy; [3]Emilia-Romagna Cord Blood Bank-Transfusion Service, S. Orsola-Malpighi Teaching Hospital, Bologna, Italy; [4]Ophthalmology Unit, University of Bologna and S. Orsola Teaching Hospital, Bologna, Italy

Age-related macular degeneration (AMD), is a multifactorial disease leading to blindness condition. Degeneration is centered in the fovea, the central part of the retina and has two forms “dry” and “wet”. A consolidated model to mimic this pathology in rats is the light damage model (LD), obtained by exposing albino rats, raised at 5 lux, for 24 hours to 1000 lux. In this model degeneration starts from an area in the dorsal side of the retina, called “hot-spot”, which expands over time. Cord blood serum (CBS) is an extract full of chemokine and trophic factors and is potentially a good candidate as neuroprotectant. It is already in use in clinical practice for corneal pathologies, we propose its use for photoreceptor’s degenerations also on the basis of case reports in glaucoma patients. Animals were treated four times/per day starting 7 days before LD and were additionally treated for 7 days after LD. Electroretinographic recordings (f-ERG) were performed at the end of this period and subsequently animals were sacrificed for histological evaluation and immunolabelling. CBS treatment mitigated the reduction in f-ERG response after LD and reduced the extension of the "hot spot". In addition, the morphology of outer nuclear layer was maintained together with a reduction in microglia migration and activation. Interestingly, the treatment did not modulate reactive gliosis and activation of self-protective mechanism (FGF2). In Conclusion, our results show that CBS-based eye drops might be successfully used to mitigate retinal neurodegenerative processes. Moreover, compared to previous studies, where only single trophic factor has been used, the effective dose used in this work, is at least one order of magnitude smaller for each of the dosed neurotrophin.

PP.38

The role of altered voltage-gated currents in motor-neuron degeneration: Analysing the spinal and bulbar muscular atrophy (SBMA) case

Jimenez Garduño AM, Martinez Rojas VA, Juarez Hernandez LJ, Musio C

UDLAP, Mexico/ IBF Trento, Italy

Voltage-gated currents are the key players of cell ionic conductance; therefore, the gating of every voltage-gated channel (VGC) present in all neurons is carefully coordinated in the membrane to set its excitability conditions. The function of the VGCs can be altered by many factors; however, oligomer accumulation originated from misfolded proteins have gained attention in the last years since they play a key role in the genesis of high prevalent neurodegenerative diseases such as Alzheimer´s, Parkinson´s and Huntington. In SBMA the Androgen Receptor (AR) exerts an abnormal amount of polyQ repeats, which causes its misfolding and cell accumulation leading to motor neurodegeneration. Lower motor neurons are well-known targets of excitotoxicity, an overstimulated state that can accelerate underlying pathological conditions and lead to degeneration. It is still unknown if excitotoxic conditions are related to oligomer accumulation, and if they play a role in the pathogenesis of SBMA. Excitotoxicity is mainly associated to excess of glutamatergic stimulus; however, alterations of VGCs can also cause a hyper-excited state with normal glutamate function. Our group first has analysed Na+, K+, Ca2+ and Cl- voltage-gated currents in SBMA cell models and has showed different alterations of each current. Our results show that the pathological conductive state predisposes neurons to be hyper-excitable. Furthermore, pharmacological rescuers that ameliorate the SBMA phenotype in animal models restore VGCs activity. These evidences reveal that VGCs are functionally altered in SBMA cells, indicating that motor neurons may be vulnerable to an excitotoxic damage in vivo. [Work funded with the contribution of Fondazione CARITRO Trento (VAMR, CM)]

PP.39

Stepping in neonates

Sylos-Labini F[1,2], Magnani S[3], Cappellini G[1,2], La Scaleia V[1,2], Fabiano A[4], Picone S[4], Paolillo P[4], Di Paolo A[5], Lacquaniti F[1,2,6], Ivanenko Y[2]

[1]Center of Space BioMedicine of the University of Rome Tor Vergata, Rome, Italy; [2]Laboratory of Neuromotor Physiology of the IRCCS Santa Lucia Foundation, Rome, Italy; [3]Sapienza University of Rome, Rome, Italy; [4]Neonatology and Neonatal Intensive Care Unit, Casilino Hospital, Rome, Italy; [5]Neonatology and Neonatal Intensive Care Unit of the Ospedale San Giovanni, Rome, Italy; [6]Department of Systems Medicine of the University of Rome Tor Vergata, Rome, Italy

Stepping on ground can be evoked in human neonates, though it is rather irregular and stereotyped heel-to-toe roll-over pattern is lacking. Such investigations can provide insights into the role of contact- or load-related proprioceptive feedback during early development of locomotion. However, the detailed characteristics of foot placements and their association with motor patterns are still incompletely documented. We elicited stepping in 33 neonates supported on a table. Unilateral limb kinematics, bilateral plantar pressure distribution and EMG activity from up to 11 ipsilateral leg muscles were recorded. Foot placement characteristics in neonates showed a wide variation. In ~25% of steps, the swinging foot stepped onto the contralateral foot due to generally small step width. In the remaining steps with separate foot placements, the stance phase could start with forefoot (28%), midfoot (47%), or heel (25%) touchdowns. Despite forefoot or heel initial contacts, the kinematic and loading patterns markedly differed relatively to toe-walking or adult-like two-peaked vertical force profile. Furthermore, while the general stepping parameters (cycle duration, step length, range of motion of proximal joints) were similar, the initial foot contact was consistently associated with specific center-of-pressure excursion, range of motion in the ankle joint, and the center-of-activity of extensor muscles. In sum, we found a variety of footfall patterns in conjunction with associated changes in motor patterns. These findings suggest the potential contribution of load-related proprioceptive feedback and/or the expression of variations in the locomotor program already during early manifestations of stepping in human babies.

PP.40

The Cerebral Microvascular endothelial cells: are they a new target for AD therapy?

Forcaia G[1], Formicola B[1], Negri S[2], Blasi P[3], Re F[1], Moccia F[2], Sancini G[1]

[1]School of Medicine and Surgery, Nanomedicine Center, Neuroscience Center, University of Milano Bicocca, Monza, Italy; [2] Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Italy; [3] Department of Pharmaceutical Sciences, University of Camerino, Perugia, Italy

The blood-brain barrier (BBB) is the interface between blood components and neural tissue within the brain and spinal cord, which regulates homeostasis of the brain microenvironment for correct neuronal activity. The BBB is composed of four main cellular elements, namely, cerebral microvascular endothelial cells (CMECs), astrocyte end-feet, microglial cells, and pericytes. After years of research, CMECs are now recognized not only as a simple anatomical and physiological barrier, but also as a highly active metabolic system. Moreover, CMEC dysfunction can trigger brain tissue damage, such as neurodegenerative diseases, and these injuries exacerbate CMEC dysfunction via a feedback loop. In brain cells P2YR expression is increased by stimulation with interleukin-1β (IL-1β) whose levels are elevated in AD, in part due to nucleotide-stimulated release from glial cells. Other results indicate that oligomeric β-amyloid peptide (Aβ1-42) increases nucleotide release from astrocytes, which would serve to activate upregulated P2YRs in neurons. Recent data suggest that P2YR upregulation by IL-1β and subsequent activation by UTP are neuroprotective, since this increases the non-amyloidogenic cleavage of amyloid precursor protein. We previously demonstrated that the administration of liposomes functionalized with phosphatidic acid and an ApoE-derived peptide (mApoE-PA-LIP) reduced brain beta-amyloid burden and ameliorated impaired memory in AD mice. The purinergic stimulation of Ca2+ signaling in the human CMEC/D3 cells acts via the G-protein-coupled P2YR. Here we put in evidence a prolonged intracellular calcium wave operated by mApoE-PA-LIP upon ATP stimulus in human CMEC/D3, which could be exploited therapeutically providing new insights in AD onset and progression.

PP.41

Regulation of Aquaporin-4 isoforms expression investigated by a CRISPR/Cas9 genome editing mouse model

Pisani F[1], Frigeri A[2], De Bellis M[1], Svelto M[1], Nicchia GP[1]

[1]Dept of Bioscience, Biotechnology and Biopharmaceutics, University of Bari "Aldo Moro"; [2] School of Medicine, Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari "Aldo Moro"

The CNS plasma-membrane water channel aquaporin-4 (AQP4) is expressed as two main isoforms, M1 and M23, whose ratio controls its structure-function relationship. In cultured cells translational mechanisms control M1/M23 ratio but the overall mechanism regulating isoforms expression *in vivo* is largely unknown. To investigate the physiological role of AQP4 isoforms and the possible existence of mechanisms for regulating their expression and crosstalk *in vivo*, we have generated a M23-null mouse model ($M23^{null}$) by CRISPR/Cas9 genome editing. M1 and M23 expression was analyzed at transcriptional, translational and post-translational levels comparing WT, heterozygous and $M23^{null}$ mice spinal cord samples. Western blot analysis shows a reduction of M1 in heterozygous and a strong reduction of M1

especially in adult M23null mice. No evidence of M1-degradation was observed in $M23^{null}$ mice. M1-specific qPCR indicates that M1mRNA transcription is unaffected in M23null. RT-PCR for a dominant negative alternative spliced AQP4 isoform excludes this splicing in $M23^{null}$. Co-transfection of M1 and M23 isoform in HEK cells and cycloheximide-based protein stability assay indicate that co-expression with M23 does not change the stability of M1 protein. *In silico* analysis of WT mice by CatRAPID software predicts a physical interaction between M23 protein and M1mRNA. All these data show that in vivo the M23 controls M1 expression and that transcription, splicing and protein stability are not involved. This indicates that some M23-dependent translational control mechanism occurs. In silico prediction suggests the existence of a potential translational feedback that could be compromised in $M23^{null}$ and that may be involved in the M1/M23 ratio control in physiological conditions in spinal cord.

PP.42

Anti-inflammatory and cognitive effects of Interferon-β1a (IFNβ1a) in a rat model of Alzheimer's disease

Di Liberto V[1], Frinchi M[1], Nuzzo D[2], Scaduto P[1], Plescia F[3], Massenti MF[3], Di Carlo M[2], Cannizzaro C[3], Cassata G[4], Cicero L[4], Ruscica M[5], Belluardo N[1], Grimaldi LM[5] Mudò G[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostic, University of Palermo, Italy; [2]Institute of Biomedicine and Molecular Immunology "Alberto Monroy" (IBIM), Consiglio Nazionale delle Ricerche (CNR), Palermo, Italy; [3]Department of Sciences for Health Promotion and Mother and Child Care, University of Palermo, Italy; [4]Experimental Zooprophylactic Institute of Sicily "A. Mirri", Palermo, Italy; [5]Neurology Department, Fondazione Istituto Giuseppe Giglio, Cefalù (PA), Italy

Aβ1-42 peptide abnormal production is associated with the development and maintenance of neuroinflammation and oxidative stress in brains from Alzheimer disease (AD) patients. Since, suppression of neuroinflammation may represent a suitable therapeutic target in AD, we evaluated the efficacy of IFNβ1a in attenuating cognitive impairment and inflammation in an animal model of AD. A rat model of AD was obtained by intra-hippocampal injection of Aβ1-42 peptide (23μg/2μl). After 6 days, 3.6 μg of IFNβ1a was given subcutaneously (s.c.) for 12 days. Using the novel object recognition (NOR) test we evaluated changes in cognitive function. Measurement of pro-inflammatory or anti-inflammatory cytokines, reactive oxygen species (ROS) and SOD activity levels was performed in the hippocampus. We showed that treatment with IFNβ1a was able to reverse memory impairment and to counteract microglia activation and upregulation of pro-inflammatory cytokines (IL-6, IL-1β) in the hippocampus of Aβ1-42 injected rats. The anti-inflammatory cytokine IL-10, significantly reduced in the Aβ1-42 animals, recovered to control levels following IFNβ1a treatment. IFNβ1a also reduced ROS and lipids peroxidation and increased SOD1 protein levels in the hippocampus of Aβ1-42 injected rats. This study shows that IFNβ1a is able to reverse the inflammatory and cognitive effects of intra-hippocampal Aβ1-42 in the rat. Given the role played by inflammation in AD pathogenesis and the established efficacy of IFNβ1a in the treatment of inflammatory diseases of the central nervous system, such as multiple sclerosis, its use may be a viable strategy to inhibit the pro-inflammatory cytokines and oxidative stress cascade associated with Aβ deposition in the hippocampus of AD patients.

PP.43

Pindolol Reduces The Inhibition Induced by 5-HT in DRN Neurons of Mice

Yaman B, Bal R

Department of Physiology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Serotonin (5-HT) has important role in the pathophysiology of the mood disorders like major depression, anxiety disorders in central nervous system. Especially dorsal raphe nucleus (DRN) neurons send serotonergic projections to postsynaptic neurons in forebrain regions like hippocampus and affect mood of individuals. These serotonergic neurons also affect themselves through 5-HT1A autoreceptors in DRN region. If there are more serotonin molecules in neuro synaptic junction and somatodendritic part of the serotonergic neurons in DRN in some conditions like usage of serotonin reuptake inhibitor drugs, these molecules affect negatively the same neurons and hyperpolarize their resting membrane potential by triggering K efflux in physiological conditions. This is undesirable and reduce therapeutic effects of antidepressants. This effect emerge through somatodendritic serotonin receptors, which are G protein coupled receptors. Pindolol is known as partial 5-HT1A receptor agonist and can prevent increased serotonin effect in DRN neurons. In the current study, we aimed to investigate effects of pindolol on the current induced by 5-HT ($I_{5\text{-}HT}$) in DRN neurons in mice. We used electrophysiological whole cell patch clamp technique in the neurons of DRN slices from 28-33 days old Balb/c mice. The recordings were performed in standard artificial cerebrospinal fluid (aCSF). The current induced by 5-HT was blocked in DRN neurons of mice by WAY100135, a 5-HT1A receptor antagonist, and pindolol blocked $I_{5\text{-}HT}$ like WAY100135. Besides pindolol also blocked outward

current induced by 8-OHDPAT, a spesific 5-HT1A receptor agonist. In conclusion, our findings suggest that effect of serotonin in DRN neurons of mice occurs via somatodendritic 5-HT1A receptors. These receptors can be blocked by pindolol and so it can be inferred that increased hyperpolarization and inhibition in serotonergic DRN neurons triggered with antidepressant usage can be prevented by pindolol, a partial 5-HT1A receptor agonist.

PP.44

Alpha-band cortico-muscular coherence predicts visual sensitivity

Tomassini A[1], Hilt P[1], Fadiga L[1,2], D'Ausilio A[1,2]

[1]Center for Translational Neurophysiology of Speech and Communication (CTNSC), Istituto Italiano di Tecnologia (IIT), Ferrara, Italy; [2]Università di Ferrara, Dipartimento di Scienze Biomediche e Chirurgico Specialistiche, Ferrara, Italy

Brain oscillations are now recognized to have a key role in regulating cortical excitability and orchestrating the interactions between relevant populations of neurons. Recent evidence suggests that oscillations-based mechanisms may structure sensorimotor integration in humans. Here, we exploited the ongoing oscillations that can be measured in the motor output (i.e., in the force) as a window into the central motor rhythms. We recorded EEG/EMG activity while participants (n=20) performed continuous isometric contraction with their right hand for 5 s; at random times during hand contraction, we briefly (16 ms) presented a visual flash at the individual luminance threshold and participants had to report whether they had seen or not seen the stimulus (50-50%, hits-misses). We found that hand force fluctuates at ~10 Hz, which is commonly reported as the main frequency of physiological tremor. This rhythm is coupled (phase coherent) with an EEG alpha rhythm, with the central rhythm driving the peripheral one. Remarkably, just before stimulus presentation (~200 ms), brainforce alpha phase synchronization is significantly stronger for hits compared to misses. Visual perception is thus not only dependent on the oscillatory activity in sensory areas but on its continuous, dynamic interplay with the ongoing motor activity. These findings outline a new and promising view on the intimate interconnection between sensory and motor functions and its possible neurophysiological substrate.

PP.45

Progressive epileptic encephalopathy associated with a novel HCN_2 mutation

Binda A[1], Murano C[1], Di Francesco JC[2,3], Castellotti B[4], Milanesi R[5], Ragona F[6], Freri E[6], Canafoglia L[2], Franceschetti S[2], Solazzi R[6], Granata T[6], Gellera C[4], Rivolta I[1]

[1] School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; [2]. Clinical Neurophysiology and Epilepsy Center, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [3]. Department of Neurology and Epilepsy Center, Milan Center for Neuroscience, San Gerardo Hospital, University of Milano-Bicocca, Monza, Italy; [4]. Unit of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy; [5]. Molecular Physiology and Neurobiology, The PaceLab, Department of Biosciences, Università degli Studi di Milano, Milan, Italy; [6]. Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

So far mutations in HCN2 gene, encoding for the hyperpolarization-activated cyclic nucleotide-gated channel 2, have been related to mild epileptic clinical phenotypes. In this study, a novel HCN2 mutation was found in a proband, now aged 7 years old, with a congenital encephalopathy characterized by drug resistant epilepsy, severe developmental delay, ataxia, dystonia and cerebral visual impairment. A convulsive status epilepticus at 5 months of age marked the onset of epilepsy. The HCN2 mutation affects an aminoacid located in the S6 transmembrane helix (p.Gly460Asp) and is carried in heterozygosis. To describe the functional consequences of the mutant channel, whole-cell patch-clamp experiments were performed in HEK293 cells expressing HCN2 wild type (WT) or p.Gly460Asp channel. A coexpression of the same amount of plasmid encoding for the wt or the mutant form of the channel mimed the heterozygous condition. A complete abolishment of the current was observed considering the mutant compared to the WT channel (-9.9±1.2 pA/pF, n=20 vs -31.2±8.4 pA/pF, n=44 respectively; $p<0.05$) and the heterozygous condition led to a significant reduction of the current density (-20.1±5.1 pA/pF n=35; $p<0.05$). WT and heterozygotic channels shared overlapping activation curves (V1/2 and k -92.9±0.3 mV and 5.6±0.3, n=29 vs -91.6±0.2 mV and 5.6±0.2, n=16 respectively) and no significant differences were present in their kinetics of both activation and deactivation. In conclusion, this is the first study linking HCN2 to progressive epileptic encephalopathy. The Gly460Asp mutation seems to act as a loss-of-function that could potentially affect the control of neuronal excitability and therefore explain the proband pathological condition.

PP.46

Dynamics of changes in heart rate variability after prolonged exposure to dark

Svorcjr P, Jarkulis V, Skrda D, Michalcak T, Kmet S, Marko A

Dep. of Physiology and Pathophysiology, Faculty of Medicine, University of Ostrava, Czech Republic

Aim was to evaluate the influence of long exposure of dark on heart rate variability (HRV) in young people. This method is currently used in psychology with an improvement of mental health in people under constant stress. So far, no evidence of changes in autonomic nervous system function were measured after a long term stay in the dark. 29 students (19 to 26 years) were placed into a room with max. darkness for 96 h. The room met all the requirements for stay, (quiet, socially isolated place). The participants received food and drinks without using of any device emitting light or showing the actual time. Orthostatic test was used for measuring power LF, HF and LF/HF ratio. The first measurement was performed the day before starting the therapy, next measurement was taken 30 minutes after completing the session, followed by two more measurements in the fourth and the seventh day after exiting the chamber. The power HF showed a significant change between the first and the second measurement (increasing activity of HF) in the horizontal position (p 0,05), with a similar trend observed during consecutive measurements. The LF/HF ratio pointed non-significantly to a modulatory influence of sympathetic and parasympathetic nervous system during the test. 96 h. of therapy and subsequent time disorientation likely influenced the parasympathetic nervous system regulation after completing the therapy. This discovery was further supported by a lowered heart rate which can also affect cardiovascular system.

PP.47

Loopomics: explaining the complexity of life by conjugating physiology and control theory

Burlando B[1,2], Martinoia S[3,2], Massobrio P[3], Palmero S[1], Blanchini F[4], Giordano G[5]

[1]Dept. of Pharmacy, University of Genova, Italy; [2]Biophysics Institute, National Research Council, Genova, Italy; [3]Dept. of Informatics, Bioengineering, Robotics and Systems Engineering, University of

Genova, Italy; [4]Dept. of Mathematics, Computer Science and Physics, University of Udine, Italy; [5]Delft Center for Systems and Control, Delft University of Technology, Netherlands

The human body consists of about 3.7´1013 cells, while a rough average of 109 chemical reactions per second occur inside each cell, yielding a total of about 1022 per second. The huge complexity of this system, and the lack of a synthetic theory, inevitably affect the possibility of explaining events and processes, thus setting limits e.g. to the understanding and management of recalcitrant diseases like allergies, autoimmune and metabolic syndromes, cardiovascular disorders, neurodegenerative processes, and cancer. Life sciences principally model the body's functioning by using open chains (i.e. open loops), but the body is a self-sustained system maintaining steady state, meaning that its processes must be conversely regulated by closed loops. Loop dynamics can lead to stable equilibrium points, such as the regulation of body temperature and blood pressure, or give rise to sustained oscillations, like hormone fluctuations, pacemaker activities, and neural oscillatory activity. We considered endocrine and neural networks from literature data and experimental recordings, and then modeled them in terms of functional agents (loop nodes) and their interactions (loop arcs), allowing us to investigate loop dynamics at different scales. We performed a structural analysis of the resulting dynamic loop network, described in terms of an ordinary-differential-equation model, and of the associated interaction matrix. Our analysis revealed the presence of candidate oscillators, each admitting a single equilibrium point that can either be stable or give rise to oscillatory instability. Such a result could represent a recurrent motif of loop arrangements, both within and among cells and organs, possibly leading to a general paradigm with direct repercussions on medicine and health care.

PP.48

DSS-induced colitis generates chronic visceral hypersensitivity in rats

López-Estévez S, Martínez V

Department of Cell Biology, Physiology and Immunology; Neuroscience Institute, Universitat Autònoma de Barcelona, Spain

Visceral hypersensitivity is a key component of functional gastrointestinal disorders in humans. This characteristic has been difficult to mimic in animal models. We assessed the validity of dextran sulfate sodium (DSS)-induced colitis in the development of long-term colonic hypersensitivity in rats and the potential mechanisms involved. Adult, female SD rats were used (n=23). Colitis was induced by exposure to a 5% solution of DSS during a 7-day period (day 0-7). Colonic sensitivity was assessed by determining pain-related visceromotor responses to isobaric colorectal distension (CRD; 12 pulses at 80 mmHg/30 s duration, 5 min interval between pulses). Colonic sensitivity was assessed before (day 0) and after colitis induction (days 17-35; every 3-4 days). Changes in the expression of immune and nociceptive markers (RT-

qPCR) were assessed in colon and lumbosacral spinal cord at days 0, 21 and 35. During colitis induction, body weight loss and clinical signs were observed. In animals with colitis, responses to CRD were increased when compared with healthy animals. This enhanced response was sustained in time, lasting up to 35 days post-colitis induction ($P<0.05$ vs. response to CRD in control rats). At spinal level, INF-γ, IL-1β and IL-10 showed a time-related upregulation in DSS-treated rats at day 35 post-induction (all $P<0.05$ vs. control). Expression of sensory related markers (cannabinoid 1, 2 and μ-opioid receptors) was also up-regulated in the spinal cord of DSS-treated rats, up to day 35 post-colitis. These data show that DSS-induced colitis generates a long-lasting state of visceral hypersensitivity, reminiscent of the state of hypersensitivity observed in humans with functional gastrointestinal disorders, and can be used as an animal model to study such a condition.

PP.49

Comparison of housekeeping genes for qPCR analysis in rat hippocampus: chronic depression model

Kurar E[1,2], Eroglu C[1,2], FarukUguz[1,3], Mehmet Ak[1,3], Koca RO[4], Solak H[4], Koç A[5], Sahin Z[6], Kutlu S[1,4]

[1]Necmettin Erbakan University Neuroscience Research and Application Center (NENRC); Konya, Turkey; [2]Department of Medical Biology, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Turkey; [3]Department of Psychiatry, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Turkey; [4]Department of Physiology, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Turkey; [5]Department of Physiology, Hitit University, Faculty of Medicine, Çorum; [6]Department of Physiology, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey.

Housekeeping genes (HKGs) are often used for normalization of quantitative real time polymerase chain reaction (RT-PCR) data. It was however suggested that expression of HKGs can be influenced by different experimental applications, which also may cause wrong normalization and interpretation of the data. Objective of this study was to select appropriate HKGs to be used in the normalization for gene expression of rat hippocampus in chronic depression model with or without sertralin treatment (an antidepressant). Chronic experimental depression model was constituted in 24 female adult rats which were divided into four groups (n=6) including control, depression, depression + 1 mg/kg sertraline and depression + 10 mg/kg sertraline. Drug infusions were subcutaneously performed for 14 days using osmotic minipumps. Hippocampal tissues were used for total RNA isolation and cDNA synthesis. mRNA level expressions of 8 HKGs were evaluated using quantitate RTPCR. Average expression stability values (M) were separately calculated for general and each groups and using BestKeeper, geNorm and NormFinder software. Both results of geNorm and NormFinder algorithms indicated similar M values in general and PGK1 was the most reliable reference gene. NormFinder suggested that PGK1 and CycA were the best combination of two genes with M=0,424 value. PGK1 and CyCA appears to be the most reliable HKGs for studying mRNA level gene expression studies of chronic depression model in rat hippocampus. Financially supported by TUBITAK (Project No:215S616).

PP.50

Expression of the glucose transporter GLUT12 in mouse models of Alzheimer's disease and aging

Gil-Iturbe E[1,2], Solas M[3,5,6], Cuadrado-Tejedo M[4,5,6], Ramírez MJ[3,6], Lostao MP[1,2,6]

[1]University of Navarra, Department Nutrition, Food Science and Physiology, Pamplona, Spain; [2]University of Navarra, Nutrition Research Centre, Pamplona, Spain; [3]University of Navarra, Department of Pharmacology and Toxicology, University of Navarra, Pamplona, Spain; [4]University of Navarra, Division of Neurosciences, CIMA, Pamplona, Spain; [5]University of Navarra, Pathology, Anatomy and Physiology Department, Pamplona, Spain; [6]IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

The brain requires high consumption of energy which mainly comes from glucose. Several glucose transporters, belonging to the GLUT family, have been identified in the brain, being GLUT1 and GLUT3 the most relevant. Interestingly, previous studies from our group revealed GLUT12 expression in the frontal cortex of human brain of young subjects; this expression increased in aged subject and was even greater in aged Alzheimer's disease (AD) patients. In AD there is an important reduction of glucose uptake, as well as GLUT1 and GLUT3 expression. However, the cause of glucose transporters alteration is still unknown. Aging is a risk for AD. The aim of this study was to investigate if the upregulation of GLUT12 in AD is related with aging and with Aβ deposition. For that, we studied GLUT12 protein expression in comparison with GLUT1, GLUT3 and GLUT4, in two mouse models of AD (Tg2576 and APP/PS1). We also evaluated the effect of age with the accelerate senesce model SAMP8, and the effect of β-amyloid plaques deposition, using mice after intracerebroventricular injection of Aβ peptide. In the frontal cortex of both AD models at 16 months, GLUT12 expression was increased; GLUT1 and GLUT3 were decreased, while GLUT4 levels did not change. In the SAMP8 mice, GLUT12 and GLUT4 were upregulated at 10 months, while GLUT1 and GLUT3 levels did not show significant changes with the

age. In the hippocampus of Aβ injected mice, GLUT12 expression increased while GLUT4 expression was not modified. Consistent with the results found in AD frontal cortex, GLUT3 and GLUT1 protein were downregulated. In summary, both beta-amyloid and aging upregulate GLUT12 protein expression. The increase of GLUT12 in AD may suggest a compensatory mechanism to counter balance the reduction of GLUT1 and GLUT3.

PP.51

Effects of antipsychotic drug administration on antioxidative defense enzymes in male rat liver

Nikolić-Kokić A[1], Tatalović N[1] Nestorov J[2], Oreščanin-Dušić Z[1], Vidonja-Uzelac T[1], Blagojević D[1], Spasić M[1], Miljević Č[3]

[1] Department of Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [2] Department of Biochemistry, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [3] Institute of Mental Health, School of Medicine, University of Belgrade, Belgrade, Serbia

Antipsychotics are medication choices in the treatment of schizophrenia. Most antipsychotics are dopamine receptor antagonists what explains clinical efficacy, but also numerous side effects. Treatment with atypical antipsychotic drugs (APD) (second-generation antipsychotics SGA), induces less pronounced adverse effects, but some are still severe including drug induced liver disease that may be attributed to oxidative stress. The aim of this study was to examine whether some APD like clozapine, ziprasidone or sertindole treatment affected the liver antioxidant defense system contributing to the common APD-induced adverse effects in liver. Male 3-month-old Wistar rats were treated for 28 days with a recommended daily dose of APD therapy. Protein level and the activity of antioxidant enzymes: superoxide dismutase - SOD, catalase - CAT, glutathione peroxidase - GPx, glutatione reductase - GR, as well as glutathione-S-transferases - GSTs activity were determined in rat liver. Treatment with clozapine led to increase in both protein level and activity of SOD, as well as GPx and GR, while CAT activity was decreased. Sertindole treatment increased protein level and activity of GPx only, and decreased CAT activity. Ziprasidone treatment did not affect antioxidative enzymes. GSTs activities were significantly increased only in rats treated with closapine. Changes in the amount and activity of SOD, GPx and GR on one hand and decreased CAT activity on the other, indicate disturbance of antioxidative balance in liver cells of treated animals, which may be one of the explanation of liver injury observed in closapine and sertindole treated patients.

PP.52

Combined Lipoic Acid and Vitamin D3 on Astrocytes as a Way to Prevent Brain Ageing by Induced Oxidative Stress and Iron Accumulation

Molinari C[1], Morsanuto V[1], Ruga S[1], Notte F[1], Gaetano L[2], Uberti F[1]

[1]Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy; [2]Contract Research Organization (MIAC AG) affiliated to the University Hospital of Basel, Basel, Switzerland

Brain ageing is a complex multifactorial process characterized by gradual and continuous loss of neuronal functions. It is hypothesized that at the basis of brain ageing as well as age-related diseases there is an impairment of antioxidant defense system leading to an increase of oxidative stress. In this study two different biological aspects involved in brain ageing and neurodegeneration have been investigated: oxidative stress and iron accumulation damage. In primary mouse astrocytes the stimulation with 50 μM lipoic acid (LA) and 100 nM vitamin D (vitD) was first investigated in a time-course study to determine the dosages to be used in combination and then in a permeability test using an in vitro Blood-Brain Barrier. In a second set of experiments the role of oxidative stress was investigated pre-treating astrocytes with 200 μM H2O2 for 30 min. The ability of vitD and LA alone and combined together to prevent or repair the damage caused by oxidative stress was investigated after 24h of stimulation by MTT test, mitochondrial membrane potential measurement and Western blot analysis. To induce neurodegeneration, cells were pretreated with 300 μM catalytic iron for 6 days and then treated with vitD and LA alone and combined for additional 6 days to investigate the protection exerted by combination, analyzing viability, ROS production, iron concentration and activation of intracellular pathways. In our study the combination of LA and vitD showed beneficial effects on viability of astrocytes, since the substances are able to cross the brain barrier. In addition, combined LA and vitD attenuated the H2O2-induced apoptosis through the mitochondrial-mediated pathway. The combination was also able to counteract the adverse conditions caused by iron, preventing its accumulation. All these data support the hypothesis of the synergistic and cooperative activity exerted by LA and vitD in astrocytes indicating a possible new strategy to slow down ageing.

PP.53

The effects of clozapine, ziprasidone and sertindole treatment on lipid profile in rats

Blagojević D[1], Nikolić-Kokić A[1], Tatalović N[1], Oreščanin- Dušić Z[1], Vidonja Uzelac T[1], Spasić M[1], Miljević Č[2]

[1]Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia; [2]Institute of Mental Health, School of Medicine, University of Belgrade, Belgrade, Serbia

Different studies reported that patients with schizophrenia had lower cholesterol levels in blood compared to healthy controls. However, it is unclear whether changed cholesterol concentration and lipid status are a consequence of changed neurotransmitter metabolism intrinsic to origin of the disease or affect central nervous system neurotransmission and influence the development of psychiatric disorders. Anyway, schizophrenia treatment with atypical antipsychotic drugs (APD) additionally influences lipid status in blood and all families of APD agents can cause severe side effects including dyslipidemia. Therefore, the aim of the present study was to evaluate effects of 28-day treatment with recommended human daily dose of APD: ziprasidone, clozapine, sertindole on 3 months old healthy male rats' levels of cholesterol, HDL, LDL, and triglyceride in the blood serum. Our results showed a decrease of both triglycerides and cholesterol in clozapine treated rats. In ziprasidone treated rats triglycerides and HDL were lower comparing to untreated controls. Treatment with sertindole had no effects on lipid blood serum levels. However, there were no changes of index of atherosclerosis in APD treated rats. Our results showed that treatment with clozapine and ziprasidone influence blood serum levels of lipids indicating altered lipid metabolism.

PP.54

Olfactory sensitivity is associated with body mass index, polymorphism in the odor binding-protein (OBPIIa) gene and inflammatory bowel disease

Sollai G[1], Melis M[1], Mastinu M[1], Magri S[2], Chicco F[2], Usai P[2], Tomassini Barbarossa I[1], Crnjar R[1]

[1]Department of Biomedical Sciences, University of Cagliari, Italy; [2]Department of Medical Sciences and Public Health, University of Cagliari, Italy

Smell strongly contributes to food hedonistic evaluation, determines food choice and its possible consumption. A reduction or loss of smell has been related to malnutrition problems. Some patients with inflammatory bowel disease (IBD), mainly Crohn's disease (CD) and ulcerative colitis (UC), present weight loss, while others gain excessive weight. We evaluated the olfactory performance in IBD patients and age-matched healthy controls (HC), and its association with BMI and polymorphism in the human odor binding-protein (OBPIIa) gene. We assessed the olfactory performance in 152 subjects (CD patients, n=44; UC patients, n=56; HC, n=52), based on the olfactory Threshold, Discrimination and Identification score (TDI score), measured using the "Sniffin' Sticks" Test. Subjects were genotyped for the rs2590498 polymorphism of the OBPIIa gene, whose major allele A was associated with a higher retronasal perception as compared to the minor allele G. Subjects classified as hyposmic, by the total TDI, showed a higher BMI than normosmic ones. CU patients exhibited a lower TDI score, but a higher BMI, than CD patients and HC. D score was the main determinant of the overall performance in CD patients, while T score was for HC and CU patients. A significant effect of the OBPIIa genotypes was found on the TDI score of HC, but not on the TDI score of CD and UC patients. Our findings show an inverse relationship between olfactory performance and BMI, and a direct relationship between smell and health status; therefore, IBD patients present a higher BMI than controls, since they have a reduced olfactory sensitivity. They seem to present olfactory disfunctions related to cognitive factors, as also suggested by the lack of relationship between olfactory performance and OBPIIa genotypes.

PP.55

SNC80, an agonist of delta-opioid receptors, differently affects neonatal hippocampal neurons from male and female rats

Moravcikova L, Moravcik R, Dremencov E, Lacinova L

Institute of Molecular Physiology and Genetics, Center of Biosciences Slovak Academy of Sciences, Slovakia

Three types of opioid receptors are currently known: mu, delta (DOR) and kappa. DORs are implied in chronic pain treatment and they may be also perspective target in the treatment of mood disorders, like depression and anxiety. The aim of our work was to investigate effect of DOR activation on excitability of hippocampal neurons in vitro. Experimental object were hippocampal neurons from newborn female or male rats cultured separately for 8-13 days in vitro. After this period half of neurons was exposed to DOR agonist SNC80 in concentrations 0.1, 1 and 10 μM for 24, 48, and 72 hours. Spontaneous and depolarization-activated generation of action potentials (APs) was assessed in whole-cell patch clamp. Spontaneous

activity was recorded for 5 min from an actual resting membrane potential of each neuron. Depolarization-activated firing was evoked from a membrane potential of -70 mV by a rectangular current pulse. SNC80 suppressed depolarization-activated AP firing in hippocampal neurons from both male and female rats. This effect was slightly more prominent in neurons from male pups. We have shown that an acute application of 0.1-10 μM SNC80 moderately suppressed depolarization-activated APs in hippocampal neurons in mixed male/female culture. Chronic effect is in line with this observation. Acute application of SNC80 increased spontaneous activity only in a concentration of 100 μM. Chronic application of SNC80 facilitated spontaneous activity in neurons from male, but not from female rats and was effective already in lower concentrations suggesting more complex underlying mechanism present only in neonatal neurons from male pups. To conclude, we have shown that different regulatory pathways related to DORs are active in neonatal hippocampal neurons from male and female pups. Supported by a grant APVV-15-0388.

PP.56

Gating properties of Cav3.3 T-type channel are modulated by highly conserved proximal region in channel carboxy terminus

Jurkovicova Tarabova B[1], Cmarko L[2], Rehak R[3], Zamponi GW[3], Lacinova L[1], Weiss N[2]

[1]Center of Biosciences, Institute of Molecular Physiology and Genetics, Academy of Sciences, Bratislava, Slovakia; [2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic; [3]Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Canada

Low voltage-activated T-type calcium channels with specific voltage dependent gating properties contribute significantly to neuronal excitability in normal and pathological conditions. We aimed to investigate the channel structural determinants responsible for the unique gating properties using series of channel constructs with deletions in carboxy terminal region. Mutants of Cav3.3 channel with the C-terminal region either entirely removed or with the conserved proximal region of 20 amino acids preserved (C short) were transfected in the HEK cell line. Patch clamp electrophysiology approach revealed that initial proximal region of the carboxy terminus contains 20 amino residues which are essential for gating of Cav3.3 channel. Deletion of this region altered voltage-dependence of activation and inactivation, inactivation kinetics, and coupling between the voltage sensing and the pore opening of the channel. Observed effects fit with a model in which the carboxy terminus stabilizes the channel in a closed state. Funding: N.W. is supported by the Institute of Organic Chemistry and Biochemistry. G.W.Z. is a Canada Research Chair and supported by the Canadian Institutes of Health Research. LL is supported by a grant VEGA 2/0143/19. NW and LL are supported by bilateral SAS-CAS project.

PP.57

Effects of Magnesium on Behavior, iNOS, nNOS and eNOS Expression in Male Rats Exposed to Anxiety

Cetin A[1], Karabulut S[2], Taskiran AS[3], Ergul M[4], Ozdemir E[3]

[1]Department of Obstetrics and Gynecology, Cumhuriyet University Faculty of Medicine, Turkey; [2]Department of Medical Physiology, Erciyes University Faculty of Medicine, Turkey; [3]Department of Medical Physiology, Cumhuriyet University Faculty of Medicine, Turkey; [4]Biochemistry Department, Faculty of Pharmacy, Cumhuriyet University, Turkey

The present study was carried out to evaluate the anxiolytic and mnemonic effects of two different doses of magnesium sulfate with behavioral tests and to investigate possible intracellular mechanisms of its effects. Fifty male Sprague Dawley rats were randomly assigned to five groups: Control, Anxiety, Diazepam, Low-magnesium (200 mg/kg) and, High-magnesium (400 mg/kg). For anxiety, each animal was exposed to odor block (cat feces) in cages for 10 min. Rats in the Diazepam and Magnesium groups received either diazepam (2 mg/kg) or magnesium sulfate by intramuscular on the 1., 2., and 5. days. The anxiety and learning were tested in an elevated T maze on 1. and 5. days, a passive avoidance box on 2. and 5. days. Finally, cortex and hippocampus samples were collected to determine iNOS, nNOS, and eNOS protein expressions. Overall, with T-maze task, a mild-moderate decrease in escape latencies in rats receiving low and high doses of magnesium ($p<0.05$); and with passive avoidance task, there was no significant effect on latencies in rats receiving low and high doses of magnesium ($p>0.05$). There was mild-moderate change in the concentrations of iNOS, eNOS, and nNOS in cortical and hippocampal tissue samples ($p<0.05$). In conclusion, magnesium affected the behavioral and biochemical test results in a mild-moderate degree.

PP.58

ELOVL5-dependent fatty acids are required for proper action potential conduction

Hoxha E[1], Balbo I[1], Ravera F[1], Spezzano R[2], Genovese F[1], Malvicini E[1], Di Gregorio E[3], Brusco A[3], Mitro N[2], Caruso D[2], Tempia F[1]

[1]Dept. of Neuroscience and NICO, University of Torino; [2] Dept. of Pharmacological and Biomolecular Sciences, University of Milan; [3] Dept. of Medical Sciences, University of Torino, Italy

ELOVL5 is the rate limiting enzyme for elongation of unsaturated fatty acids with 18 carbon atoms. Mutations of ELOVL5 cause the spino-cerebellar ataxia type 38, indicating an important role in the nervous system. Since membrane fluidity and stability are strongly dependent on the specific contents of acyl groups in phospholipids, we assessed the effect of Elovl5 lack on the lipidic profile by lipidomic analysis of peripheral nerve and cerebellum of Elovl5 knock-out mice. The results revealed an unbalance between fatty acids longer than 18 carbon atoms relative to shorter ones. In addition, the ratio of saturated to unsaturated fatty acids was strongly increased. We hypothesized that the altered composition of myelin phospholipids might affect the conduction of action potentials along myelinated nerve fibers. The sciatic nerve conduction velocity was significantly reduced without change in the amplitude of the nerve compound potential, suggesting a myelin defect without a concomitant axonal degeneration. To study a central myelinated axon, we recorded the antidromic potential of the cerebellar Purkinje cell. The conduction velocity was significantly reduced, showing that not only peripheral but also central axons are affected by the lack of Elovl5 activity. These results indicate that the deletion of Elovl5 causes an alteration of myelin in central and peripheral axons. As a first assessment of myelin structure we analyzed the levels of the main proteins associated with myelin sheaths. In the cerebellum of Elovl5 knock-out mice, MBP was significantly reduced, while CNPase and PLP were expressed at the same levels as in control littermates. Further studies are needed to evaluate peripheral nerve myelin proteins and the ultrastructure of central and peripheral myelin.

PP.59

Establishing the model of tMCAO induced stroke in TFF3-/- knockout mice

Mihaljevic Z[1], Misir M[2], Radmilovic M[3], Justic H[3], Grgic S[3], Matic A[1], Susnjara P[1], Kozina N[1], Drenjancevic I[1]

[1]Institute and Department of Physiology and Immunology, Faculty of Medicine Josip Juraj Strossmayer University of Osijek, Osijek, Croatia; [2]Department for Neurology, Osijek University Hospital, Osijek, Croatia; [3]Department of Histology and Embryology, School of Medicine University of Zagreb, Zagreb, Croatia

Arachidonic acid (AA) metabolism and products affect vascular reactivity mechanism. TFF3-/- mice have protective phenotype of favorable ratio of ω-6/ω-3 fatty acids and modified metabolism of AA. However, the role of TFF3 peptide in hypertension, stroke and vascular function is still underinvestigated. High salt (HS) intake is one of the major risk factors for the development and progression of arterial hypertension directly proportional to the risk of stroke. This study aimed to establish the method of transient middle cerebral artery (t-MCAO) occlusion-reperfusion stroke model in WT and TFF3-/- mice in our laboratory and to asses the effect of HS diet on stroke volume. WT and TFF3-/- mice aged 12-14 weeks were divided into 4 groups: (WT_LS, TFF3-/-_LS- fed standard chow); and groups fed HS (4% NaCl) diet for 7 days prior the surgery, WT_HSD and TFF3-/-_HSD. Animals were anaesthetized with 2% isoflurane and heated to avoid hypothermia. After midline neck incision, silicon coated 6-0 monofilament was inserted via external carotid artery into internal carotid artery, and then into the circle of Willis, thus occluding MCA for 60 min.n 24h after surgery mice were anaesthetized with ketamine and midazolam and decapitated. The brains were cut into 2-mm-thick coronal sections, stained with 2% 2,3,5-triphenyltetrazolium chloride, fixed in 10% buffered formalin solution and scanned. Infarct volumes were measured using ImageJ imaging software and expressed as a percentage of the ischaemic hemisphere. All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (directive 86/609) and were approved by institutional Ethical Committee. Result of this pilot study (N=3 /group) show successfully established model of tMCAO in mice, but no significant differencies in cortical and total infarct size, due to small sample. Partially supported by Croatian Science Foundation under the project #IP-2014-09-6380: “Impaired Vasorelaxation and Endothelial Leukocyte Interaction (ELI) in Development of Atherosclerotic Lesions - V-ELI Athero“ and Faculty of Medicine Osijek VIF2018-MEFOS-09-1509 grant (PI Drenjancevic I).

PP.60

Compensatory changes in skeletal muscle and spinal cord of mice carrying motoneuronal loss induced by cholera toxin-B saporin

Vicario N[1], Giunta MAS[1], Spoto G[1], Calabrese G[1], Vecchio M[1], Gulisano M[2], Leanza G[2], Parenti R[1], Gulino R[1]

[1]Department of Biomedical and Biotechnological Sciences, University of Catania, Italy; [2]Department of Drug Sciences, University of Catania, Italy

Despite the relevant research efforts, the causes of amyotrophic lateral sclerosis (ALS) are still elusive and no effective cure is available. Many authors suggest that ALS is caused by a network failure instead of being a cell-autonomous disease confined to motoneurons. Although motoneuronal loss is the main hallmark of ALS, other cell populations including muscle and glial cells are involved, but understanding their relative role in disease onset and progression requires further investigation. In particular, little is known about plastic changes of the degenerating motor system. These compensatory events are unable to slow down the neurodegeneration but their elucidation and possible use as a therapeutic target represents an important aim of ALS research. Herein, by using a mouse model of spinal motoneuron depletion induced by injection of cholera toxin-B saporin in the gastrocnemius muscle, we investigated the possible occurrence of compensatory changes in both skeletal muscle and spinal cord. The results showed that muscles became atrophic after lesion and displayed electromyographic activity similar to that observed in ALS patients. Conversely, modifications of muscle fiber morphology were different from that observed in ALS models, thus suggesting that muscular effects of disease may in part be primary effects instead of being caused by denervation. Notably, we found plastic changes in the spared motoneurons possibly sustaining a functional restoration, which could be similar to the compensatory changes occurring in disease. These events may be at least partially driven by glutamatergic signaling, and supported by astrocytes contacting the surviving motoneurons.

PP.61

Subretinally njected P3ht nanoparticles fully rescue vision in a rat model of retinal dystrophy

Maya-vetencourt JF, Manfredi G, Mete M, Colombo E, Bramini M, DiFrancesco ML, Shmal D, Eleftheriou C, Di Maria F, Cossu V, Emionite L, Ticconi F, Marini C, Sambuceti G, Pertile G, Lanzani G, Benfenati F

Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Genova, Italy

Inherited retinal dystrophies and late stage age-related macular degeneration are among the most prevalent causes of legal blindness for which effective medical treatments have not yet been identified. Retinal prostheses have been proposed to stimulate the inner retinal network, although problems such as sensitivity, resolution, wiring or external cameras have limited their application. Here we show that subretinally injected conjugated polymer nanoparticles (NPs) can persistently rescue visual functions in a rat model of Retinitis pigmentosa (RP). P3HT-NPs spread out over the whole subretinal space and promoted the light-dependent activation of spared retinal neurons, yielding a full recovery of subcortical, cortical and behavioral visual responses in the absence of retinal inflammation. By conferring sustained light-sensitivity and high spatial resolution to degenerate retinas after a single injection, these NPs represent a new avenue in retinal prosthetics with potential large-scale applications not only in RP, but also in age-related macular degeneration.

PP.62

Functional connectivity underlying leftward visuospatial bias: an EEG study

Brignani D[1], Bagattini C[1], Mazza V[2], Ferrari C[1], Carlo M[1,2]

[1]IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy; 2Center for Mind/Brain Sciences, University of Trento, Rovereto, Italy

Neurologically healthy individuals usually show a behavioral bias that favors the processing of stimuli appearing in the left visual field (i.e., pseudoneglect). This phenomenon has been ascribed to the right hemisphere dominance for visuospatial attention, which has recently found anatomical foundation in a larger fronto-parietal network in the right than in the left hemisphere. We have recently reported a preserved leftward bias during an enumeration task in healthy elderly (HE) and in mild cognitive impairment patients (MCI), which vanished in mild Alzheimer's disease patients (AD). The present study aimed at investigating the neural mechanisms subtending the alteration of pseudoneglect in the same sample of subjects. EEG was recorded in 14 HE, 15 MCI and 14 AD, while they performed a multiple objects enumeration task. Functional connectivity was estimated in theta (3-7 Hz) frequency band by means of Partial Directed Coherence, which provides strength and direction of the causal links between different brain areas. Connectivity analyses disclosed higher fronto-parietal connections in the right hemisphere as compared to the left hemisphere in HE and MCI, but not in AD patients. The divisibility index, which describes the degree of interhemispheric segregation between parietal areas, increased going from normal aging, through MCI, to AD, resulting significantly different in AD as compared to HE. No significant differences emerged when considering other local or global graph indices. The results of the present study confirm that the key role of the right parietal areas in visuospatial bias has to be intended in relation both to (i) a connectivity advantage of the right fronto-parietal network, and (ii) parietal (possibly inhibitory) interhemispheric connections.

PP.63

Local neural population dynamics rely on specific connectivity patterns in monkey pre-supplementary motor area

Albertini D[1,2], Gerbella M[1,2], Lanzilotto M[1], Livi A[1], Maranesi M[1], Ferroni CG[1], Bonini L[1,3]

[1]Department of Medicine and Surgery, University of Parma, Parma (Italy); [2]These authors equally contributed to the work; [3]Lead Contact: luca.bonini@unipr.it

Information about objects' position, contextual rules and other agents is essential for planning and withholding actions in social contexts. The pre-supplementary motor area F6 plays a role in the processing of all these types of information. Yet, its intrinsic functional organization remains unclear as well as the possible underlying connectional specificities. Here, we addressed these issues by combining chronic neuronal recordings along the rostro-caudal extent of monkeys' area F6 and local tracer injections into the specific functionally-characterized sites. Specifically, we studied F6 neural population with a task in which monkeys viewed and then grasped (or refrained from grasping) objects, and when they observed a human doing the same task. We found a rostro-caudal functional gradient, with the rostral part that preferentially encodes the presence of objects and experimenter in the monkey's peripersonal space and the caudal one showing higher motor and action observation responses. This distribution of functional properties fits well with the stronger direct connections of rostral sites with the lateral prefrontal and pregenual anterior cingulate cortex whereas caudal sites are most tightly linked with dorsal and ventral premotor areas and the motor putamen. The observed anatomical and functional rostro-caudal gradients may explain the multiplicity of functional signatures ascribed to pre-SMA, with the rostral parts showing preferential tuning to spatial and contextual information relative to the caudal ones, which in turn exhibit stronger tuning to self and others' action processing.

PP.64

Quantification and characterization of nitric oxide neurons in human corpus callosum

Sagrati A[1], Lorenzi T[1], Montanari E[2], Tagliabracci A[2], Barbaresi P[1], Morroni M[1], Fabri M[1]

[1]Dip. Med. Sper. e Clin., Univ. Politecnica Marche, Ancona, Italy; [2]Dip. Med. Sci. Biomed. e San. Pubb., Univ. Politecnica Marche, Ancona, Italy

Previous papers showed the presence of nitric oxide synthase (NOS) positive neurons in the corpus callosum (CC) of monkey (Rockland and Nayyar, Front Neural Circuits, 10.3389, 2012) and rat (Barbaresi et al., Brain Behav, 4: 317–336, 2014). Recently, we have confirmed the presence of NO producing neurons in the human CC, and analyzed their distribution and morphology. In order to quantify the nNOS immunopositive neuronal cells in the whole CC, 60μm-thick frozen sagittal serial sections from 3 autoptic specimens were reacted using an antibody against neuronal NO synthase (nNOS). Moreover, western blot was performed in the same specimens to confirm the quantitative data. The number of nNOS immunopositive neurons was counted by two blinded observers using light microscopy. As previously reported, the nNOS-positive neurons were found along the antero-posterior and medio-sagittal extention of the CC, especially in the peripheral zone. The neurons were more abundant in the body and splenium at 4 mm from the medial line. Up to date, 24 neurons were counted in the anterior CC, 408 in the callosal body and 276 in the splenium. Their morphologies were confirmed to be bipolar/fusiform and round also in the central and posterior regions of the CC; in the same callosal regions, some nNOS-positive nerve fibres associated with blood vessels were observed. The large number of neurons found in the human CC confirm our previous data indicating that these cells could play an active role in blood flow regulation in human white matter. Further analysis will be necessary to determine if the nNOS positive neurons are involved in the BOLD (blood oxygenation level dependent) effect reported in previous MRI studies (Fabri et al., World J Radiol, 6: 895-906, 2014).

PP.65

Characterization and quantification of neurons in human indusium griseum

Lorenzi T[1], Sagrati A[1], Montanari E[2], Tagliabracci A[2], Barbaresi P[1], Fabri M[1], Morroni M[1]

[1]Dip. Med. Sper. e Clin., Univ. Politecnica Marche, Ancona, Italy; [2]Dip. Med. Sci. Biomed. e San. Pubb., Univ. Politecnica Marche, Ancona, Italy

Our preliminary results indicate that human indusium griseum (IG) contains neurons, in accordance with a previous Golgi study in rat (Wyss et al., J Comp Neurol, 219:251-272, 1983) and with a very recent paper in humans (Rasonja et al., Cereb Cortex, doi: 10.1093/cercor/bhz004, 2019). Our study also showed that some neurons are immunopositive to the neuronal nitric oxide synthase (nNOS), the enzyme responsible for the synthesis of nitric oxide. Given the novelty of this finding, we decided to characterize the IG neurons

quantifing them and analyzing their distribution and morphology. To this purpose, immunohistochemistry on frozen sagittal serial sections (60μm) from 3 autoptic specimens of corpus callosum (CC) including the overlying IG were reacted using an antibody against nNOS. In order to confirm the quantitative data, western blot was performed in the IG of the same specimens. Immunohistochemical staining revealed the presence of many nNOS-immunopositive neurons in human IG, located along both rostral-caudal and medio-lateral directions. In particular, they are more numerous at 1 mm from the medial line and their number peaks in the body. They showed different morphologies and sometimes were arranged at the boundary between IG and CC, more densely packed in proximity to the pial arteries penetrating into the CC. The significant presence of nNOS-immunopositive neurons suggests that IG is not a merely rudimentary tissue (Humphrey, J Anat, 101:655-676, 1967), but it likely plays a functional role in the adult brain. The distribution of neurons suggests they might have a role in the communication between IG and CC, as already hypothesized by recent immunohistochemical findings in rat IG (Barbaresi, Neurosci Res, 130:23-38, 2018), as well as in the neurovascular regulation.

PP.66

Pain control by proprioceptive and exteroceptive stimulation at the trigeminal level

Zampino C[1], Ficacci R[1], Checcacci M[2], Franciolini F[1], Catacuzzeno L[1]

[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy; [2]Azienda Sanitaria Locale ROMA 1, Via Marco Polo 93, 00154 Roma, Italy

The Gate Control Theory of pain, published more than half a century ago to explain nociceptive modulation of peripheral sensory input, assumes inhibition of incoming nociceptive (pain) information produced by mechanical stimulation. To verify the presence of such a gate control mechanism at the level of the human trigeminal system, we evaluated the effects on pain sensation of a proprioceptive trigeminal stimulation induced by mandibular extension. We found that such a stimulation, applied for 7 minutes, was effective in increasing the threshold and tolerance of tooth pain induced by electrical activation of dental nociceptors. Moreover the antinociceptive effect lasted for several minutes after the proprioceptive stimulus had ceased. We also tested whether an exteroceptive palatal stimulation superimposed on the proprioceptive stimulation would increase the effects on tooth pain perception of human volunteers. We observed that the exteroceptive stimulation significantly increased the antinociceptive effect induced by the sole proprioceptive stimulation. The physiological mechanisms and the possible implications of these observations are discussed.

PP.67

The effect of trazodone on bladder detrusor smooth muscle

Burunsuz O, Ozdengul F

NEU Meram Faculty of Medicine, Department of Physiology

Trazodone is used to treat depression. It may help to improve mood, appetite, energy level as well as decrease anxiety and insomnia related to depression. Trazodone works by helping to restore the balance of a certain natural chemical (serotonin) in brain. We aim to determine the effects and mechanism of action of trazodone on contractility of rat bladder smooth muscle. Experimental studies were carried out in Experimental Medicine Research and Application Center and Laboratory of Physiology Department in Necmettin Erbakan University. 16 male wistar albino rats of 8-20 weeks, weighing 200-250 g have been used for study. Rats were euthanized by cervical dislocation after light ether sedation. The abdomen was opened by median line and the bladder was removed and taken into Krebs solution. The bladder was opened with a longitudinal incision in the direction of apex from the neck to prepare two muscular strips of 2x10 mm in the vertical direction. The strips were placed by applying 1 g of tension to assemble in isolated organ bath. All contractions were recorded. Following a 45-minutes period, tonic contractions were induced by application of acethylcholin (ACh) at 10-5 M concentration and waited for 20 minutes, trazodone doses were given cumulatively. The effects were recorded. Spontaneous and ACh induced bladder smooth muscle contractions did not show any significant effects with the application of 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M doses of trazodone ($p > 0.05$). A statistically significant inhibition was observed in 10^{-4} M and 10^{-3} M doses ($p < 0.05$). Trazodone has shown a significant inhibitory effect on in vitro bladder smooth muscle contraction, especially at 10-4 M and 10-3 M concentrations. When prescribing medication, doctors must be careful about high doses of some antidepressants.

PP.68

Colon inflammation increased the excitability of rat pyramidal neurons of motor cortex.

Carrascal I[1,3], García-Miranda P[1], Vázquez-Carretero MD[1], Coutteau A[1], Ilundáin AA[1], Castro C [2,3], Calonge ML[1], Peral MJ[1], Nunez-Abades P[1,3]

1Departamento Fisiología, Facultad de Farmacia, Universidad Sevilla, Spain; 2Departamento Fisiología, Facultad de Medicina, Universidad Cádiz, Spain; 3Instituto de Investigación e Innovación Biomédica de Cádiz (INiBICA), Universidad Cádiz, Spain

Inflammation is an important inducer of the development of neurodegenerative diseases and peripheral inflammation greatly affects the central nervous system via humoral and nervous pathways. The concept of the brain-gut axis has long been visualized as the mediator of stress-related gastrointestinal symptoms but the intestinal inflammation may also alter neuron membrane properties contributing to or causing their functional death. The aim of this work was to evaluate inflammatory parameters and the electrophysiological changes on motor cortex neurons induced by intestinal inflammation, using a rat model of ulcerative colitis. Two animal groups were compared, one as a control and the animals of the second group were treated with 5% dextran sulfate sodium (DSS) dissolved in their drinking water, during 7 days. Inflammatory markers were evaluated in the brain and colon by real-time PCR and the electrophysiological properties of rat motor cortex neurons were determined by whole cell patch-clamp recordings in current clamp mode performed on brain slice preparations. The results revealed significant differences between the two rat groups under study. Compared to the control group, DSS-treated rats exhibited in motor cortex and colon increased mRNA levels of IL6 and iNOS. The motor neurons showed an increase in input resistance, frequency gain and maximal discharge frequency and a decrease in resting membrane potential and rheobase. These changes result in an increase in neuronal excitability. We conclude that intestinal inflammation induces neuroinflammation that could contribute to neuronal degeneration via an increase in membrane excitability.

PP.69

Reversible Hyperphosphorylation of Tau protein in the Enteric Nervous System during Synthetic Torpor

Luppi M[1], Stanzani A[2], Chiocchetti R[2], Giancola F[3], De Giorgio R[4], Hitrec T[1], Squarcio F[1], Occhinegro A[1], Piscitiello E[1], Cerri M[1], Amici R[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2]Department of Veterinary Medical Sciences, University of Bologna, Italy; [3]Department of Medical and Surgical Sciences, University of Bologna, Italy; [4]Department of Medical Sciences, Nuovo Arcispedale S.Anna, University of Ferrara, Italy.

Thauopathies are a wide class of neurodegenerative diseases (ND) sharing the hyperphosphorylation of Tau protein (P-Tau) in the central nervous system. A similar process is observed in hibernating mammals and in the synthetic torpor (ST), a torpor-like state induced in the rat, a non-hibernator: though in these cases P-Tau does not lead to ND, reversing back to normal when returning to euthermia. Since the involvement of the enteric nervous system (ENS) in tauopathies is still poorly understood, we took advantage of the ST model to assess the accumulation of P-Tau in the ENS and its eventual reversibility after 6h or 38h of recovery of euthermia. Male Sprague-Dawley rats were repeatedly injected (100nL) in the Raphe Pallidus with the GABAA agonist muscimol (1mM), or vehicle for controls, inducing ST. Following this procedure, rats were sacrificed and the intestinal tract was extracted. Myenteric plexus whole-mount preparations of the small intestine were processed for immunohistochemistry. To assess P-Tau, the monoclonal mouse anti-AT8 antibody (1:200) was used. Co-localization with choline acetyl-transferase (ChAT; 1:50) was also assessed. A high AT8 immunoreactivity (IR) was found in ST in the ENS. During recovery, P-Tau reversed completely into the non-phosphorylated form after 38h and in most enteric neurons after 6h, although a subpopulation of these neurons showed a persistence of high AT8 IR. These P-Tau positive neurons rarely co-localized with ChAT. Our data show that in non-hibernating mammals P-Tau can be expressed and reversed in ENS. Unexpectedly, some non-cholinergic neurons are less prone to P-Tau resolution. Understanding what happen to these neurons may suggest a way to better clarify which process lead neurons to be either resilient or prone to ND.

PP.70

Which structure in the central nervous system integrates bilateral corticobulbar output to lower facial muscles?

Ginatempo F[1], Manzo N[2], Rothwell JC[3], Deriu F[1]

[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy; [2]IRCCS NEUROMED, Pozzilli, IS, Italy; [3]Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK

The crucial role of the corpus callosum in the execution of movements involving both body sides, particularly the hands, is well known (Ferbert et al. J Physiol, 1992), whereas proximal muscles are more likely to involve cortico-reticulospinal pathways (Brinkman et al. Science,1972). Previous studies carried out on the primary motor cortex innervating lower facial muscles (fM1) showed that cortical projections to the facial motor nucleus are bilateral, but how and at which level bilateral movement are coordinated is still unknown.

This work investigated interhemispheric connections between the fM1s using TMS double coil protocols. IHI was investigated in 10 healthy subjects using paired-pulse TMS in the depressor anguli oris (DAO), upper trapezius (UT) and first dorsal interosseous (FDI) muscles. Conditioning stimuli (CS) of 90-130% resting motor threshold (RMT) preceded test motor evoked potentials (MEP) by 7 interstimulus intervals (ISIs) ranging 4-12 ms. In the DAO, we also examined IHI at 1-2 ms ISIs. IHI was detected in the UT (CS 130% RMT;ISI 8 ms; p=0.02) and FDI (CS 120% and 130% RMT, at 8-10 ms ISIs; p=0.004), but not in DAO at any ISI, instead, there was facilitation at 1-4 ms ISIs and 110-130% RMT CS. In the DAO, conditioned responses at 1-4 ms ISIs were significantly larger than both test MEPs and the response induced by the CS alone. In the DAO there was no evidence of IHI even though this was clear in hand and axial muscles. Control experiments excluded a trancallosal origin of the facilitation observed at the shortest intervals. We suggest that coordination of the facial muscles of both sides mainly involves brainstem circuits engaged by corticobulbar output from fM1, rather than interhemispheric connections.

PP.71

Binge drinking have different effects on sociability and preference for social novelty

Bagosi Z, **Simon B**, **Karasz G**, **Csabafi K**, **Szakács J**, **Ibos KE**, **Szabó G**

Department of Pathophysiology, Faculty of Medicine, University of Szeged, Hungary

Binge drinking is an increasing public health issue that is defined as consuming a large amount of alcohol in a short period of time. The aim of the present study was to determine the effects of binge drinking on social behavior. For this purpose, a classical animal model of binge drinking was used, in which male C57BL/6 mice were exposed to drinking in the dark. Hence, the circadian rhythm of the mice was changed for 14 days, then their water was switched to alcohol of 20% for 4 days. The mice had access to alcohol in the dark, for 2 hours per day on the first 3 days, and for 4 hours on the 4th day. On the last day, the mice were investigated in a three-chamber social interaction test arena. In order to investigate the sociability and the preference for social novelty of the mice, two different tests were performed. First, the tested mice had to choose between a stranger mouse and an unfamiliar object. Second, the choice had to be made between a stranger and the previously familiarized mouse. The number and the time of social interaction of the tested mice with the stranger increased in the first test, suggesting enhanced sociability. However, the number and the time of social interaction with the stranger decreased in the second test, indicating reduced preference for social novelty. Thus, the present study demonstrates that binge drinking have different effects on sociability and preference for social novelty in mice. Grants: EFOP-3.6.2-16-2017-00006

PP.72

Investigating the effect of kisspeptin-13 fragments on anxiety and nociception in rodents

Ibos KE, **Csabafi K**, **Szakács J**, **Bagosi Z**, **Szabó G**

Department of Pathophysiology, Faculty of Medicine, University of Szeged, Hungary

Kisspeptins (KP) are mammalian neuropeptides of the RF amide family that are known as major regulators of the reproductive axis. In our previous experiments, KP-13 induced anxiety in rats and decreased the nociceptive threshold in mice. The aim of the current study was to investigate the effect of two KP-13 fragments on anxiety and pain perception. KP-13(1-5) and KP-13(6-13) were synthesized by solid phase peptide synthesis. All anxiety-related experiments were conducted in male Wistar rats. Following icv treatment with 0.1, 1 or 10 μg of KP-13(1-5) or KP-13(6-13), computerized open field test was carried out to assess anxiety-like behavior. After icv administration of KP-13(1-5), the hypothalamic expression of arginine vasopressin (AVP) and corticotropin releasing factor (CRF) genes were also measured by quantitative real-time polymerase chain reaction (RT-qPCR). Moreover, the effect of KP-13(1-5) on GABA release from the amygdala was investigated using ex vivo superfusion. Nociception was assessed using the tail flick test in male C57BL/6 mice after icv treatment with either KP-13(1-5) or KP-13(6-13). In the open field test, the 0.1 μg and 1 μg doses of KP-13(1-5), as well as 0.1 μg of KP-13(6-13) significantly decreased central ambulation time and distance, indicating anxiety-like behavior. KP-13(1-5) also modified the function of anxiety-related brain regions: it induced a significant downregulation of hypothalamic CRF expression and suppressed GABA release from the amygdala. None of the fragments had any significant effect on nociception. Although both fragments induced anxiety similarly to KP-13, they failed to influence nociception, for which differences in receptor binding affinity might provide an explanation. This work was supported by EFOP-3.6.2-16-2017-00006.

PP.73

Different processes of Concurrent Motor Inhibition are active during Joint Action: Evidence from TMS study

Cardellicchio P[1], Dolfini E[1,2], Vescovo E[2], Fadiga L[1,2], D'Ausilio A[1,2]

[1]IIT@UniFe Center for Translational Neurophysiology, Istituto Italiano di Tecnologia, Ferrara, Italy; [2] Section of Human Physiology, University of Ferrara, Ferrara, Italy.

Interaction in a social context requires reciprocal exchange of information between subjects and mutual adaptation of actions. At present, several neurophysiological mechanisms underlying interaction are still under investigation. Here we present a study where subjects were required to perform a simple interactive goal-directed action: opening a bottle held by another individual (Joint Action, JA). In a control condition, the bottle was held by a mechanical holder (vice clamp, no-JA). During the tasks (reaching phase) we administered transcranial magnetic stimulation (TMS) over subjects' primary motor cortex to assess: (i) corticospinal excitability (CSE), (ii) cortical silent period (cSP) and (iii) short-interval intracortical inhibition mechanisms (sICI) by recording the motor evoked potentials (MEPs) from the Opponens Pollicis muscle. Data analysis performed on MEPs demonstrated the absence of any differential modulation of CSE for the JA vs no-JA comparison. Conversely, a significant modulation of the two inhibitory indexes was present in JA condition with respect to the no-JA one, both in terms of reduced sICI and prolonged cSP. These results suggest that intracortical indexes of inhibition might represent sensitive markers of mutual co-adaptation during joint action.

PP.74

Kisspeptin-13's anxiogenic action might be mediated by central vasopressin release

Csabafi K, Ibos K, Filkor K, Szakács J, Bagosi Z, Szabó G

Department of Pathophysiology, University of Szeged, Hungary

Kisspeptins, key regulators of the reproductive axis, are encoded by the Kiss1 gene and act through kisspeptin 1 (KISS1R). In our previous study kisspeptin-13 (KP-13), one of the shorter derivatives, induced anxiety-like behavior in rats and evoked an increase in corticosterone secretion. In the present experiments, we investigated the possible mechanism of KP-13's anxiogenic effect in adult male Wistar rats. KP-13 (2 μg/2 μl) was injected intracerebroventricularly (icv) and 2 h later hypothalamus, amygdala and hippocampus samples were obtained, in which we measured the relative gene expression of the corticotropin-releasing hormone (CRH) and vasopressin (VP) system via qPCR. Furthermore, the behavior of animals, pretreated with KISS1R, CRH or VP receptor antagonists prior to KP-13, were recorded via elevated plus maze and open field tests. Our results showed that KP-13 upregulated the relative gene expression of VP in amygdala and hypothalamus, CRH receptor 1 in the hippocampus and VP1a receptor in the hypothalamus. In addition, KISS1R and VP receptor antagonist pretreatment was able to abolish KP-13's anxiogenic effect in behavioral tests, whereas CRH blockage had little effect. In conclusion KP-13's anxiogenic action might be mediated by central release of the stress responsive VP, rather than CRH via KISS1R activation. Indeed, increased amygdalar VP expression may alter amygdala processing to induce anxiety and trigger downstream activation of the hypothalamic-pituitaryadrenal axis in rats. Grants: EFOP-3.6.2-16-2017-00006.

PP.75

Effects of central salusin-beta infusion on pituitary-thyroid axis in rats

Tekin S[1], Demir I[1], Özyalin F[2], Sandal S[1]

[1] Department of Physiology, Inonu University Medical School, Turkey; [2]Department of Medical Biochemistry, Inonu University Medical School, Turkey

Salusin-β is a newly identified bioactive peptide, which is widely expressed in many tissues including hypothalamus, pituitary and the endocrine system. Pituitary-thyroid axis is related to energy expenditure and metabolism rate. Effects of salusins on energy expenditure and basal metabolism rate are still unknown. The aim of the present study was to investigate the potential roles of salusin-β on pituitary-thyroid axis in rats. Forty Wistar-albino male rats were randomly divided into four groups, including ten rats in each group as follows: Group 1: Control; Group 2: Sham (artificial cerebrospinal fluid, aCSF); Group 3: 2 nmol salusin-β; Group 4: 20 nmol salusin-β. Salusin-β or aCSF were intracerebroventricularly infused via osmotic mini pumps for seven days. The end of the experiments, rats were decapitated and blood samples were collected and serum TSH, free-T3 and T4 levels were determined. The serum TSH and free-T3 levels in salusin-β-infused groups were found to be significantly increased compared to sham and control groups ($p<0.05$), whereas serum free-T4 levels was significantly higher in 2 nmol salusin-β-treated group compared to sham group ($p<0.05$). The study results indicated that centrally infused salusin-β may play a

role in the regulation of energy expenditure and basal metabolism rate by affecting pituitary-thyroid axis. Acknowledgement: This study was supported Inonu University BAP (Project number: TSG-2017-952).

PP.76

Oxaliplatin-induced cytosolic acidification alters TRP and K_2P channel activity in sensory neurons

Dionisi M[1], Riva B[1], Ruffinatti AF[1], Potenzieri A[1], Lim D[1], Carozzi VA[2,3], Cavaletti G[2], Marmiroli P[2], Genazzani AA[1], Distasi C[1]

[1]Department of Pharmaceutical Sciences, University of Piemonte Orientale, Novara, Italy; [2]Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-

Bicocca, Monza, Italy;[3]Young Against Pain Group, Monza, Italy

Oxaliplatin (OHP), a platinum-based chemotherapeutic agent, causes peripheral neuropathy (OIPN) characterized by an acute cold-induced syndrome accompanied by cramps, paresthesias/dysesthesias in the distal limbs and perioral region. The mechanisms underlying OHP-induced increase of nociceptor excitability are not fully understood; however, one common mechanism appears to be changes in ion channel expression and function. Here we have combined different approaches in order to investigate, in mouse dorsal root ganglia (DRG) neurons, the impact of OHP treatment on the molecular targets that are known to participate in the processing of noxious stimuli. We found that therapeutically relevant OHP concentrations lead to a cytosolic acidification of DRG neurons. It is likely that different classes of channels could be affected by this alteration of pH homeostasis. In particular, path-clamp single channel recordings suggest the involvement of members of the Transient Receptor Potential channel family, namely TRPA1 and TRPV1, and of the two-pore domain K+ (K2P) channel family. These channels produce background currents that act to regulate membrane resting potential and excitability. Moreover, TREK-1, TREK- 2 and TRAAK channel activity is tightly regulated by different stimuli, including changes of cytosolic pH. Furthermore, we found these channels undergoes up-regulation following OHP treatment. In light of this, the hyperexcitability underlying the OINP can be explained as the resultant effect of multiple regulations on different targets in response to the OHP alteration of pH homeostasis. This provides useful insights for the understanding of the pathogenesis behind this neuropathy in order to propose effective therapeutic pain management. Funded by PRIN 2017, PI G. Cavaletti.

PP.77

Role of physiochemical properties on the residence time of serotonin 7 (5-HT_7) receptor ligands

Penna E[1,4], Niso M[3], Lacivita E[3], Perrone-Capano C[2], Crispino M[1], Leopoldo M[3,4]

[1]Department of Biology, [2]Department of Pharmacy, University of Napoli Federico II, Napoli, Italy; [3]Department of Pharmacy-Drug Sciences, University of Bari Aldo Moro, Bari, Italy; [4]Biofordrug srl, Bari, Italy

The serotonin receptor 7 (5-HT7R) is a G protein-coupled receptor (GPCR) involved in many physiological processes and in neurological and neurodevelopmental disorders such as Fragile X syndrome and Rett syndrome, which are characterized by abnormal neuronal connectivity and consequent intellectual disabilities. To unveil the molecular pathways linking 5-HT7R to these diseases, it is important to study the pharmacology of 5-HT7R by using appropriate pharmacological tools, such as N-(4-cyanophenylmethyl)-4-(2-diphenyl)-1 piperazinehexanamide (LP-211), a brain penetrant and selective 5-HT7R receptor agonist. Usually, the pharmacological properties of an agonist are defined by the affinity for the target receptor (Ki) and by the agonist potency (EC50). In recent years, there is interest also to investigate how a given ligand kinetically interacts with the target, namely the residence time (RT), because it has been proposed that persistent ligand binding can be exploited in drug discovery to achieve more selective response profiles. Previous studies on LP-211 and related analogs have suggested that the residence time of this class of 5-HT7R ligands might be structure-dependent. By assessing the residence time of a series of 5-HT7R ligands characterized by different lipophilic properties (ClogP), we have found that it is not the overall lipophilicity of the molecule that determines its dissociation rate, but rather the lipophilicity at a specific position of the scaffold. Future studies will be focused on the effects of 5-HT7R agonists characterized by different residence time on neuronal morphology in animal models of neurodevelopmental diseases.

PP.78

Oxidative Damage of Polyunsatured Fatty Acid Plays a Key Role in the Pathogenesis and Progression of Krabbe Desease

Graziano ACE[1], Signorini C[2], Pannuzzo G[1], Durand T[3], Galano JM[3], Oger C[3], Leoncini S[4], Cortelazzo A[4], Lee JCY[5], Hayek J[4], De Felice C[4,6], Cardile V[1]

[1]Department of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Italy; [2]Department of Molecular and Developmental Medicine, University of Siena, Italy; [3]Institut des Biomolécules Max Mousseron, (IBMM), CNRS, Université de Montpellier, ENSCM, France; [4]Child Neuropsychiatry Unit, Azienda Ospedaliera Universitaria Senese, Siena, Italy; [5]The University of Hong Kong, School of Biological Sciences, Hong Kong Special Administrative Region; [6]Neonatal Intensive Care Unit, Azienda Ospedaliera Universitaria Senese, Siena, Italy

The gray and white matter of the nervous system are rich in docosahexaenoic acid and adrenic acid, respectively, and under non-enzymatic oxidative stress release isoprostanoids, i.e. F4-neuroprostanes (F4-NeuroPs) and F2-dihomo-isoprostanes (F2- dihomo-IsoPs). Krabbe disease (KD) is a rare genetic demyelinating syndrome characterized by deficiency of the enzyme β-galactosylceramidase, lysosomal psychosine accumulation, and loss of myelin-forming cells. Oxidative stress is implicated in the pathogenesis of KD, but the precise mechanisms involved remain unknown. The aim of this research was to investigate the formation of isoprostanoids in brain tissue of twitcher mice that represent a well-established KD model. According to the genotype determination, three groups of mice were selected: wild-type control mice (n = 13), heterozygotes mice (carriers of GALC mutations, n = 14) and homozygous twitcher mice (n = 13). Measurement of F2- dihomo-IsoP and F4-NeuroP levels were performed on whole brain tissue obtained at day 15 and day 35 of the life cycle. Brain isoprostanoid levels were significantly higher in the twitcher mice compared to the heterozygous and wild-type control mice. However, F2- dihomo-IsoP and F4-NeuroP levels did not differ in brain of day 15 compared to day 35 of the heterozygote mice. Interestingly, isoprostanoid levels were proportionally enhanced with disease severity (F2-dihomo-IsoPs, rho = 0.539; F4-NeuroPs, rho = 0.550; p values 0.0003; n = 40). Our findings are the first to show the key role of polyunsaturated fatty acid oxidative damage to brain grey and white matter in the pathogenesis and progression of KD. This shed new insights on the biochemical indexes of KD progression, and potentially provide information for novel therapeutic targets.

PP.79

Modulating attentional capture via Transcranial Magnetic Stimulation (TMS) of right TPJ

Lega C[1], Ferrante O[1], Santandrea E[1], Cattaneo L[1,2], Chelazzi L[1,2]

[1]Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Italy; [2]Istituto Nazionale di Neuroscienze (INN), Italy

In visual search, salient, yet task-irrelevant, distractors in the stimulus array interfere with target selection. This is due to the unwanted shift of attention to the salient stimulus -- the so-called attentional capture effect, which delays deployment of attention onto the target. Although powerful and automatic, attentional capture by a salient distractor is nonetheless modulated by cross-trial contingent history: The distractor cost is typically more robust when there is no distractor on the previous trial, compared to when there is one. Here we used transcranial magnetic stimulation (TMS) to shed light on the causal role of two crucial nodes of the ventral attention network, namely the Temporo-parietal Junction (TPJ) and Middle Frontal Gyrus (MFG), in the exogenous control of attention and its history-dependent modulation. Participants were to discriminate the direction of a target arrow while ignoring a task-irrelevant salient distractor, when present. Immediately after display onset, 10 Hz triple-pulse TMS was delivered either to TPJ or MFG on the right hemisphere. Results demonstrated that stimulation of right TPJ – but not of right MFG, significantly modulated attentional capture as a function of the type of previous trial, by enhancing the cost associated with the distractor when the preceding trial was a distractor-absent trial and decreasing the cost when the preceding trial was a distractor-present trial. These findings indicate that TMS of right TPJ exacerbates the effect of the recent history, perhaps reflecting enhanced updating of the predictive model that dynamically governs proactive distractor-filtering mechanisms. More generally, the results attest to a role of TPJ in mediating the history-dependent modulation of attentional capture.

PP.80

Influence of Propranolol and Clonidine on Epileptogenic Threshold in Neocortex and Hippocampus

Saralidze E[1], Diasamidze I[1], Kobaidze I[2], Khuchua L[3]

[1]Batumi Shota Rstaveli State University,Batumi,Georgia; [2]Ivane Javkhishvili, Tbilisi State University,Tbilisi,Georgia; [3].Institute Of neurology.Tbilisi, Georgia

The goal of the work was investigation of clonidine and propranolol influences on the epileptiform dicsharges in the hippocampus and neocortex. Materials and Methods. In vivo experiments were carried out on chronic Wistar rats. Under ketalar anesthesia Stimulating (bipolar) and recording (unipolar) electrodes were implanted in the neocortex and dorsal hippocampus (DH) according to stereotaxic coordinates by Paxinos and Watson atlas.epileptiform discharges (EDs) were evoked with electrical stimulation (30Hz) either of neocortex or dorsal

hippocampus.Intraperitoneal injection of clonidine, β-adrenoreceptor stimulant (1mg/kg) and propranolol β-adrenoblocker (0,5mg/kg) was performed after establishing a minimal threshold stimulation sufficient for elicitation stable epileptiform discharges either in neocortex or in hippocampus. The brain electrical activity was recorded with electroencephalograph. Results. Propranolol injection decreased epileptiform discharges elicited by cortical stimulation, while hippocampally-induced discharges, after propranolol injection – increased. This result may be due to suppressed inhibitory capacity of β-adrenoreceptors. influence of clonidine injection was just an opposite to that of propranolol – cortically-induced epileptiform discharges increased, while the hippocampally-induced ones- decreased. Conclusions 1. Clonidine decreases the neocortical seizure threshold both after intraperitoneal and intracranial injection which means that increases its readiness for seizures 2. Clonidine increases the hippocampal threshold both after intra cranial and intraperitoneal injections and decreases the seizure duration 3. The propranolol intraperitoneal and intracranial injection increases the neocortical epileptic threshold and shortens of seizure duration 4. The propranolol intraperitoneal and intracranial injection decreases the hippocampal epileptic threshold which express in longer seizure activity

PP.81

Exosomes from Virus-Affected Airway Cells Enter Brain and Suppress Microglial Mitochondria

Blazauskaite D[1], Jankauskaite L[2], Balion Z[2], Jekabsone A[1,2].

[1]Institute of Neuroscience, Lithuanian University of Health Sciences, Lithuania; [2]Institute of Pharmaceutical Technologies, Lithuanian University of Health Science, Lithuania

Upper respiratory tract infections are the most common, and approximately 80% of them are caused by viruses. Exosomes from virus-primed cells may have viral genetic material or other inflammatory factors and transmit the inflammatory signal away from periphery to the brain because they can pass blood-brain barrier. Immune cells including microglia respond to viral infection via mitochondrial antiviral signalling protein inducing changes in mitochondrial function and increased production of ROS. However, it is not clear whether exosomes from virus-affected airway cells might induce similar immuno-metabolic changes. In this study, we demonstrate that exosomes isolated from airway cell culture primed with virus mimetic Poly (I:C) enter brain within several hours after intranasal administration. In mixed neuronal-glial cell cultures, microglia internalise the exosomes faster compared to other cells. Incubation with exosomes from Poly (I:C)-primed airway cells significantly reduces the activity of microglial mitochondria (measured by Seahorse XFp analyzer) and increases intracellular ROS production (evaluated as DCFH-DA fluorescence intensity). In conclusion, exosomes from virus-affected airway cells can enter brain and induce metabolic changes in microglia.

PP.82

A TrkB agonist rescues impairments in synaptic plasticity and dedritic morphology in the perirhinal cortex and restores visual recognition memory in a mouse model of CDKL5 deficiency disorder

Ren E[1], Roncacè V[2], Trazzi S[1], Fuchs C[1], Medici G[1], Gennaccaro L[1], Loi M[1], Galvani G[1], Ye K[3], Rimondini R[3], Aicardi G[2,4], Ciani E[1]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy; [2]Department for Life Quality Studies, University of Bologna, Italy; [3]School of Medicine, Emory University, Atlanta, USA; [4]Department of Medical and Clinical Sciences, University of Bologna, Italy; [5]Interdepartmental Center "Luigi Galvani" for the Integrated Study of Biophysics, Bioinformatics and Biocomplexity, University of Bologna, Italy.

Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is a severe X-linked neurodevelopmental encephalopathy caused by mutations in the *CDKL5* gene and characterized by early-onset epilepsy and intellectual and motor impairments. No cure is currently available for CDD patients. *Cdkl5* knockout (KO) mouse models, recently created to investigate the role of CDKL5 in the etiology of CDD, recapitulate various features of the disorder. Previous studies have shown alterations in synaptic plasticity and dendritic pattern in the cerebral cortex and in the hippocampus, but the knowledge of the molecular substrates underlying these alterations is still limited. Here, we have examined for the first time synaptic function and plasticity, dendritic morphology, and signal transduction pathways in the perirhinal cortex (PRC) of this mouse model. We found that long-term potentiation (LTP) was impaired, and that the TrkB/PLCγ1 pathway could be mechanistically involved in this alteration. PRC neurons in mutant mice showed a reduction in dendritic length, dendritic branches, PSD-95-positive puncta, GluA2-AMPA receptor levels, and spine density and maturation. These functional and structural deficits were associated with impairment in visual recognition memory. Interestingly, an in vivo treatment with a TrkB agonist (the 7,8-DHF prodrug R13) to trigger the TrkB/PLCγ1 pathway rescued defective LTP, dendritic pattern, PSD-95 and GluA2-AMPA receptor levels, and restored visual recognition memory in *Cdkl5* KO mice. Present findings

demonstrate a critical role of TrkB signaling in the synaptic development alterations due to CDKL5 mutation, and suggest the possibility of TrkB-targeted pharmacological interventions.

PP.83

Investigation of Metabolic Response to Virus and Virus-Primed Cell Exosomes in Astrocytes with Triple Alzheimer's Disease Mutation

Vydmantaitė G[1], Jankauskaitė L[2], Balion Z[1,2], Lim D[3], Morkūnienė R[4], Jekabsone A[1,2]

[1]Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania; [2]Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Lithuania; [3]Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Italy; [4]Department of Drug Chemistry, Faculty of Pharmacy, Medical Academy, Lithuanian University of Health Sciences, Lithuania

Viral infections might accelerate neurodegeneration during Alzheimer's disease (AD). Upper airway viral infections are the most common, and exosomes from infected cells might carry the inflammatory signal to the brain. In AD, neuronal loss first appears in hippocampus, and astrocytes are important mediators of neuronal dysfunction and death. Mitochondria are key players in antiviral immuno-metabolic responses. Thus, in our study, we aimed to investigate mitochondrial function and reactive oxygen species (ROS) production in response to virus-mimicking polyinosinic:polycytidylic (Poly (I:C)) sequence and Poly(I:C)-primed airway cell exosomes in immortalized hippocampal astrocytes containing three AD-related mutations (APPswe/Tau-P301L/PS-1M146V, further referred to as TG). TG astrocytes had suppressed mitochondrial function and glycolysis (measured by Seahorse XFp analyser) and higher ROS production (evaluated according to DCFDA and MitoSOX fluorescence) compared to the isogenic wild type (WT) control. TG also demonstrated different response to treatment with Poly (I:C) and Poly (I:C)-primed airway cell exosomes. There were no mitochondrial response to Poly (I:C) in TG, whereas WT cells reacted by decrease in oxygen consumption rate (OCR) and burst in mitochondrial ROS. Surprisingly, TG astrocytes had increased OCR and extracellular acidification rate (ECAR) after treatment with Poly (I:C)-primed exosomes. These results suggest that TG astrocytes have altered mitochondria and therefore react differently to virus and virus-primed exosomes comparing to normal astrocytes.

PP.84

Deletion of calcineurin from GFAP-expressing astrocytes impairs neuronal excitability and reproduces features of neurological diseases.

Tapella L[1], Soda T[2], Mapelli L[2], Ponzoni L[3], Bortolotto V[1], Bondi H[1], Farruggio S[4], Raina G[4], Ummarino S[4], Manfredi M[5], Di Ruscio A[4], Verpelli C[3], Sala M[3], Marengo E[5], Grossini E[4], Grilli M[1], Genazzani AA[1], D'Angelo E[2], Moccia F[6], Lim D[1]

[1]Department of Pharmaceutical Sciences, Università del Piemonte Orientale (UPO), Novara, Italy; [2]Department of Brain and Behavioral Sciences, University of Pavia, Italy; [3]Department of Pharmacology, University and CNR of Milan, Italy; [4]Department of Translational Medicine, UPO, Novara, Italy; [5]Depatment of Sciences and Technological Innovation, UPO, Novara, Italy; [6]Department of Biology and Biotechnology ''Lazzaro Spallanzani'', University of Pavia, Italy

Astrocytes perform important housekeeping functions in the nervous system including maintenance of adequate neuronal excitability, although the regulatory mechanisms are currently poorly understood. The astrocytic Ca^{2+}/calmodulin-activated phosphatase calcineurin (CaN) is implicated in the development of reactive gliosis and neuroinflammation, but its roles in healthy brain is unknown. We have generated a mouse line with conditional knockout (KO) of CaN B1 (CaNB1) in glial fibrillary acidic protein (GFAP)-expressing astrocytes (astroglial calcineurin knock-out, ACN-KO). Here we report that postnatal and astrocyte-specific ablation of CaNB1 did not alter normal growth and development as well as adult neurogenesis. However, beginning from 6 mo of age ACN-KO mice show increased risk to develop spontaneous epileptic seizures followed by premature death. Yet, we found that at 1 mo of age specific deletion of astrocytic CaN selectively impairs intrinsic neuronal excitability in cerebellar granule cells (CGCs) and hippocampal CA1 pyramidal neurons. This impairment is due to inactivation of astroglial Na^+/K^+ ATPase. Shotgun mass spectrometry proteomics analysis of hippocampal synaptosomes showed altered expression of astroglial as well as neuronal proteins. The specific overrepresented GO terms were*: synaptic vesicle cycle, mitochondrion, extracellular matrix* and *focal adhesion*. Overrepresented KEGG pathways included: *oxidative phosphorylation, Alzheimer's, Parkinson's* and *Huntington's diseases*. WB and pcr validation of the in silico analysis is now underway. In conclusion, astrocyte-specific CaN KO shows features of several neurological and neurodegenerative disease, indicating that alteration of its activity may be involved in the early stage pathogenesis of brain diseases.

PP.85

TRP channels expression in Chronic Low Back Pain

Fozzato S[1], Baranzini N[2], Bossi E[2], Roseti C[2], Cinquetti R, Grimaldi A[2], Campomenosi A[2], Surace MF[2,3]

[1]PhD School of Experimental and Translational Medicine, Insubria University- Department of Medicine and Surgery, University of Insubria, Varese, Italy; [2]Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy; [3]Interdisciplinary Research Centre for Pathology and Surgery of the Musculoskeletal System, Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy.

Chronic Low Back Pain (CLBP) is an inflammatory condition that may originate from an injury, disease or stress on tendons, ligaments and discus of the spinal structure. It is known that neuroinflammatory processes are pathologic hallmarks of CLBP that lead to the release of proinflammatory molecules that increase nociceptors sensitization, pain hypersensitivity or hyperalgesia. Transient Receptor Potential (TRP) channels are known to act as receptors of various stimuli in peripheral sensory neurons and in other somatic structure. Numerous studies highlighted the activation and/or sensitization of these channels during inflammation as the major mechanism underlying neuropathic and inflammatory pain. In order to investigate the role played and to classify TRPs channels in samples from 6 patients affected by CLBP, the TRPs expression was measured and morphological, ultrastructural and immunohistochemical alterations were analyzed. Immunofluorescence and expression analyses showed a significant increase in the levels of TRPs (A1, V1, V2, V4 and M8) in the pathological capsule compared to control tissues. Interesting, in each patient analyzed, we found an over-expression of TRPV4, independently by the location and number of affected sites. Moreover, using silver impregnation, it was shown that in CLBP patients the capsular connective tissue appeared degraded and infiltrated by sensitive unmyelinated nervous fibers. The findings confirm the involvement of TRP channels, in particularly of the TRPV4 and TRPM8 in CLBP pathological condition suggesting that these channels could represent a target for new therapeutic approaches.

PP.86

Changes in static perimetry during CHAMBER REST: a pilot study

Michalčák T[1], Škrda D[1], Malůš M[2], Vavřina Z[1], Ekrtová T[1], Jarkuliš V[1], Švorc PJ[1].

[1]Dep. of Physiology and Pathophysiology, University of Ostrava, Czech Republic; [2] Dep. of Psychology, University of Ostrava, Czech Republic

The aim of this study is to evaluate the effect of CHAMBER REST on visual field in young people. The results of this experimental therapeutic method, which is based on staying in complete darkness to improve health of people living under constant stress, suggest that it may positively influence the sensitivity of the retina. We evaluated 14 students were placed into a special room with maximal darkness for 96 hours. The first measurement was performed the day before starting the therapy. The next measurement was taken 30 minutes after completing the therapeutic session, followed by two measurements in the 4th and the 7th day after exiting the darkness. Measurements were obtained for both left and right eye by using the OCULUS Centerfield 2, in cooperation with the Macula Threshold test. The Mean Defect (MD) visual field index, which is the most important index describing the mean loss of sensitivity and the reduction of the visual field, was used for the evaluation. In this study, the results of the MD index (measured 1 day before starting the therapy and 7 days after finishing it) were compared. 71 % of students showed an improvement of the MD index in both eyes, 21 % improved only in one eye and no improvement was found in 7 % of the students. A statistical analysis proved these results to be statistically significant. Results demonstrate the positive effect of long exposure of darkness on the retina and the visual field in young people.

PP.87

Selection for action in Fruitfly

Frighetto G[1,4], Zordan MA[2,4], Castiello U[1], Megighian A[3,4]

[1]Department of General Psychology, University of Padova, Italy; [2]Department of Biology, University of Padova, Italy; [3]Department of Biomedical Sciences, University of Padova,

Italy,[4]Padova Neuroscience Center, University of Padova, Italy

The mechanism of action selection is a widely shared fundamental process required by animals to interact with the environment and adapt to it. A key step in this process is the filtering of many "distracting" sensory inputs which may disturb action selection. Because it has been suggested that, beyond sharing common mechanisms, action selection may also be processed by shared circuits in vertebrates and invertebrates we wondered whether invertebrates showed the ability to filter out "distracting" stimuli to maintain a goal directed action, as seen in vertebrates. Therefore, we studied

action selection in wild-type Drosophila melanogaster, by investigating their reaction to the abrupt appearance of a visual distractor during an ongoing locomotor action directed to a specific visual target. We found that flies tended to shift the original trajectory towards the distractor, thus acknowledging it's presence, but did not appear to commit to it, suggesting that an inhibition process took place in order to continue to carry out the original goal-directed action. To some extent flies appeared to take into account the level of salience of the abrupt distractor appearance as a basis for the ensuing motor program. However, they did not engage in a complete change in their initial motor program in favour of the distractor. These results provide interesting insights into the selection-for-action mechanism, in a context requiring action-centered attention which might have appeared rather early in the course of evolution.

PP.88

Changes in brain NOS activity depending on duration of social isolation

Vrankova S, Benko J, Galandakova Z, Cebova M, Murinova J, Riecansky I, Pechanova O

Centre of Experimental Medicine, SAS, Institute of Normal and Pathological Physiology, Bratislava, Slovakia

Nitric oxide (NO) concentration is sensitive to redox balance whose disruption has been proposed to play a role in pathophysiology of schizophrenia. Post-weaning social isolation is a well established developmental model, which is based on neurodevelopmental hypothesis of schizophrenia. The aim of this study was to elucidate some behavioural and biochemical changes using Sprague-Dawley rats reared in social isolation. At the age of 21 days, animals were randomly assigned into four groups. Two experimental groups were subjected to either 10 week or 29 week isolation; in control groups rats were reared socially. At the end of the experiment open-field test and prepulse inhibition (PPI) were carried out. Furthermore, we assessed NOS activity, protein expression of nNOS and iNOS isoforms and concentration of conjugated dienes (CD) in different brain areas. There was a significant effect of social isolation on PPI only after 10 week isolation. We found a decrease in NOS activity after 10 week isolation in both cerebellum and cortex. Paradoxically, 29 week isolation had an opposite effect - NOS activity increased almost two-fold in both regions. Ten week isolation caused significant decrease in nNOS expression only in cerebellum, while the expression of iNOS was increased only in hippocampus. Additionally, 10 week isolation raised CD concentration in whole-cortex homogenate. Decrease of NOS activity after 10 week isolation might have been caused by chronic stress during isolation, which has been previously observed in other studies. Increased oxidative state might have sinked part of NO as it readily reacts with superoxide radical by creating peroxynitrite. Subsequent increase of NOS activity might have been needed to compensate for this loss. VEGA2/0151/18, APVV-14-0932

PP.89

Neuronal alterations in retinas with defective expression of the full length dystrophin

Catalani E[1], Coazzoli M[2], Zecchini S[3], Giovarelli M[2], Perrotta C[2], De Palma C[3], Clementi E[2,3], Cervia D[1]

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Università degli Studi della Tuscia, Viterbo, Italy; [2]Department of Biomedical and Clinical Sciences "Luigi Sacco" (DIBIC), Università degli Studi di Milano, Milano, Italy; [3]Unit of Clinical Pharmacology, University Hospital "Luigi Sacco"-ASST Fatebenefratelli Sacco, Milano, Italy

Duchenne Muscular Dystrophy (DMD) patients have a cognitive impairment and disordered CNS architecture. They also show atypical retinal electrophysiology and colour defects although the impact of dystrophin alterations on singular retinal population is unknown. Using mdx mice, a model of DMD exhibiting defective expression of the full length dystrophin (Dp427), we found no evident changes in the thickness of the whole retina/singular layers and cell density. However, LC3 and p62 autophagy markers were over-expressed in OPL/INL and GCL of mdx retinas, indicative of a defective autophagic turnover; similar results were obtained analysing the active-caspase 3 staining to detect apoptosis. Accumulated LC3 co-localized with: i) CtBP2-positive photoreceptor synapses and mGluR6-positive postsynaptic membranes of bipolar cells, in OPL; ii) PKC-/Mab115A10-positive bipolar cells, gat-1-/dab-1-positive amacrine cells, in INL; and iii) beta tubulin-III-positive ganglion cells, in GCL. No LC3 signal was detected in calbindin-positive horizontal cells of INL. The majority of LC3 positive cells stained with active-caspase 3. Ultrastructural analyses of OPL/INL revealed deep morphologic abnormalities in photoreceptor synapses of mdx retinas that were disorganized and altered in shape; synapses and neurons, likely bipolar and amacrine cells with damaged mitochondria, contained an higher number of autophagosomes. These data indicated that neurons of mdx mice display dysfunctional autophagy and are committed to dye by apoptosis, in line with the role of apoptosis/autophagy system in different retinal pathologies. The most compromised retinal region is the OPL/INL, which mainly expresses Dp427, thus

further corroborating its involvement in neuronal networks and synapses formation.

PP.90

Cellular brain edema induced by water intoxication in rat experimental model.

Pokorný J[1], **Marešová D**[1], **Kozler P**[1]

[1] Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

A new model of the cellular brain edema, induced by water intoxication (WI) is presented. The basic principle of the model is an osmotic imbalance in the cell membrane followed by an intracellular flow of sodium and simultaneous accumulation of water leading to the subsequent increase of blood-brain barrier permeability. The adequacy of the model was tested in specified conditions with clearly expressed results. The model enabled to understand both the mechanism of cellular edema as well as its accompanying effects. The assumption that WI brings about cellular edema with increased BBB permeability was proved by intracellular accumulation of intravital dye with a large molecular size; by increased brain-water content confirmed by measuring the dry/wet weight ratio along with decrease in CT density and by elevated intracranial pressure (ICP) due to the expanding volume, determined by continuous monitoring the ICP. The structural lesions caused by cellular edema were proved by identification of the myelin disintegration; and the impaired nervous functions was demonstrated by behavioural changes in the of open field test. Presented experimental model can foster the future studies of pathophysiology of cellular brain edema and it can be also suitable for testing neuroprotective agents. Supported by Q35/LF1.

PP.91

p11 in cholinergic interneurons of the nucleus accumbens is essential for dopamineresponses to rewarding stimuli.

Hanada Y[1], **Kawahara Y**[1], **Ohnishi Y**[1], **Shuto T**[1], **Kuroiwa M**[1], **Sotogaku N**[1], **Greengard P**[2], **Sagi Y**[2], **Nishi A**[1]

[1]Department of Pharmacology, Kurume University School of Medicine, Japan; [2]Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, USA

p11, a member of the S100 protein family, regulates depression-like behaviors and responses to antidepressants. A recent study showed that p11 expressed in cholinergic interneurons (CINs) of the nucleus accumbens (NAc) is a key regulator of depression-like behaviors. Dopaminergic neurons projecting to the NAc are responsible for reward-related behaviors, and their function is impaired in depression. The present study investigated the role of p11 in NAc CINs in dopamine responses to rewarding stimuli. The extracellular dopamine and acetylcholine (ACh) levels in the NAc were determined in freely moving male mice using in vivo microdialysis. Rewarding stimuli (cocaine, palatable food and female mouse encounter) induced an increase in dopamine efflux in the NAc of wild-type mice. The dopamine responses were attenuated (cocaine) or abolished (food and female mouse encounter) in constitutive p11 KO mice. The dopamine response to cocaine was accompanied by an increase in ACh NAc efflux, whereas the attenuated dopamine response to cocaine in p11 KO mice was restored by activation of nicotinic or muscarinic ACh receptors in the NAc. Dopamine responses to rewarding stimuli and ACh release in the NAc were attenuated in mice with deletion of p11 from cholinergic neurons (ChAT-p11 cKO mice), whereas gene delivery of p11 to CINs restored the dopamine responses. Furthermore, chemogenetic studies revealed that p11 is required for activation of CINs in response to rewarding stimuli. p11 in NAc CINs plays a critical role in activating these neurons to mediate dopamine responses to rewarding stimuli. The dysregulation of the mesolimbic dopamine system by dysfunction of p11 in NAc CINs may be involved in the pathogenesis of depressive states.

PP.92

M_4 muscarinic receptor regulation of locomotor activity biorhythm is caused by core clock changes and striatum, thalamus, motor cortex and intergeniculate leaflet are involved.

Riljak V, **Janisova K**, **Myslivecek J**

Institute of Physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Understanding the complex interplay between gastroenteric information (from food or bacteria) and brain functioning requires a deep analysis of the neuronal substrate where gut-derived information is processed in the brain. In this work we analysed the CNS responses to intraluminal administration of molecules of bacterial origin, in order to understand which networks the brain employs to analyse microbiota-related information travelling along the gut-brain axis. We mapped cortical and subcortical responses to intraluminal stimulation in mice by *functional ultrasound imaging*, performed through a cranial window exposing the whole brain in the medio-lateral direction, and spanning a length of ±2 mm

around bregma in the rostrocaudal direction. The stimulation was performed by injecting LPS from *E. coli*, the short fatty acid Propionate, various fractions from the cultivation of Lactobacillus *JB-1*, water or glucose 10%. We found that the administration of bacterial molecules in the gut lumen evokes responses mainly from hypothalamic and limbic regions, with activation of all major nuclei of the amygdala. In this regard, bacteria-related inputs resemble much closely water-induced responses, which are known to activate extensive homeostatic control networks, involved in fluid balance and blood pressure management, than glucose-induced responses, which are mainly limited to interoceptive sensory areas, such as the Insular cortex. Moreover, bacterial molecules did also elicit pronounced activity in the hippocampus. These results show for the first time that the mammalian brain can detect the presence of different bacteria in the gastrointestinal tract through interoceptive circuits, and can produce different responses depending on the nature of the bacterial input.

PP.93

Cortical and subcortical representations of interoceptive inputs from Microbial populations in the gut.

Sanchini G, Gelmini S, Brocci F, Quadraro D, Esposti F

Vita-Salute San Raffaele University, Neuroscience Division, Milan, Italy

We have shown previously that deletion of M4 muscarinic receptors (MR) increases locomotor activity and changes biorhythm parameters in females, not males. Further, muscarinic drug effects differed on the morning and on the evening. Here, we searched for the mechanisms that are responsible for locomotor activity biorhythm changes. We performed biorhythm analysis in two experiments: in the experiment 1, the mice (C57Bl/6NTac and M4 MR -/- mice (KO) on the same background) were first exposed to standard LD regime (12/12 light/dark cycle, light on at 7:00 AM) for 8 days and then they were exposed to constant darkness (for 24 hours/day, DD regime) for other 16 days. In the experiment 2 were the mice (after standard LD regime) exposed to DD regime and to one light pulse (300 lx, 1h, administered at zeitgeber time 14, onset of subjective night) on day 9. In the experiment 1, the biorhythm activity curves differed, period (t, duration of diurnal cycle) was shorter in DD regime. Moreover, day mean, night mean and their difference were higher in KO animals. Also, mesor (midline value) was higher in KO. The time, in which the maximal slope occurred was lowered in WT and KO and was lower in KO than in WT. In the experiment 2, there were no differences in biorhythm parameters between WT and KO. In vitro autoradiography showed that M4MR proportion represents 24% in the motor cortex, 50% in the striatum, 69% in the thalamus, and 48% in the intergeniculate leaflet. The M4MR densities were negligible in other brain areas, like suprachiasmatic nuclei, involved in biorhythm regulation. These results suggest that core clock output is changed and that the structures involved in biorhythm regulation in WT and KO are the most probably the same.

Poster Session II (1/7)

EUROPEAN YOUNG PHYSIOLOGISTS

PP.94

The effect of surfactant on the airway smooth muscle after lipopolysaccharide exposure

Topercerova J[1], Kolomaznik M[2], Kopincova J[1], Nova Z[1], Urbanova A[2], Mokra D[2], Mokry J[2,3], Calkovska A[1].

[1]Department of Physiology; [2]Division of Respirology, Martin Biomedical Centre; [3]Department of Pharmacology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Slovakia

Surfactant function may be negatively influenced by bacterial wall component lipopolysaccharide (LPS). LPS may directly or indirectly modify the airway smooth muscle (ASM) tone, possibly interacting with the effects of pulmonary surfactant. The aim of the study was to investigate the effects of LPS on the ASM reactivity and to elucidate the role of surfactant in this interaction. Variable response of LPS was controlled by indomethacin and potential relaxing effect of surfactant on the ASM was studied using leukotriene and histamine receptor antagonists. The experiments were performed by method of tissue organ baths of adult guinea pigs (healthy or exposed to LPS in vitro conditions) and animals intraperitoneally injected with LPS at a dose 1 mg/kg of b.w. once a day during 4day period. Exposure of tracheal strip to surfactant at 1 mg of phospholipids/ml caused significant ASM relaxation of healthy ($P<0.05$) and LPS-treated animals ($P<0.001$). Indomethacin potentiated tracheal reactivity to metacholine, but reduced the response of ASM exposed to LPS in vitro ($P<0.05$). Antagonists of leukotriene receptors montelukast and histaminine-H1 receptors mepyramine significantly decreased reactivity of tracheal tissue to metacholine in healthy and LPS-treated animals ($P<0.001$). The exogenous surfactant has relaxing effect on the ASM, but does not reverse LPS-induced smooth muscle contraction. Indomethacin inhibits production of bronchodilator prostaglandins and results in an increase of metacholine response of ASM exposed to LPS in vitro. The results indicate involvement of leukotriene and H1 receptors in contractile mechanisms of the airways in presence of LPS. Supported by projects VEGA 1/0055/19 and APVV-17-0250.

PP.95

Oxidative imbalance and kidney damage: new study perspectives from animal models to hospitalized patients

La Russa D[1,2], Montesanto A[3], Pellegrino D[2,3].

[1]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Italy; [2]LARSO, University of Calabria, Italy; [3]Department of Biology, Ecology and Earth Sciences, University of Calabria, Italy.

Chronic kidney disease (CKD) is a major public health problem worldwide and its main consequences include the loss of renal function leading to end-stage renal disease, an increased risk of cardiovascular disease, a significant increase in morbidity and mortality, and a decrease in health related quality of life. The present research work was based on translational approach to study the role of many CKD risk factors such as hypertension, oxidative stress/inflammation, obesity, and hyperuricemia with the aim of identifying new molecular mechanisms of kidney damage to prevent it by successful behavior modifications. For this purpose, both human and animal models were used. In animal models, we analyzed the mechanisms of renal damage induced by hypertension (Spontaneously Hypertensive Rat) and obesity (Cafeteria diet rats) showing that the redox disequilibrium in plasma and tissue is extremely important in the renal alteration in terms of both oxidative damage (lipid peroxidation, altered expression antioxidant enzymes) and apoptotic pathways (intrinsic/extrinsic) activation. In hemodialysis patients, we explored the correlation between the global oxidative balance and both inflammatory markers and cardiovascular risk showing a strong correlation between oxidative index and both blood levels of C-reactive protein and previous cardiovascular events. In addition, in hyperuricemic patients, we detected a significant alteration in redox balance. This multilevel approach has allowed us to individually and synergistically analyze some aspects of the complex pathogenic mechanism of CKD, in order to clarify the role of the new amplifying risk factors for CKD and to prepare an effective personalized prevention plan by acting on both modifiable and non-modifiable risk factors.

PP.96

Role of orexin neurons in the lateral hypothalamus in torpor onset in mice

Hitrec T[1], Amici R[1], Bastianini S[1], Berteotti C[1], Chiavetta P[1], Lo Martire V[1], Luppi M[1], Martelli D[1], Occhinegro A[1], Squarcio F[1], Stanzani A[2], Tupone D[1], Zoccoli G[1], Cerri M[1].

[1]Department of Biomedical and Neuromotor Sciences-Physiology, [2]Department of Veterinary Medicine, University of Bologna.

Torpor is an energy-saving physiological state, characterized by a transient decrease in metabolic rate and core temperature. The mechanism underlying torpor occurrence is unknown. Orexin (ORX) neurons in the lateral hypothalamus (LH) are involved in wakesleep and food intake regulation, and in thermogenesis, the latter by modulating the activity of the raphe pallidus (RPa), a key brainstem thermoregulatory area. RPa has also been suggested as a possible target of the food intake-promoting effects of ORX. The aim of this experiment was to assess whether ORX neurons in the LH projecting to the RPa play a role in torpor onset in mice. Twenty eight C57BL/6J female mice (17-24g), adapted to an ambient temperature (Ta) of 28 C, were assigned to one of the following experimental groups: i) Torpor (n=5): the torpid state was induced by a 36-h fasting, followed by an acute exposure to low Ta (15 C); ii) Cold Exposure (n=5): mice were only acutely exposed to Ta 15 C; iii) Fasting (n=10): mice were only fasted. iv) Control (n=8): no changes in the ambient conditions were made. For tracing neural projection to the RPa, all animals were pre-injected in this area with a retrograde tracer (Cholera Toxin-b subunit, CTb). Mice from the Torpor group were sacrificed 90 min. after torpor onset, while the sacrifice of other animals was time-matched. Animals were transcardially perfused and their brains extracted for immunohistochemical detection of cFos, CTb and ORX. Triple stained neurons (cFos+/CTb+/ORX+) were found in both Fasting and Torpor groups, but not in Cold Exposure group. The results show that ORX neurons projecting to the RPa may have a role in hunger or food-seeking behaviour, rather than in thermoregulation.

PP.97

Investigation of the Possible Antiproliferative and Hepatoprotective Effects of an alcoholic extract from Thymbra spicata (Lamiaceae) aerial parts

Khalil M[1, 2], Khalifeh H[2], Baldini F[1], Daher A[2] Voci A[1] Vergani L[1*]

[1]Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, Italy; [2]Department of Biology, Laboratory Rammal Rammal (ATAC), Lebanese University, Beirut, Lebanon.

In Mediterranean area, thyme-like plants such as Thymbra spicata (Lamiaceae) are widely used in traditional medicine. This study investigates the

possible antitumor, antioxidant and antisteatotic activity of a polyphenol-enriched extract from T. spicata collected in South Lebanon. Aerial parts were subjected to extraction with ethanol 100%. The chemical composition of the extract (TE) was determined by gas and high-performance liquid chromatography (HPLC) coupled to mass spectrometry. TE was rich in different phenolic compounds, especially carvacrol (36.8%). The direct anti-tumor activity was assessed on two cancer cell lines (MCF7 and PC3), and on a normal cell line (MCF10A) as control, by MTT assay at increasing concentrations of TE. The IC50 values were 80±5.6 μg/mL for MCF7, 89±8.6 μg/mL for PC3, and >150 μg/mL for MCF10A cells. The radical scavenging activity was assessed by DPPH assay (IC50 values of about 24.5±1.09 μg/mL for all cell lines). The possible antisteatotic and hepatoprotective activity of TE was investigated in vitro on a cellular model of hepatosteatosis consisting of FaO hepatoma cells exposed to a mixture of oleate/palmitate. TE at 1.5 μg/mL ameliorated both lipid accumulation and oxidative stress in steatotic FaO cells. The present findings show that at high doses (>50 μg/mL) T. spicata extract exerted a significant dose-dependent inhibition of cancer cell proliferation, higher than that of pure carvacrol. At lower doses (<5 μg/mL), TE reduced steatosis and ameliorated the oxidative stress condition in steatotic hepatocytes. Our results suggest potential applications of Thymbra spicata as nutraceutical compound able to integrate therapy of metabolic disorders or selected tumors.

PP.98

Proinflammatory effects of phorbol-12-myristate-13-acetate on Caco-2 cells monolayers at different stages of spontaneous enterocyte-like differentiation in the presence or absence of the dipeptide carnosine: analysis of differential cytoskeletal morphology and gene expression

Mazzei A[1], **Croce F**[1], **Giampetruzzi L**[2], **Francioso L**[2], **Siciliano P**[2], **Verri T**[1], **Barca A**[1]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, IT; [2]CNR Institute for Microelectronics and Microsystems Lecce, IT.

GI inflammation involves pathological processes affecting the gut epithelial barrier that are poorly reversible. A direct target of such processes is the enterocyte monolayer, which absorptive function is challenged by inflammation-induced cytoskeleton dynamics. Here, we evaluate the proinflammatory effects of PMA (phorbol-12-myristate-13-acetate) on Caco-2 intestinal cells at two different stages of spontaneous differentiation i.e. undifferentiated (7 dps, days post seeding) and differentiated enterocyte-like cells (>21 dps) in the presence/absence of the natural dipeptide carnosine (CAR) as potential anti-inflammatory molecule. By analysing actin cytoskeleton in 7 dps Caco-2 monolayers versus 21 dps monolayers, we revealed opposite effects of PMA which respectively induced disruption vs. intensification of actin rings and fibers; in both cases, simultaneous administration of PMA and CAR showed counteracting effects. Remarkably, the mRNA expression analysis of the ACTB (actin b) gene mirrored the morphological evidences and the counteraction of CAR. Moreover, the mRNA expression analysis of the AIF-1 gene (Allograft Inflammatory Factor 1) revealed opposite trends in 7 dps- versus 21 dps-grown Caco-2 with respect to PMA effects, and the same holds true for the SLC15A4/PHT1 gene involved both in intestinal peptide absorption and inflammatory responses; nevertheless, AIF-1 mRNA were not affected by CAR, whilst SLC15A4/PHT1 mRNA showed CAR-dependent up-regulation regardless of the differentiation stage. Overall, our results describe cytoskeletal and gene expression modulations which hint a model to distinguish physiological and inflammatory responses based on the differentiation stage of the Caco-2 monolayer and on the effects of CAR as differentially protective substrate.

PP.99

Omega 3 fatty acids and hepatic insulin resistance: focus on ER stress and mitochondrial dynamics markers

Di Gregorio I[1], **Busiello RA**[2], **Lepretti M**[1], **Migliaccio V**[2], **Lionetti L**[1]

[1]Department of Chemistry and Biology "A. Zambelli", University of Salerno, Fisciano; [2]Department of Biology, University of Naples "Federico II", Italy

Omega 3 Poly-Unsaturated Fatty Acids (PUFA-ω3) have a protective and therapeutic role to prevent hepatic insulin resistance (IR). In this study, the protective effect was evaluated through: 1) hepatic insulin signaling pathway markers (phosphorylated protein kinase B on Ser473, pAKT/PKB); 2) endoplasmic reticulum (ER) stress marker (phosphorylated transcription factor p-eIF2); 3) mitochondrial dynamics marker (Mitofusin 2, Mfn2). These parameters were evaluated into 3 Wistar rats groups, so treated for 6 weeks: 1- N rats, treated with a standard diet (10.6% fats); 2- L rats, treated with a high fat diet, rich in lard (40% fats); 3- F rats, treated with a high fat diet rich in fish oil, major PUFA-ω3 source (40% fats). Hepatic pAKT, p-eIF2 and Mfn2 levels were determined by western blotting analysis. L group exhibited hepatic insulin resistance (as showed by

pAKT content) associated with ER stress (as showed by increased p-eIF2 content). Furthermore, we observed increased hepatic insulin sensitivity (as showed by reduced pAKT content) associated to ER stress reduction (reduced p-eIF2 content) in F group compared to L group. A fundamental role seems to be played by Mfn2, that increased in F vs L group, preventing not only mitochondrial integrity, but also eIF2 phosphorylation. In this way, fish oil may have positive effect in the prevention of ER stress and IR onset.

PP.100

Physiological adaptation to chronic exposure to high fat diet and environmental pollutant (DDE) in rat testis.

Migliaccio V[1,2], Sica R[2], Scudiero R[2], Simoniello P[3], Putti R[2], Lionetti L[1].

[1]Department of Chemistry and Biology, University of Salerno, Italy; [2]Deptment of biology, University Federico II, Naples, Italy; [3]Deptment of science and technology, University Parthenope, Naples, Italy

Fat intake induces metabolic disorders associated to obesity. Moreover, a wide range of hydrophobic chemicals, arrive to the body with contaminated foods acting as endocrine disruptors. Examples are represented by the organochlorine (OC)s at which belongs Dichlorodiphenildichloroethylene (DDE). These chemicals undergo to biomagnification producing oxidative stress, metabolic and hormonal disorders in mammalian. Our work was carried out to evaluate the adaptive responses in adult rat testis to which has administrated a non-toxic dose of DDE (10mg/Kg b.w.), for 28 days, alone or associated to high fat diet (HFD). 4 animal groups were used: N (Normal diet); D (HFD); D+DDE and N+DDE. Morphology of tubules was detected by using H&E stain. Lipid peroxidation, antioxidant activity and serum testosterone levels were measured with specific kits. Western blot analyses were done to test BAX, PCNA and androgen receptor (AR) protein levels. Finally, apoptotic cells were stained with caspase 3 immunohistochemistry. The results showed, in D group, increased lipid peroxidation, reduced antioxidant capacity, reduction of testosterone and AR levels, cellular damages and apoptosis. These results were found much more evident in DDE-treated groups, where the highest tissue damages occurred. In opposition to the rised negative effects, cellular proliferation increases in all groups, particularly in N+DDE. In conclusion, we assume that HFD and DDE produce cellular stress lead to antioxidant impairment, hormonal alteration, testicular damages and apoptosis more evident in presence of the pesticide. The increased cellular proliferation could be used to counterbalance the damages occurred following treatments, maintaining a pool of tubules that follow a physiological differentiation and maturation

PP.101

Effects of Weekend Warrior and Continuous Exercise Models on Depression Induced Cognitive Impairment

Öztürk ÇÇ[1], Ataoğlu SN[2], Arvas A[2], Tokol H[2], Yaprak H[2], Gürel S[2], Levent HN[3], Akakın D[3], Karademir B[4], Çakır B[1], Çakır ÖK[5].

[1]Department of Physiology, Maltepe University Turkey; [2]Marmara University Turkey; [3]Department of Histology & Embryology, Marmara University, Turkey; [4]Department of Biochemistry, Marmara University, Turkey; [5]Department of Physiology, Marmara University, Turkey

Depression is an important psychological disorder that goes on with cognitive decline. Exercise improves depressive mood. Our aim was to show the role of weekend warrior and continuous exercise models on cognitive impairment in depression and to exhibit the possible underlying mechanisms. Male rats (n=36) were separated as; Sedentary (SED), weekend warrior (WW), continuous exercise (CE) groups. Then the groups were divided into subgroups according to depression induction with chronic mild stress (CMS) procedure (n=6/group). CMS and exercise protocols continued for 6 weeks. Cognitive functions were evaluated by object recognition, anhedonia by sucrose preference, anxiety levels by Porsolt, open field and elevated plus maze, and fear conditioning by passive avoidance tests. Following decapitation, brain tissue glutathione (GSH) levels, myeloperoxidase (MPO), superoxide dismutase (SOD) and catalase (CAT) activities were measured, and histological damage was evaluated. The data was analyzed by ANOVA and student's t tests. Cognitive function and sucrose preference was decreased with CMS and increased in both CMS-induced exercise groups ($p<0.05$-0.001). The increased freezing time with CMS was decreased in CMS+WW group ($p<0.05$). The suppressed SOD and CAT activities in SED+CMS group and also GSH content were improved via both exercises ($p<0.05$-0.001). MPO activity was increased with CMS and returned back with both exercises ($p<0.05$). The latency decreased with CMS ($p<0.05$) and increased with CE ($p<0.05$). The rearing number was decreased with CMS and improved with CE ($p<0.05$). Neuronal damage was alleviated with both exercises. CMS-induced memory decline was improved by both exercise protocols via suppressing inflammatory process, anxiety level and by improving antioxidants.

PP.102

Experimental Parkinson Disease (PD) Alters Severity of Colitis in Rats

Secil F[2], Can HE[2], Karakoc HF[2], Dalgic N[2], Boyukyilmaz S[2], Karamahmutoglu T[4], Ozdemir ZN[1], Arabacı S[1], Sen LS[1], Karagoz A[2], Onat F[4], Akakın D[5], Alican YI[3], Imeryuz N[1,3]

[1]Physiology, Marmara University, Istanbul, Turkey; [2]Marmara University, Istanbul, Turkey; [3]Internal Medicine, Gastroenterology, Marmara University, Istanbul, Turkey; [4]Pharmacology, Marmara University, Istanbul, Turkey; [5]Histology, Marmara University, Istanbul, Turkey

Prevalence of PD is increased in humans with inflammatory bowel disease.We investigated whether PD alter the severity and mechanism of gut inflammation induced by trinitrobenzenesulfonic acid-ethanol(TNBS-E) in rats.PD/sham PD was induced in male Sprague Dawley rats by 6OHDA(0.8 mg/0.4 mcl/rat)/saline(S) injection within the medial forebrain bundle stereotaxically.Control(C) rats received no injection.Efficacy of the procedure was tested by rotation response to apomorphine.Colitis was induced by intrarectal administration of TNBS(30mg/ml;1ml) in 50% ethanol/saline 5 weeks after PD induction.The rats sacrificed 3 days later.Locomotor activity was measured in 6OHDATNBS-E(n=8),6OHDA-S(n=6),S-TNBS E(n=7),S-S(n=6),C-TNBS-E(n=7),C-S(n=6) groups 15 days before and 3 days after induction of colitis.Brain and colon were excised.Severity of colitis was evaluated macroscopically and microscopically.Myeloperoxidase(MPO) activity,malondialdehyde(MDA) and glutathione(GSH) levels determined in frozen colon and brain samples.Data were expressed as mean±SD,and compared by ANOVA.6OHDA treatment increased time spent at rest,decreased horizontal and vertical movement ($p<0.001$).Locomotor activity worsened by time,but did not change by TNBS-E.TNBS-E colitis was milder in 6OHDA treated rats macroscopically($p=0.03$) and microscopically ($p=0.02$).Colon MPO activity increased,GSH and MDA levels decreased in TNBS-E treated rats($p=0.003$ all),but 6OHDA treatment had no effect.Evaluation of brain is going on.Experimental PD alleviates TNBS-E induced colitis by reducing lipid peroxidation.Our study supports bidirectional relationship between brain and gut.Mechanisms of dopaminergic control in response to gut injury require further clarification.

PP.103

The splanchnic anti-inflammatory pathway requires the brain stem but not the hypothalamus

Occhinegro A[1], Farmer DGS[2], McKinley MJ[2], McAllen RM[2], Martellia D[1,2]

[1]Department of Biomedical and Neuromotor Science, University of Bologna, Italy; [2]Florey Institute of Neuroscience and Mental Health, Parkville, Australia

A neural reflex, the inflammatory reflex, controls inflammation developed in response to an immune challenge. The efferent motor arm of the inflammatory reflex, the splanchnic anti-inflammatory pathway (SAIP), travels in the splanchnic sympathetic nerves and, when activated, damps down inflammation. Following the finding that high spinal section did not prevent the increase of splanchnic sympathetic nerve activity induced by lipopolysaccharide (LPS), we hypothesized that the inflammatory reflex might be a simple spinal reflex. However, while in sham spinalized rats, bilateral section of the greater splanchnic nerves (SplancX) induced a dramatic increase in tumor necrosis factor (TNF), no such increase was seen if rats had previously been spinalized at C1 level. Spinalization alone was sufficient to enhance TNF plasma levels in response to LPS, indicating that the source of drive to the SAIP is supraspinal. To trace that supraspinal source of drive, we next transected or sham-transected the neuraxis, caudal to the paraventricular nucleus of the hypothalamus (PVN). The PVN contains premotor neurons with direct projections to the sympathetic preganglionic neurons in the spinal cord, from which the splanchnic nerves originate. They thus could be a source of drive to the SAIP. We studied two groups of anaesthetized rats whose brains were either sham or fully transected caudal to the PVN. We injected LPS (60 µg/kg i.v.) and collected blood samples 90 minutes after to measure TNF plasma levels. The results showed that brain transection caudal to the PVN did not alter the TNF response to LPS when compared with that in sham-transected animals. We conclude that the reflex control of the SAIP resides within the brain stem, rostral to the spinal cord but caudal to the PVN.

PP.104

Digoxin, A HIF 1 Pathway Inhibitor possesses significant effects on Glycemic Balance and Redox Status in a Rat Model of Type One Diabete Mellitus

Silvester N, Waweru P, Bukachi F

Department of Medical Physiology, University of Nairobi, Kenya

Oxidative stress is known to play a critical role in the initiation and progress of type 1 diabetes mellitus (T1DM). The HIF 1 pathway is critical in the body's adaptation and maintenance of redox balance during oxidative insult. This study investigated the effects of HIF 1 inhibition using digoxin on metabolic indices in T1DM. Thirty rats were randomized into five experimental groups: high dose test (digoxin), low dose test (digoxin), blocker [ethyl 3,4-dihydroxybenzoate (EDHB)], mechanism of action group (digoxin low dose and EDHB) and negative (normal saline). Animals in the low dose test group had: significant decreases in fasting blood glucose, improvements in insulin sensitivity, decreases in liver malondialdehyde concentration and serum catalase activity. Animals in the high dose test group however displayed opposite characteristics. EDHB antagonized the beneficial effects of low dose digoxin confirming the involvement of HIF pathway. Thus, HIF 1 pathway inhibition using low dose digoxin, improves glycemic and redox balance, and may prove therapeutically beneficial in management of type 1 diabetes mellitus patients.

PP.105

Effects of Antiepileptic Agents on Contractility of Detrusor Muscle Isolated from Wistar and Absence Epileptic WAG/Rij Rats

Salihoglu AK, **Kurt A**, **Ayar A**

Department of Physiology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey.

BACKGROUND: Considering the reports of urinary retention secondary to antiepileptic use, we aimed to investigate possible effects of valproic acid, levetiracetam and phenytoin on agonist-induced contractions of detrussor isolated from healthy Wistar and absence epileptic Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. METHODS: Isolated bladder strips were suspended in organ bath containing modified Krebs solution, bubbled with 95% O2 + 5% CO2 at 37°C, and isometric contractions were recorded. After equilibrium under resting tension of 1 g, contractions were stimulated by cumulatively applied carbachol (CCh; 0.1, 0.3, 1 and 3μM). CCh (3 μM) induced contractions were regarded as 100%, and effects of cumulatively applied valproate or levetiracetam or phenytoin was assessed on area under the curve (AUC) and peak amplitude of contractions. Data were compared by using Friedman test and Mann-Whitney U test. RESULTS: CCh caused significant contractile response in a dose dependent manner; both mean peak and AUC values of contractions of detrusor from WAG/Rij rats (n=24) was significantly higher than those from Wistars (n=21, $p<0.001$). In bladder strips of WAG/Rij rats; the mean AUC of CCh-induced contractions (considered as 100%) was inhibited to 79±3, 71±3, 63±4 and 51±4 % by 0.1, 0.3, 1 and 3μM valproate; 81±3, 73±4, 65±5 and 53±8 % by 100μM, 300μM, 1mM and 3mM levetiracetam; and 65±6, 37±7 and 21±5 % by 10μM, 30 μM, 100 μM phenytoin (n=8 for each), respectively. CONCLUSIONS: Although the tested concentrations are rather high in consideration of their therapeutic use, the inhibition by these antiepileptic agents may be of clinical relevance as they may impair micturition process by a peripheral effect.

Poster Session II (2/7)

Animal and Environmental Physiology

PP.106

A physiological approach to investigate the stress syndrome development in the Mediterranean mussel, *Mytilus galloprovincialis*.

Capolupo M, **Franzellitti S**, **Valbonesi P**, **Fabbri E**

Department of Biological, Geological, and Environmental Science, University of Bologna, Italy

The biochemical and cellular processes underlying the physiological responses induced by environmental factors in marine bivalves have been subject of several studies. Nonetheless, little is known regarding their evolution over a temporal gradient. In this study, we followed the response induced by an anthropogenic stress factor, i.e. burnt oil residues, in marine mussels (*Mytilus galloprovincialis*) over a 14-day exposure. A series of parameters were measured including general health status markers, as lysosomal membrane stability (LMS), neutral lipids (NL) and lysosome to cytoplasm volume ratio (LYS/CYT); lipid peroxidation products, as malondialdehyde (MDA) and lipofuscin (LF); the activity of enzymes involved in antioxidant processes (catalase, CAT), phase II metabolism (glutathione S-transferase, GST), and synaptic transmission (acetylcholinesterase, AChE). Results showed a rapid LMS decrease (day 4) and a strong increase of NL and LYS/CYT from day 2 to 12, indicating a protracted general stress condition. MDA showed a bell-shaped trend with significant changes after 4-8 days, while LF levels peaked at days 2 and 10. CAT was rapidly down- regulated after 4-6 days, likely reflecting a concurrent effect of lysosomal disorders and lipid peroxidation status on the antioxidant defences. GST activity showed a rapid up-regulation at day 2, followed by a progressive significant decrease, suggesting a time-dependent inhibition due to enhanced catabolic rate. AChE also showed an overall decrease, although no clear trend of

alteration was observed over time. Data suggest that stress-induced lysosomal alterations and pro-oxidant condition may lead to metabolic and neurological disorders in mussels, which might be deleterious for their physiological fitness in the long term.

PP.107

Physiological mechanisms of early embryo development in the bivalve *Mytilus*: influence of environmental factors

Miglioli A[1,2], Balbi T[1], Kapsenberg L[3,4], Gattuso J-P[3], Dumollard R[2], Canesi L[1]

[1]Dept. of Environmental, Earth and Life Sciences, University of Genoa, Italy; [2]Sorbonne University, CNRS, Laboratoire de Biologie du Developpement de Villefranchesur-mer, France; [3]Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche surmer, France; [4]Institute of Marine Sciences, Spain

In marine organisms, early life stages are highly sensitive to environmental perturbations, in particular those of calcifying species, that are facing new challenges from acidification to exposure to emerging contaminants. In bivalves, primary shell develops from the formation of a shell field, that secretes the shell matrix, the scaffold for $CaCO_3$ deposition. However, the physiological processes of development of the first shelled embryo, when the blueprint for calcification is established, are poorly understood. The mussel *Mytilus* is widely utilized to evaluate physiological responses to environmental stressors. Data are summarized on the phenotypical and molecular changes occurring in early development. from fertilization to 24 and 48 hpf, evaluated by light and confocal microscopy, transcriptomics, *in situ* hybridization. Main changes in transcription of genes involved in neuroendocrine signaling and shell formation were observed, with a key role of tyrosinase in shell matrix deposition. The molecular targets of estrogenic compounds 17β-estradiol, bisphenol A were also identified. The estrogen-induced phenotypical changes were similar to those induced by low pH, that affected the formation of the shell field and soft-tissues, independent from calcification. The results show that the formation of the first shelled embryo is the most sensitive stage of mussel development and will help identifying common/specific molecular targets for different environmental stressors.

PP.108

Heat stress-dependent morpho-functional changes in the gills of the Antarctic *Trematomus bernacchii* and *Chionodraco hamatus*

Garofalo F[1], Santovito G[2], Amelio D[1]

[1]Department of Biology, Ecology and Earth Sciences (B.E.S.T.), University of Calabria, Italy; [2]Department of Biology, University of Padova, Italy

In the global warming scenario, the effects of temperature changes on morpho-functional traits of the Antarctic marine species received great attention. In the present work, we investigated, on the gills of the Antarctic red-blooded *Trematomus bernacchii* and the haemoglobinless *Chionodraco hamatus*, the impact of heat stress on the morphology, the NOS/NO system, the heat shock response and the antioxidant defense. In both species, heat stress induced morphological modifications mainly represented by oedema of secondary lamellae and epithelial lifting. By immunolocalization we detected, in the unstressed gills of both Antarctic species, the presence of HSP-90, HSP-70, Xantine Oxidase, Heme Oxigenase and NOS and described their localization pattern. We also observed that heat stress affected the expression of these effectors, with changes that are species-specific. Although more efforts are needed to better understand the morpho-functional response to heat stress of Antarctic teleost gills, our preliminary data are of interest in view of the expected ocean-warming in which, the increment of water temperature can deeply influence both physiology and adaptive mechanisms of Antarctic stenotherm fish.

PP.109

Too warm or not too warm... Is the antioxidant system of Antarctic fish ready to face climate changes?

Tolomeo AM[1], Carraro A[1], Bakiu R[2], Toppo S[3], Gerdol M[4], Irato P[5], Bonato M[5], Corrà F[5], Pellegrino D[6], Garofalo F[6], Ferro D[7], Place SP[8], Santovito G[5]

[1]Department of Women's and Children's Health, University of Padova, Italy; [2]Department of Aquaculture and Fisheries, Agricultural University of Tirana, Albania, [3]Department of Molecular Medicine, University of Padova, Italy; [4]Department of Life Sciences, University of Trieste, Italy; [5]Department of Biology, University of Padua, Italy; [6]Department of Biology, Ecology and Earth Sciences, University of Calabria, Italy; [7]Department of Molecular and Cellular Biology, University of Arizona, USA; [8]Department of Biology, Sonoma State University, USA.

Antarctic fish, due to the short- and long-term stability of thermal conditions in the Southern Ocean over the last million years, are considered highly stenothermal with limited evolutionary potential to cope with climate changes. Recently, warm acclimation experiments performed on some notothenioid species have indicated a significant capacity for these fish to elevate

their heat tolerance, over the environmental CTmax, through acclimation. This result suggests that thermal plasticity may be universal throughout the Antarctic ichthyofauna. However, the physiological and genetic bases of their heat tolerance has been poorly studied. In the present work we described the molecular characterization of mitochondrial peroxiredoxins (Prdx) in the Antarctic emerald rockcod *Trematomus bernacchii* and gene expression of these antioxidant enzymes in various tissues, in response to short-term thermal stress. The obtained data are the first on the molecular and functional characterization of the genes encoding Prdx3 and Prdx5 of Antarctic fish, and constitute a further contribution to study these enzymes in specific ecological contexts such as Antarctica. Our expression analyses may be important for predicting climate change responses in this organism. In fact, the obtained results revealed rapid and specific responses of the Antarctic rockcod to wormer temperature. The presence of a Prdx (*prdx3* gene) whose expression is activated by increased temperatures, may be a condition that limits the stenothermy of *T. bernacchii*, macking this species less vulnerable to moderate environmental temperature changes and other environmental perturbations associated with global climate changes. (Supported by P.N.R.A. and M.I.U.R. grants).

PP.110

Innate immunity in bivalve molluscs: specificity of hemocyte responses to different environmental *Vibrio* species

Balbi T, **Vezzulli L**, **Canesi L** [5]

Department of Earth, Environment and Life Sciences-DISTAV, University of Genoa, Italy.

Invertebrates represent 97% of animal species and are widespread in any ecosystem. Despite they lack adaptive immunity, they have developed a potent and complex innate immune system showing many commonalities with that of vertebrates. The mechanisms of immune specificity is central to the invertebrate ability to maintain the physiological homeostasis in diverse environments. In bivalve molluscs, circulating hemocytes, resembling in structure and function the mammalian monocyte/macrophage lineage, mediate the responses to a variety of environmental stressors. Due to their filter-feeding habit, marine bivalves can accumulate large numbers of bacteria, in particular *Vibrio* species abundant in coastal waters. Persistence of vibrios in bivalve tissues largely depends on their sensitivity to the bactericidal activity of the hemolymph, resulting from complex interactions between bacteria, circulating hemocytes and soluble hemolymph components. In particular, the mussel *Mytilus galloprovincialis* is endowed with extremely powerful immune defenses, that make this species particularly resistant towards a wide range of microorganisms. In this work, the mechanisms involved in mussel innate immunity were investigated in vitro and in vivo using different experimental approaches; the results obtained in response to different *Vibrio* species and strains (*V. aestuarianus*, *V. splendidus*, *V. tapetis*, *V. coralliilyticus*), revealing different strategies to cope with potential pathogens, are summarized. The results provide evidence for the specificity of *Mytilus* immune response towards distinct strains and underline how research on host-pathogen interactions can greatly help understanding the mechanisms at the basis of innate immunity in bivalve molluscs, from the molecular to the organism level.

PP.111

Mechanisms of physiological protection in early embryo stages of marine mussels

Wathsala RGH, **Fabbri E**, **Capolupo M**, **Valbonesi P**, **Franzellitti S**

Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy

Ontogeny of protective systems at early stages of bivalves is crucial because those stages are considered more sensitive to the environment. In this study, in vitro fertilizations of gametes from naturally-spawning Mediterranean mussels were performed to follow transcriptional profiles of transcripts involved in protective processes across embryo development from fertilized oocytes (30 min post fertilization, pf) to fully developed D-shape shelled veligers (48h pf). Transcripts encoding ABC transporters increased up to 48h pf in agreement with a general reinforcement of protective systems needed to counteract the increased interaction of larvae with the environment. More complex profiles were observed for immune-related and lysosomal transcripts, likely in relation with their functional role during embryo development. Modulation of these protective mechanisms was evaluated with larvae grown in the presence of styrene (0.1 and 10 μg/L) or 3-μm polystyrene microparticles (PS-MPs, 50 and 500 particle/mL). PS-MPs showed significant reduction of transporter efflux activity and down-regulation of related gene transcription. Both styrene and PS-MPs induced a decrease in mRNA levels of genes encoding lysosomal enzymes and different modulation of immune-related transcripts. The effects observed with MP exposure are likely to be part of a generalized response triggered by particle ingestion and stimulation of digestive and immune systems,

whose developments in marine bivalves are inextricably linked together.

PP.112

Nanoparticles as a tool for unravelling the physiological mechanisms of innate immunity in the marine bivalve *Mytilus*

Auguste M, **Balbi T**, **Canesi L**

Department of Earth, Environment and Life sciences DISTAV, University of Genoa, Italy

In the last decades, the expansion of nanotechnologies for a variety of applications, including nanomedicine, has led to question about the interactions and mechanisms of action of nanoparticles-NPs, at the cellular level. Beside their beneficial or potential adverse biological effects, NPs can represent an interesting tool for studying the interactions with the physiology of target cells, especially with those of the immune system. In particular, the large spectrum of properties of NPs (core composition, size, shape and surface charge) offers an infinity of options for appreciating the different mechanisms involved in the interactions with the innate immune response, the first line of defense against non self- material. In the immune cells, the hemocytes, of the marine bivalve *Mytilus,* resembling the mammalian monocyte/macrophage lineage, NPs have been shown to trigger immunomodulatory effects by interfering with the mechanisms responsible for the physiological defence response. Through *in vitro* short-term exposure to NPs, investigation of several functional immune parameters (e.g. lysosomal function, phagocytosis, ROS and NO production, enzyme release) can give valuable information about the specificity of the immune response towards different types of NPs. Moreover, NP behavior in physiological exposure medium has been shown to depend on NP surface charge and interactions with soluble hemolymph proteins, thus revealing unexpected, although conserved roles for humoral-based immune response. The results underline how comparative studies on the interactions occurring at the nano-bio-interface represent an opportunity to understand the conservation of the mechanism of innate immunity from lower organism to humans. Work supported by the EU H2020 project PANDORA (GA 671881).

PP.113

Chronic exposure to Calypso on digestive cells in *Mytilus galloprovincialis*

Pagano M[1], **Stara A**[1,2], **Capparucci F**[1], **Faggio C**[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy. [2]Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Czech Republic.

Calypso is a widely used insecticide that is very effective against a wide spectrum of pests and for this reason it is present in water surface. Its main component is thiacloprid, a neonicotinoid. Neonicotinoids are widely used in agriculture. Currently in Europe three important neonicotinoids (imidacloprid, clothianidin and thiamethoxam) have been banned for environmental risk due to the negative effects they have on the life of bees. Studies on these insecticides have been carried out on vertebrates and invertebrates, and have highlighted lethal concentrations. However, there is lack of data on how the filtering aquatic organisms respond to the presence of these pollutants. *Mytilus galloprovincialis* is likely to be normally exposed to water used for agriculture and, normally used for human food, could accumulate this pollutant and create damages to humans. The main objective of the study was, after carrying out preliminary tests to assess the lethal dose of Calypso with *M. galloprovincialis*, the mussels expose for 20 days at two different concentrations of this insecticide (7.77 mg/L and 77.7mg/L) and take as target tissue the digestive cells, evaluating both the cell viability of hepatocytes and their volumetric regulation. The most significant result indicated that, after 20 days of treatment, the use of this insecticide could be dangerous for aquatic organisms and for the overall ecosystem, in fact, digestive cells of animals exposed to the lowest concentration were alive, instead of the cells exposed to highest concentration show high mortality. Calypso caused damages in a concentration dependent manner, showing serious consequences on their homeostatic capacity. Both concentrations of pollutants jeopardized the mechanisms involved in cell volume regulation. In conclusion, chronic exposure to this insecticide alters the physiology of these animals, thus can have a negative effect on the whole ecosystem. Work supported by the Ministry of Education, Youth and Sports, Czech Republic, project CENAKVA (LM2018099), and project Development of University of South Bohemia: International Mobility MSCA IF (no. CZ.02.2.69/0.0/0.0/17_050/0008486).

PP.114

Evaluating bivalve cytoprotective responses and their regulatory pathways in a climate change scenario.

Franzellitti S, **Fabbri E**

Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy

Marine mussels possess finely tuned protective mechanisms to cope with their living coastal environments, that are characterized by fluctuating physical/chemical parameters and elevated levels of natural and anthropogenic toxins. Elucidating how these mechanisms respond under environmental pressures to control animal acclimatory capacities is a challenging task. In this study, mussels were exposed to different concentrations of the metal copper (Cu) or the antibiotic oxytetracycline (OTC) at temperatures matching projected scenarios of future seawater temperature increases. The effects of thermal stress on Cu- or OTC- induced transcriptional modulation of a 70-kDa heat shock protein (HSP70) and of the ABC transporter P-glycoprotein (P-gp) was assessed along with the cAMP/PKA signaling pathway regulating both gene transcriptions. At the physiological temperature (16 C), Cu and OTC affected the regulatory pathway with bimodal changes of cAMP levels and PKA activities in gills of exposed animals. These results agree with reports showing Cu as a modulator of the mussel cAMP signaling, and tetracyclines as exerting non-antibacterial related effects in mammalian cells. A correlation between OTC- or Cu- induced changes of PKA activity and expression of stress-related transcripts *HSP70* and *P-gp* was observed. Temperature increases (up to 24 C) altered *P-gp* and *HSP70* responses to the pollutants and disrupted their relationship with cAMP/PKA modulation, leading to non-related concentration-related trends. On the whole, the results indicate that temperature may influence the regulatory pathways of cellular responses to pollutants (metals or antibiotics) commonly found

PP.115

Nitrite stress and arginase activity in freshwater aquatic animals: similarities and differences between fish and shrimp

Napolitano G[1], **Venditti P**[2], **Fasciolo G**[2], **Agnisola C**[2]

[1]Department of Science and Technologies, University of Naples "Parthenope", Italy; [2]Department of Biology, University of Naples "Federico II", Italy

In the last few decades, there has been a gradual increase in nitrite environmental stress due to human activities. In freshwater animals, nitrite can accumulate in body fluids owing to its high affinity with the branchial chloride transporter. Increases in nitrate levels can have consequences on the body metabolism by altering the homeostasis of nitric oxide and by determining the formation of methaemoglobin with consequent reduction of the oxygen uptake capacity. Nitric oxide homeostasis is dependent from the arginase activity. This enzyme competes with NOS (nitric oxide synthase) for the substrate (arginine) and is a major determinant of urea production and excretion in ammoniotelic organisms as well as a modulator of the arginine-NO homeostasis. Here we report the effects of the acute exposure to submaximal concentrations of nitrite (1-2 mM) on the urea excretion, as well as muscle urea levels and arginase activity in two fish species (*Danio rerio* and *Amatitlania nigrofasciata*) and one shrimp species (*Caridina multidentata*). In all species, nitrite exposure induces a significant increase in the urea production and excretion, associated with a stimulation of muscular arginase activity, suggesting that this enzyme may be at the centre of the response to nitrite stress in aquatic animals.

PP.116

ERK1/2 modulation of cell proliferation and migration in trophoblast cells exposed to bisphenols as endocrine disruptors

Valbonesi P, **Profita M**, **Fabbri E**.

Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy

Successful pregnancy requires effective proliferation, differentiation and invasion of villous trophoblast cells into the endometrium, where they remodel the maternal vascular system and establish maternal-fetal circulation. Insufficient migration and shallow invasion may lead to spontaneous abortion, fetal intrauterine growth restriction, and pre-eclampsia. Bisphenol A (BPA) is the prototype of environmental chemicals, with a well-recognized estrogen-like activity and wide diffusion in several items, including polycarbonate plastics and cans used for food and beverages. It transfers across the placental-blood barrier and is widely found in amniotic fluid, placental tissue, and umbilical cord blood. As we previously observed, proliferation of HTR-8/SVneo cells, derived from extravillous trophoblast cells, is enhanced by BPA through the ERK1,2 pathway. In the present study BPA exposure reduced the migratory ability of HTR-8/SVneo cells, which decreased to 78% and 47% compared with controls, in the presence of 10^{-7} and 10^{-5} M BPA, respectively. Both the exposure to 10 μM U0126, a specific inhibitor of ERK1,2 activation, and the treatment with tamoxifen, an anti- estrogenic drug, abolished the negative effect of BPA on cell migration. Furthermore, Bisphenol S (BPS), touted as a potential safer alternative to BPA, triggers an estrogenic response via ERK1,2 pathway on HTR-8/SVneo cells.

Overall, BPA and BPS induce the phosphorylation of ERKs suggesting a possible role as estrogen-disruptors affecting the reproductive success via non-genomic pathways. Further experiments are needed to clarify downstream effectors of ERK1,2 activation leading to up- and down- regulation of proliferation and migration, respectively.

PP.117

Seasonal variations in lipid content and protein degradation enzymes pattern in reared rainbow trout organs moderate by natural dietary supplement

Kantserova N, **Lysenko L**, **Fokina N**, **Sukhovskaya I**, **Nemova N**

Institute of Biology KarRC RAS, Petrozavodsk, Russia

Rainbow trout, a widely cultured fish species, whose growth and survival always are threatened by different factors particularly summer increase in water temperature. It is known that this uncontrollable environmental factor disturbs physiological (growth, behaviour, appetite) and biochemical (heat shock response, fatty acid composition of membranes, etc.) processes in fish. To optimize rainbow trout production dietary supplements including natural substances are used commonly. The feeding trial was aimed to evaluate the effect of dihydroquercetin and arabinogalactan dietary supplement with proposed immunostimulant and related biological activities on lipid content and composition and growth-related protein degrading enzymes in rainbow trout. Fish growing in cages were fed either a commercial diet (control) or a supplemented diet during the summer-autumn season. Positive correlation in muscle and hepatic total lipid and triacylglycerol accumulation with ambient water temperature was found in both groups. Maximum water temperature corresponded with an increased content of essential saturated fatty acids, moreover, the observed phenomenon was more pronounced in the control group. Summer increase in water temperature led to suppression (more substantial in the control group) of calpain and proteasome-dependent protein degradation indicating a total decrease in net protein metabolism in an unfavorable period. Based on our observations, we suppose that the natural dietary supplement improving fish tolerance to the fluctuations in the nature environment is promising to be used in rainbow trout production. The study was financially supported by the Russian Science Foundation, project no. 17-74-20098.

PP.118

A physiological approach to assess the impact of endocrine disruptors, from invertebrate to human models

Fabbri E[1], Acconcia F[2], Canesi L[3], Cerra MC[4], Senese R[5], Verri T[6]

1Dept. of Biological, Geological and Environmental Sciences, University of Bologna, Italy; 2Dept. of Science, University of Roma, Italy; 3Dept. of Earth, Environment and Life Sciences, University of Genoa, Italy; 4Dept. of Pharmacy and Health and Nutrition Sciences, University of Calabria, Italy; 5Dept. of Environmental, biological and pharmaceutical sciences and technologies, University of Campania, Italy; 6Dept. of Biological and Environmental Sciences and Technologies, University of Salento, Italy.

Endocrine Disruptors (EDs) are exogenous chemicals that interfere with the endocrine system and may cause adverse health effects. The difficulties encountered so far to identify EDs and define the risks to humans and wildlife strongly suggest that investigations must be performed with a new focus, based on main features of endocrine physiology: i) effects of hormones (Hs) are exerted at very low concentration through specific receptors and coupled pathways; ii) some Hs may act through more than one receptor; iii) Hs may act through different/multiple mechanisms in different physiological systems; iv) Hs produce different effects during animal life cycle. Same properties are shared by EDs and us such have to be investigated. Our laboratories demonstrated that natural and environmental estrogens affect embryo development in mussels and zebrafish, and impair cardiac activity leading to expression of cellular stress markers in seabass. BPA in particular is known to impair many steps of amphibians metamorphosis. We showed that it induces lipid accumulation in rat liver, and mimics the effect of E2 reducing ER intracellular levels and activating ER - dependent gene transcription in human cell lines; it also induces breast and trophoblast cell proliferation. Although the involvement of intracellular receptors was expected, the different models showed that membrane receptors, including Gprotein coupled receptors, are also targets, and MAPK, PI3K, calcium and cAMP – dependent pathways are involved. Data highlighted action mechanisms of EDs, suggested the taxonomic conservation of some routes, and provided the basis to establish key-events shared by Hs and EDs. Further work addressing comparative aspects from invertebrates to humans is needed to develop full understanding.

PP.119

Ambient Water Temperature Promotes Temperature Response Including Lipid Modifications and Oxidative Stress in Fish

Sukhovskaya I[1], Fokina N[1], Kochneva A[1], Kurpe S[2], Kantserova N[1]

[1]Institute of Biology of KarRC RAS, Petrozavodsk, Russia ; [2]Petrozavodsk State University, Russia

The effect of seasonal variations in water temperature on oxidative stress markers and lipid composition of liver and skeletal muscle of rainbow trout was studied since May to July 2018. In poikilothermic organisms like fish, up to two-fold water temperature increase (from 7.5 to 14.3 oC) led to substantial changes in physiology and biochemistry including temperature-dependent variations in lipid composition, membrane fluidity, and reactive oxygen species generation resulting in oxidative stress response. Oxidative stress in fish was shown by the antioxidant enzyme activities and reduced glutathione (GSH) content. Energy reserves were estimated by the accumulation of individual lipid classes, such as phospholipids, triacylglycerols, and cholesterol, and their ratio. Oxidative stress response in fish fed with natural supplement, dihydroquercetin, were less pronounced as those fish had relatively high hepatic glutathione-S-transferase activity and muscle GSH reserves unaffected by temperature raise. Despite similar growth rate in rainbow trout fed both diets, their hepatic and muscle lipid reserves increased with temperature by a different manner, with the prevalence of total lipids such as phospholipids and cholesterol in fish fed standard diet. Excessive lipid accumulation in over-feeding fish led to steatosis, a common disease in cultivated fish. Thus, dietary supplement with the natural bioactive compound could prevent temperature-dependent metabolic disorders and oxidative stress response in reared fish. In our work, the promising results on moderate temperature-dependent responses in widely cultivated fish species were obtained. The study was supported by the Russian Science Foundation, project no. 17-74-20098.

PP.120

SoLute Carrier (SLC) genes expression along the rostro-caudal axis of adult teleost fish gut: a publicly available datasets analysis

Piccinno G[1,§], Del Vecchio G[1,4], Barca A[1], Mazzei A[1], Vacca F[2], Cinquetti R[2], Murashita K[3,4], Gomes AS[4], Bossi E[2], Rønnestad I[5], Verri T[1]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Italy; [2]Department of Biotechnology and Life Sciences, University of Insubria, Italy; [3]Research Center for Aquaculture Systems, National Research Institute of Aquaculture, Japan; [4]Fisheries Research and Education Agency, Japan; [5]Department of Biological Sciences, University of Bergen, Norway; [§]Present address: University of Trento, Italy

SLC genes display different expression profiles along the rostral-caudal axis of the gut depending on species, digestive requirements and specialization of the sequential domains. Here, we report on the longitudinal expression profiles of SLC genes in the intestine of two simple-gutted teleost fish, zebrafish and Ballan wrasse (*L. bergylta*), using a subset of publicly available data from the GEO repository (zebrafish: [GSE20884]; Ballan wrasse: [GSE93191]). We explored intra-species/intra-dataset concordances/discordances in the expression profiles through Spearman simple correlation, which coefficients, even if heavily affected by the compositionality of the data, may be informative of which genes are co- or counter-expressed. Through hierarchical clustering on the correlation coefficients we defined groups of concordant and discordant profiles of SLC genes. An online app (freely available at: https://gianmarcopiccinno.shinyapps.io/zebrafish_wrasse_v2/) was developed that allows rapid display of the longitudinal patterns of the genes differentially expressed in the two datasets. All the analyses were performed through R programming language. To define differentially expressed genes, we performed multiple One-Way ANOVA tests, whose p-values were subjected to Benjamini-Hochberg correction. Genes were considered differentially expressed if the corresponding corrected p-value from the ANOVA test was lower than 0.05. The plots were prepared using the R package ggplot2. The online app was produced through the R package shiny, and is hosted by free servers of RStudio. Our tools allow comparative analysis of the longitudinal profiles of the genes expressed in simple guts, and can be extended to implement any new similar datasets.

PP.121

Metabolic enzymes activity and lipid profile in Atlantic salmon (*Salmo salar* L.) reared under different photoperiod regimes

Churova M, Shulgina N, Murzina SA, Pekkoeva S, Voronin V, Krupnova M, Nemova N

Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences, Russia

The study was conducted to characterize the growth rates, energy metabolism level and lipid profile in Atlantic salmon Salmo salar L. reared under different photoperiod regimes in fish hatchery. The effects of photoperiod regimes LD16:8 (16 h light:8 h dark) and LD24:0 (24 h light:0 h dark) during 4 months (July-October) on growth, the activities of metabolic enzymes (cytochrome c oxidase, COX and lactate dehydrogenase, LDH, and aldolase) and lipid and fatty acids (FA) content in muscles of Atlantic salmon parr (at age 1+) were investigated. Received results were

compared with the obtained for parr of salmon reared under LD24:0 from May, so the effect of such light regime was prolonged. The significant differences of aerobioc and anerobic enzymes activities between studied groups were established. Changes in storage lipids and certain FAs in relation to different light regimes were detected: the content of these lipids and their FA constituents – 18:1(n-9), 16:1(n-7), 20:1(n-9), 22:1(n-11) were decreased in salmon under LD24:0 regime in Autumn. The levels of docosahexaenoic and arachidonic FAs were higher in fish reared under LD24:0 regime. These results may indicate adaptive reactions of parr to different light regimes and preadaptation of salmon reared under constant light to smoltification. The study was supported by the grant of the Russian Science Foundation No 19-14-00081.

PP.122

Orofacial Stimulation Test after application of Tapentadol in rats

Rokyta R[2], Fricova J[1,2]

[1]Department of Anesthesiology, Resuscitation and Intensive Care, First Faculty of Medicine, Pain Management Center, Charles University, Prague, Czech Republic; [2]Department of Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

INTRODUCTION: Tapentadol has dual synergistic agonistic effects (MOR/NRI) on μ opioid receptors (MOR) and noradrenaline reuptake inhibition (NRI). We used tapentadol in our animal model of orofacial pain, in which we evaluated the anti-nociceptive effects of tapentadol in experimentally (thermal and mechanical stimulation) induced orofacial pain. The Orofacial Stimulation Test, developed by Ugo Basile, measures hypersensitivity to thermal or mechanical stimulation of the trigeminal area. In the experiment, rats voluntarily contact a thermal or a mechanical stimulator with their unshaved vibrissal pad in order to access a food reward. Twenty adult laboratory rats (average weight = 345 grams) were tested. Tapentadol was applied intraperitoneally (i.p.) at doses of 1 mg/kg or 2 mg/kg. During the first day of the experiment, rats had an opportunity to get acquainted with the environment and look for food and find food, without pain. Each group was administered alternately physiological solution 0.1 ml/100 g i.p. or tapentadol 1 mg/kg i.p. one hour before testing. In the sham group, saline (0.1 ml/100 grams) was injected intraperitoneally. Dosing was done one hour before testing. The first group of animals was tested with the help of thermal stimulation (70 C). Mechanical testing was performed using Von Frey Hairs. Test periods lasted 10 minutes for all groups. RESULTS: We assessed tapentadol at 1 mg/kg and 2 mg/kg and compared it to a physiological solution. Tapentadol at 1 mg/kg had no effect on the thermal and mechanical stimulation. Tapentadol at 2 mg/kg prolonged the sensitivity of rats to mechanical stimulation. Of special note, there were differences between the results of experiments held in September/December vs. February, with more intensive anti-nociceptive effects observed in February.Average time of drinking (in milliseconds) is demonstrated. CONCLUSION:Tapentadol is the latest discovered molecule in a strong opioid group. It has a dual effect (MOR / NRI), agonistic effect on μ opioid receptor (MOR) and selective noradrenaline reuptake inhibitor (NRI). Tapentadol also activates alpha2 receptors in the spinal cord. We proved that the tapentadol, antinociceptive effect is realised only after the dosage of 2 mg per kilogram of tapentadol.

PP.123

Effetcs of Metoclopramide on motility of duodenum and colon in rats

Kacar E, Yardimci A, Ulker N, Coban E

Department of Physiology, Firat University, Turkey

Metoclopramide is an antiemetic agent and it is used in combination with hyoscine butylbromide in gastroenteritis which is generally occur with emesis. The effects of metoclopramide on intestinal motility have not been investigated in studies conducted to date. In our study, we aimed to investigate the possible effects of metoclopramide on acetylcholine-induced duodenum and colon contractions. The second goal of our study was to investigate the effect of metoclopramide on the colon and duodenum with hyoscine butylbromide, which reduces intestinal motility. Adult male Sprague-Dawley rats (n=8) were used in this study. Firstly, duodenum and colon (nearly 10 mm in length) was quickly removed and mounted in (tension 1 g) isolated tissue baths After equilibration, acetylcholine (10-4 M) was given to the all baths. After ten minutes, metoclopramide (35 μM) was given and incubated 10 min. After one hour rinsing, same procedure was repeated with hyoscine butylbromide (15 μM) and finally with together. Statistical analysis of the results was performed by Paired-Sample T-test. In duodenum, frequencies were significantly reduced in metoclopramide and with hyoscine butylbromide ($p<0.05$). Peak to peak amplitude values were significantly increased in both metoclopramide ($p<0.01$). Area values were significantly increased only metoclopramide with hyoscine butylbromide ($p<0.05$). In colon, all frequency values were significantly increased ($p<0.05$) and peak to peak amplitude and area values were significantly decreased ($p<0.05$). As a result, metoclopramide has inhibitory effect on

duodenum contractions while it has activator effects on colon contractions.

PP.124

Effects of PFOA and PFBS exposure in the soil invertebrate *Dendrobaena veneta* (Annelida)

Irato P[1], Bonato M[1], Corrà F[1], Dolfatto E[1], Guidolin L[1], Pietropoli E[1], Santovito G[1], Tallandini L[1], Vantini A[2]

[1]Department of Biology, University of Padua, Italy; [2]Regional Agency for the Environment, ARPA Veneto, Italy

Among the most worrying emerging pollutants, of great interest, are the mobile and persistent organic contaminants (PMOC, Persistent Mobile Organic Compounds), pollutants that have considerable persistence within the water cycle, degrade slowly, are very mobile in the water matrix and in biological tissues. Exposure to PMOCs can lead to serious health effects. A specific subclass of PMOC are the Perfluoroalkyl substances (PFAS), widely applied in a variety of industrial and consumer products since 1949. Due to their unique properties, they have been used in a wide variety of technological and industrial application, such as surfactants in fluoropolymer production, paper, textile, and household products. Aim of the present work is to study, in the earthworm *D. veneta*, bioaccumulation patterns and cellular responses in coelomocytes (mortality and lysosomal membrane stability), and at tissue level (glutathione peroxidase and metallohioneins, MTs), after the exposure to two PFAS (PFOA and PFBS) for short (72 h) and longer (14, 28, 42 days) times. The exposures are carried out in soil microcosms prepared with glass containers filled with 300 ml of soil humidified at 30% with PFOA or PFBS spiked water. Different accumulation patterns are observed for PFOA and PFBS both in the soft tissues and in coelomocytes, the main immunodefensive system cells of the organism, with a higher PFBS bioaccumulation in both compartments. In the exposed organisms, with both compounds, a significantly higher mortality in the coelomocytes, than in the controls is detected. Additionally, significant decreases of the lysosomal membrane stability are observed in these cells, and MT total level decrements in soft tissues. Further studies are running to explore the mechanisms underlying these results.

PP.125

Total oxidant and antioxidant activities in milk with various somatic cell count intervals during discrete cow and buffalo lactation periods

Yigit AA[1], Cinar M[2], H. Macun C[3], Ozenç E[4], Kabakci R[1], Yazici E[4], Kalender H[3], Bastan A[5], Salar S[5]

[1]Department of Physiology, University of Kirikkale, TURKEY; [2]Department of Biochemistry, University of Kirikkale, TURKEY; [3]Department of Obstetrics and Gynecology, University of Kirikkale, TURKEY; [4]Department of Obstetrics and Gynecology, University of Afyon Kocatepe, TURKEY; [5]Department of Obstetrics and Gynecology, University of Ankara, Turkey

Milk includes somatic cells that originate from the mammary epithelial cells (25%) and the macrophages, polymorphonuclear neutrophils cells (PMNs), lymphocytes and erythrocytes in the blood (75%). Although somatic cells have a protective effect against infectious organisms, their accrual alters the raw milk composition. Milk also, contains prooxidative and antioxidative compounds. This study first investigated the total oxidant and antioxidant capacity in cow and buffalo milk, with various somatic cell count levels, having the same lactation numbers, Second, it determined whether there is an association between the total oxidant and antioxidant capacity and the lactation number, for the same somatic cell count level. Quarter milk samples, collected from Holstein cows and Anatolian buffaloes, were separated into somatic cell count levels of $2\ 10^5$, $2\ 10^5$-$5\ 10^5$, $5\ 10^5$ -10^6 and 10^6 cells mL-1 for cow milk, and $2\ 10^5$, $2\ 10^5$-$4\ 10^5$, $4\ 10^5$-10^6 and 10^6 cells mL^{-1}, for buffalo milk. Next, each group was subdivided, according to the lactation number (cows: 1-2nd, 3-4th, 5-6th; buffaloes: 1-4th, 5-8th, 9-12th), and total oxidant and antioxidant capacity of the milk were measured. For the same lactation numbers, total oxidant capacity increased in the cow and buffalo groups with an somatic cell count 106 cells mL-1 ($p<0.05$). Conversely, total antioxidant capacity decreased in cow milk with an somatic cell count >5 105 cells mL^{-1}. In buffalo milk, total antioxidant capacity decreased in parallel with the increased somatic cell count. Among the same somatic cell count groups, total oxidant and antioxidant capacity were not affected by the lactation number, in cow and buffalo milk. An increased somatic cell count caused an increased total oxidant capacity and decreased total antioxidant capacity level, for the same lactation number. No relation existed among total oxidantmcapacity, total antioxidant capacity and lactation number, for the same somatic cell count level. Somatic cell count may be used as an indicator of total oxidant and antioxidant capacity in cow and buffalo milk.

PP.126

Effect of different lighting regimes on the growth and expression level of muscle-specific genes in yearlings (1+) Atlantic salmon (*Salmo salar* L.).

Shulgina N, Churova M, Krupnova M, Nemova N

Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences, Russia.

Photoperiod is associated to phenotypic plasticity of somatic growth in several teleost species. However, the molecular mechanisms underlying this phenomenon are currently unknown. This study was conducted to evaluate the features of the genes expression of myogenic regulatory factors (MRFs: MyoD paralogs, Myf5, MyoG), myosin heavy chain (MyHC) and MSTN paralogs in the white muscles of Atlantic salmon Salmo salar L. (1+) reared on fish hatchery in different lighting conditions — regimes LD16:8 (16 h light: 8 h dark) and LD 24:0 (24 h light: 0 h dark), for 4 months (July – October) and LD 24:0 for 6 months from May. Salmon reared in plant lighting regime (PL) were used for comparison. Results revealed no differences in the weight of fishes between the groups at the end of the 4 months experiment. But the positive effect of constant lighting on weight gain was shown in longer experiment. According to the molecular - genetic analysis the significant differences in MRFs genes expression were revealed. In autumn, the season when fishes growth rate generally decreased, the individuals from tanks with additional lighting showed higher mRNA levels of MyHC and MRFs expression, that were associated with a high mRNA level of MSTN paralogues, the negative muscle growth regulators. The study demonstrated that there are certain season-related patterns in simultaneous expression of several muscle-specific genes. In general, the findings show that additional lighting affects on the regulatory mechanisms of muscle growth processes in fishes. The study was supported by the grant of the Russian Science Foundation № 19-14-00081.

Poster Session II (3/7)

Endocrine Physiology

PP.127

Functional evaluation of chronic complications in type 2 mellitus diabetes

Toth E[1], Gotia SL[2], Gotia SR[2], Borza C[1]

[1]Department of Pathohysiology, University of Medicine and Pharmacy "V. Babes", Romania; [2]Department of Physiology, University of Medicine and Pharmacy "V. Babes", Romania

Type 2 mellitus diabetes (MD) is characterized by acute and chronic complications (micro and macro-angiopathy, neuropathy and trophic modifications). The aim of this study was to evaluate the chronic complications correlated with the duration of the disease in a group of 68 type 2 MD patients. The patients mean age was 52-78 years (42 male and 26 female). The study group was characterized by the the next parameters: clinical signs, Echo doppler aspect, blood and functional test (The Neuropathy Disability Score). Pointed out a high prevalence of vascular insufficiency (58%) – Echo aspect – third stage. This vascular insufficiency determinated trophic modifications (21%) and skin infections (13%). In 78% of cases with severe vascular insufficiency, the Neuropathy Disability Score which expressed the impairment of sensations, was higher than 6 which is predictive for foot ulcerations. In this subgroup of the patients, the skin temperature, colour and local sensations were abnormal. These aspects were observed in patients with a duration of disease higher than 15 years and an abnormal dynamic value of glicated hemoglobin. The duration of MD is important for the development of systemic chronic complications. The management of metabolic balance, elimination of risk factors for co-morbidities and an active medical education of these patients will decrease the appearance of chronic complications (angiopathic, neuropathic and trophic type).

PP.128

The effects of Nesfatin-1 on rat acute pancreatitis model: role of melanocortin receptors

Buzcu H[1], Özbeyli D[2], Yüksel M[2], Tuğçe Çilingir Kaya O[3], Kasimay Çakir O[1]

[1]Department of Physiology, Marmara University Turkey; [2]Department of Medical Laboratory Techniques, Marmara University, Turkey; [3]Department of Histology & Embryology, Marmara University, Turkey

Nesfatin-1, a recently discovered peptide, was shown to have anti-inflammatory effects. The aim of this study is to investigate the possible anti-inflammatory role of Nesfatin-1 and its underlying mechanism in acute pancreatitis model. Cerulein was applied intra-peritoneally to induced acute pancreatitis in Sprague-Dawley rats, except for the control groups. Nesfatin-1 was administered 5 minutes before the application of cerulein to determine its anti-inflammatory role. In order to investigate the underlying mechanism, oxytocin receptor antagonist (Atosiban), melanocortin receptor antagonist (HS024), or ghrelin receptor antagonist (Cortistatin) were administered. Five minutes after Nesfatin-1 administration, two doses of cerulein were applied one hour apart. The rats were sacrified 12 hours after the first cerulein dose. Microscopic damage scoring, malondialdehyde and glutathione levels, myeloperoxidase activity, luminol and lucigenin chemiluminescence in pancreas and amylase, lipase, trypsinogen-2 levels in serum were evaluated.

Oxidative damage was decreased with Nesfatin-1 treatment in the acute pancreatitis model. The administration of HS024 reversed the effect of Nesfatin-1, via increasing lipase, amylase, trypsinogen-2, MDA, MPO, and lucigenin levels, atosiban elevated MPO activity, luminol and lucigenin chemiluminescence levels and cortistatin increased lusigenin and luminol chemiluminescence. Although receptor antagonists reversed the effect of nesfatin-1 on biochemical parameters, no significant difference was found in histological scoring. Our results indicated that Nesfatin-1 had an anti-inflammatory effect on acute pancreatitis model via mainly effecting melanocortin receptors.

PP.129

Treadmill exercise improves control over the HPA and HPG axes response to restraint stress in male rats

Kelestimur H[1], Bulmus O[2], Yardimci A[1], Tancan E[1], Serhatlioglu I[3], Kacar E[1], Gulcu Bulmus F[4]

[1]Department of Physiology, University of Firat, Turkey; [2]Department of Physiotherapy and Rehabilitation, University of Firat, Turkey; [3]Department of Biophysics, University of Firat, Turkey; [4]Vocational School of Medical Services, University of Firat, Turkey.

In stressful situations, whether treadmill exercise has modulatory effects on the hypothalamic-pituitary-adrenal (HPA) and the hypothalamic-pituitary-gonadal (HPG) axes is not well known. The purpose of this study was to investigate the exercise- associated changes in the endocrine response to chronic restraint stress in male rats. The Sprague–Dawley male rats, three months old, were used for this aim. The animals were classified into four groups (n=10) which were the control, stress, stress + exercise and exercise groups. A restraint apparatus was used to induce the chronic stress. Treadmill exercise was performed for 5 weeks. The rats in exercise groups were subjected to moderate-intensity exercise on a motor-driven treadmill continuously for a period of 5 weeks between 09.00 am and 10.00 am. At the end of the study, the animals were decapitated and blood samples were taken for hormone analyses. Restraint produced significant increases in plasma corticosterone and insulin responses in the rats. The running rats not exposed to restraint stress also had higher plasma corticosterone levels. Treadmill exercise prevented the increases in these hormones in the rats exposed to restraint. Restraint caused a reduction in plasma LH levels, which was also reversed by treadmill exercise. Plasma testosterone levels were significantly higher in the running group compared with the other groups. These findings suggest that treadmill exercise may improve control over the HPA and HPG axes response to stress while it causes corticosterone response itself in the rats not exposed to restraint. Further studies are needed to explore the mechanisms by which treadmill exercise causes different endocrine responses in stress and sedentary animals.

PP.130

Changes in neuroglia morphology and animal behavior under thyroid dysfunction

Noda M[1], Niiyama T[1], Aoi K[1], Kuroiwa M[2], Shuto T[2], Kitahara Y[2], Nishi A[2]

[1]Graduate School of Pharmaceutical Sciences, Kyushu University, Japan; [2]Department of Pharmacology, Kurume University School of Medicine, Japan

Thyroid hormones (THs) are essential for the development and function of the central nervous system (CNS). In the CNS, circulating thyroxine (T4) crosses blood- brain barrier via specific transporters and is taken up to astrocytes, becomes L-tri-iodothyronine (3, 3', 5–triiodothyronine; T3), an active form of TH, by type 2 de- iodinase (D2). T3 is released to the brain parenchyma from astrocytes (gliaendocrine system). In adult CNS, both hypo- and hyper-thyroidism, the prevalence in female being >10 times higher than that in male, may affect psychological condition and potentially increase the risk of cognitive impairment and neurodegeneration including Alzheimer's disease (AD). We have previously reported that non-genomic effects of T3 on microglial functions and its signaling and sex- and age-dependent effects of THs on glial morphology in the mouse brains of hyperthyroidism. Behavioral changes also showed sex-dependence. For example, using young mice with hyperthyroidism, male mice showed increased locomotor activity, while female mice showed depressive behavior without changes in locomotor activities. Synaptic spine in male and female hyperthyroidism was also analyzed. Male mice showed increase in spine density without any changes in spine volume, while female mice showed more significant increase in spine density with decreased spine volume. Results using aged mice or opposite thyroid dysfunction, hypothyroidism, will be also shown and discussed. These results may help to understand physiological and/or pathophysiological functions of THs in the CNS and how hypo- and hyper-thyroidism affect psychological condition and cognition.

PP.131

Comparative assessment of renal function in the male Saharan Libyan jird *Meriones libycus* (Lichtenstein, 1823) during the breeding and non-breeding season

Aknoun-Sail N[1], Zatra Y[1], Kheddache A[1], Benmouloud A[1], Exbrayat JM[2], Khammar F[1], Amirat Z[1], Khaldoun M[1]

[1]Faculty of Biological Sciences, University of Science and Technology Houari Boumediene (USTHB), Algeria; [2]Reproduction and Comparative Development, EPHE, Catholic University of Lyon, France

The kidneys play a crucial role in the regulation of homeostasis; its function is more solicited in the desert species undergoing various environmental variations. In this study, we first compare the structure of the kidney and then evaluate some renal markers including serum creatinine and urea during breeding and non-breeding season in the male Libyan jird *Meriones libycus*. In addition, we analyze the involvement of sex androgens in the renal regulation by immunohistochemical study of the androgen receptors. The results show that the kidney weight is slightly higher during non-breeding versus breeding season. Histological analysis shows that the glomeruli are more reduced during the non-breeding season with enlarged spaces of Bowman's capsule. The cells of the distal tubules are flattened in non-breeding season leaving more prominent peritubular spaces. Serum creatinine and urea levels are higher during non-breeding. The immunohistochemical study shows that the renal tissue is positively labeled with the androgen receptors; moreover the staining intensity is more important sexually active jirds. Furthermore, the analysis shows that the labeling is present in the tubules and absent in the glomeruli. This suggests that the kidney undergoes structural and physiological changes throughout the sexual annual cycle. The modifications which may be sex androgens-dependent occur mainly at secretion and tubular reabsorption levels but not at glomerular filtration level.

PP.132

Liver local hypothyroidism and altered substrate metabolism in a mouse model of congenital hypothyroidism

Giacco A[1], Iervolino S[1], Peluso T[1], Roberto L[2], Cioffi F[1], Goglia F[1], Ambrosino C[1,2], Moreno M[1], Silvestri E[1]

[1]Department of Science and Technology, University of Sannio, Italy; [2]IRGS, Biogem, Italy

Hypothyroidism has been strongly associated with non-alcoholic fatty liver disease (NAFLD), but the pathogenic mechanisms appear complex and not reducible exclusively to the impaired thyroid hormone (TH) signalling. Here, by using an established mouse model of human congenital hypothyroidism [double heterozygote for both Titf1- and Pax8-null mutations (DHTP) mice], we studied the intra-hepatic mechanisms leading to altered substrate metabolism in conditions of low TH and high Thyroid Stimulating Hormone (TSH). At the liver level, we focus attention on the expression of key factors in TH signalling, genes of lipid, cholesterol and glucose handling, hepatokines and markers of mitochondrial biogenesis. When compared to wild type (WT) controls, DHTP mice showed reduced levels of Thyroid hormone receptor β (TRβ) and type 1 iodothyronine deiodinase (DIO 1) as well as of known TH target genes such as Sterol regulatory element-binding protein 1 (SREBP1C), Acetyl-CoA carboxylase (ACC1), fatty acid synthase (FAS) and Low-Density Lipoprotein Receptor (LDLr). On the other hand, livers of DHTP mice showed increased expression levels of Peroxisome proliferator-activated receptor α (PPARα), Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α), Carnitine Palmitoyltransferase I (CPT1) and SPOT 14 (S14). A trend of increase was observed for Glucose 6-phosphatease (G6Pase). Thus, the liver of DHTP mice is characterized by a local hypothyroidism with alterations in transcription factors and enzymes involved in substrate metabolism. Of note, the increased expression of the lipogenic gene SPOT 14 is not correlated to the intra-hepatic levels of TH. Other signals could be involved (e.g., extra-hepatic ones) such as glycaemia, insulineamia and/or TSH itself.

PP.133

Random matrix analysis of calcium oscillations in pancreatic beta-cell collectives

Korošak D[1,2], Slak Rupnik M[1,3,4]

[1]Institute for Physiology, University of Maribor, Slovenia; [2]Faculty of Civil Engineering, Transportation Engineering and Architecture, University of Maribor, Slovenia; [3]Center for physiology and pharmacology, Medical University of Vienna, Austria; [4]Alma Mater Europaea - European Center Maribor, Slovenia

Even within small organs like pancreatic islets, different endocrine cell types and subtypes form a heterogeneous collective to sense the chemical composition of the extracellular solution and compute an adequate hormonal output. Erroneous cellular processing and hormonal output due to challenged heterogeneity result in various disorders with diabetes mellitus as a flagship metabolic disease. Here we attempt to address the aforementioned functional heterogeneity with comparing pairwise cell-cell cross-correlations obtained from simultaneous measurements of cytosolic calcium responses in hundreds of islet cells in an optical plane to statistical properties of correlations predicted by the random

matrix theory (RMT). We find that the bulk of the empirical eigenvalue spectrum is almost completely described by RMT prediction, however, the deviating eigenvalues that exist below and above RMT spectral edges suggest that there are local and extended modes driving the correlations. We show that empirical nearest neighbor spacing of eigenvalues follows universal RMT properties regardless of glucose stimulation, but that number variance displays clear separation from RMT prediction and can differentiate between empirical spectra obtained under non-stimulated and stimulated conditions. We suggest that RMT approach provides a sensitive tool to assess the functional cell heterogeneity and its effects on the spatio-temporal dynamics a collective of beta cells in pancreatic islets in physiological resting and stimulatory conditions.

Poster Session II (4/7)

Nutrition, Gut Microbiota, and Health

PP.134

Obesity and obesity-associated muscle wasting in patients on peritoneal dialysis

Di Lauro T, Di Maro M, Trio R, Salomone E, Di Martino R, Di Lauro M, Sacco E, Colantuoni A, Guida B

Department of Clinical Medicine and Surgery, Physiology Nutrition Unit, Federico II University of Naples, Italy

The aim of the present study was to investigate the prevalence of obesity and obesity-associated muscle wasting in peritoneal dialysis (PD) patients. Body composition was assessed with BIA and BIVA in 88 PD patients (53.4±13.1 years; 67% male). Patients with obesity and/or with reduced muscle mass were identified using FMI and SM/BW cutoff values, respectively. Inflammatory status was assessed by measuring CRP and fibrinogen blood levels. Our results indicate that 44.3% of the patients showed a reduced muscle mass (37.5% moderate and 6.8% severe). The prevalence of obesity was 6.1%, 81.8% and 100% in patients with normal, moderately and severely reduced muscle mass ($p<0.05$). 15.2% of the patients with normal muscle mass, 18.4% of those with moderately reduced and 66.7% of those with severely reduced muscle mass were diabetic. The prevalence of severe muscle mass loss was higher in diabetics than in non-diabetic patients (22.2% vs 2.8%, $p<0.05$). As compared with patients with normal body composition patients with obesity-associated muscle wasting showed higher fibrinogen (613.9±155.1 vs 512.9±159.5 mg/dL, $p<0.05$) and CPR (1.4±1.3 vs 0.6±0.8 mg/dL, $p<0.05$) blood concentrations. In conclusion, obesity and diabetes were strongly associated with muscle mass loss in our PD patients. It remains to be established whether in PD patients preventing obesity with nutritional interventions can also halt the occurrence of muscle mass loss.

PP.135

Reelin expression in the progression from human inflammatory disease to colon cancer

Serrano-Morales JM, García-Miranda P, Vázquez-Carretero MD, Calonge ML, Ilundáin AA, Peral Rubio MJ

Departamento de Fisiología, Universidad de Sevilla, Spain

Intestinal inflammation could initiate colorectal adenoma and its progression to carcinoma. We previously reported up-regulation of reelin in mouse inflamed colon and down-regulation in human colon adenocarcinoma, and that reelin protects the colon from inflammation and tumorigenesis (1-3). The present work evaluates reelin expression during the transition from colitis to colon cancer and investigates the mechanism(s) involved. Human samples of healthy colon, ulcerative colitis, polyps, adenomas and adenocarcinomas were provided by the "Biobanco Hospital Virgen del Rocío-IBIS" and total RNA was extracted from each sample. Reelin and DNMT-1 mRNAs relative abundances were measured by real-time PCR. The results revealed that, as compared with healthy colon, reelin mRNA abundance is increased in ulcerative colitis, slightly increased in polyps and that it decreases as disease severity progresses towards adenomas and adenocarcinomas. Since DNA methyltransferase 1 (DNMT-1) reduces reelin expression by methylation of its promoter (1), DNMT-1 expression was also evaluated in the same human colon samples. DNMT-1 mRNA abundance decreased in colitis samples, slightly decreases in polyps and increases in adenomas and adenocarcinomas. In conclusion, the up-regulation of reelin in colitis and its down-regulation during transition from inflammation to colon cancer is due, at least in part, to opposed changes in DNMT1 expression. The results also suggest that changes in colon reelin abundance could be used as a biomarker to predict colon pathology progression. 1. Carvajal et al. (2017) Biochim Biophys Acta.1863: 462-473. 2. Serrano-Morales et al. (2017) Mol Carcinog. 56: 712-721. 3. Carvajal et al. (2017) Biochim Biophys Acta.1863: 2126–2134.

PP.136

Nutritional approach to control inflammation and support the remission in Crohn's Disease Patients: a pilot study

Rizzello F[1,*], Spisni E[2,*], Imbesi V[1], Valerii MC[2], Gionchetti P[1]

[1]Dept. of Medical and Surgical Sciences, University of Bologna, Italy; [2]Dept. of Biological, Geological and Environmental Sciences, University of Bologna, Italy; [*]equally contributed to the work

Western diet is characterized by the abundant consumption of red meat, cheese, refined sugars and ultra- processed foods to the detriment of fiber, raw fruit and vegetables. In the recent years there has been increasing evidence that this kind of diet may be responsible of an asymptomatic low-grade inflammatory process that starts from the colon, also mediated by the alteration of the intestinal microbiota ecology, to become systemic. This phenomenon may contribute to the onset of metabolic diseases such as diabetes and obesity, and chronic inflammatory diseases. The idea that a correct diet depends exclusively on the control of caloric intake is a concept that has been totally overcome in recent years, while the more modern vision frames nutrition as a tool to support therapies for different types of diseases. Crohn's disease is an idiopathic chronic intestinal inflammatory disease, which alternates phases of remission with exacerbations, characterized by diarrhea, abdominal pain and rectal bleeding often associated with extraintestinal manifestations such as joint pain. In addition to a broadly characterized genetic background that predisposes certain individuals, environmental factors such as stress and nutrition seems to play a major role to the onset and chronicity of the disease. The different therapeutic switches to which patients with active disease are subjected prevent, to date, to have clear data on the real impact of nutrition in the progress of the disease. To understand this, we studied a population represented by Crohn's patients in clinical remission, thus not requiring therapeutic adjustments. They present high blood values of Protein C Reactive (PCR), indicating that the phlogosis was still in progress. The aim of this pilot study is to evaluate the anti-inflammatory effect of a balanced Mediterranean diet (according to the LARN and the WHO guidelines) in patients with Crohn's disease in clinical remission with altered C Reactive Protein (PCR> 0.50 mg / dL) values. To this aim, patients were consecutively enrolled and divided into 2 groups: patients on the first group followed a balanced healthy diet according to LARN and WHO guidelines for three months, patients on the second group followed their routinely diet. The administered diet excludes all ultra- processed foods (NOVA 4 classification), refined sugars and limited the amounts of red meat consumption. Fibers, vitamins, macro- and micro-nutrients intake were balanced according to guidelines. CRP, lipid profile, glycaemia, blood vitamins symptoms were evaluated at the beginning at the end of the experimental period.

PP.137

Effect of a food supplement containing Palmrose essential Oil on microbiota composition and inflammatory profile of Irritable Bowel Syndrome patients: a randomized double blind-placebo controlled trial

Rizzello F[1,*], Ricci C[2,*], Valerii MC[3], Spisni E[3], Imbesi V[1], Gionchetti P[1]

[1]Dept. of Medical and Surgical Sciences, University of Bologna, Italy; [2]Dept. of Clinical and Experimental Sciences, University of Brescia, Italy; [3]Dept. of Biological, Geological and Environmental Sciences, University of Bologna, Italy; [*]equally contributed to the work

Essential oils are volatile compounds extracted from plants that include several aromatic and aliphatic constituents of low molecular weight, generally belonging to the chemical families of terpenes and terpenoids Typically, an essential oil contains dozens of compounds, of which 2 or 3 at high concentration (20 to 80% of the total). Geraniol is a natural monoterpene present in essential oils with antibacterial activity and highly selective for pathogens respect to commensals colon bacteria. In Cymbopogon Winterianus Jowitt (Palmrose Oil) Geraniol is the most represented compound with a concentration ranging from 70 to 80%. In previous studies we showed that oral geraniol at 120 mg $kg^{(-1)}$ die dose was able to counteract intestinal dysbiosis, systemic inflammation and histological damage in mice with sodium dextran-induced colitis. In a pilot study with 19 Irritable Bowel Syndrome (IBS) patients we showed that geraniol at 8 mg $kg^{(-1)}$ die dose was able to modulate intestinal dysbiosis and relieve IBS-related symptoms. Moreover, we showed that, in rats, geraniol absorption on a fibrous matrix resulted in a decreased absorption on the small intestine and an increased availability in the colon, where it exerts its effects. The aim of this study is to evaluate the effect of a Palmrose Oil -based supplement in patients with Irritable Colon Syndrome. Patients enrolled were diagnosed with IBS according to the Roma III criteria and were randomized in a 1: 1 ratio to receive the food supplement or placebo for 4 weeks. Supplement consisted of Palmrose oil titred at 80% in Geraniol, adsorbed on ginger ryzoma powder. Placebo was prepared by using corn starch. The supplement was administred at a dose ranging from 900-mg to 1800 mg /die basing on body weight. Blood and fecal samples were collected before and after 4 weeks treatments to evaluate the effect of Geraniol on fecal microbiota ecology and on plasma chemokines (MIP-1b, MCP-1, CXCL10 and Baff). IBS-related symptoms have been evaluated by using the validated IBS-VAS questionnaire.

PP.138

Electroencephalogram (EEG) aspect in chronic alcoholism

Borza C[1], Gotia SL[2], Gotia SR[2]

[1]Department of Pathohysiology, University of Medicine and Pharmacy "V. Babes", Romania; [2]Department of Physiology, University of Medicine and Pharmacy "V. Babes", Romania

Neuronal excitability is modified and brain damage is induced by chronic alcohol consumption. The aim of this study was to evaluate the aspect of EEG in a group of 46 patients with chronic alcoholism correlated with the metabolic disturbances and psychiatric symptoms. The patients mean age was 47-75 years (18 female si 28 male). The study group was characterized by the clinical signs, EEG aspect, lab tests values and the aspect of the psychiatric functional evaluation tests. Expressed a high prevalence of EEG modifications (67% of the patients). The EEG aspect was a lessional type with a low amplitude of cerebral waves, an anterior migrated rhythm and during activation of the recording (hyperventilation and intermittent light stimulation, 6-12 Hz) rare, irritative type, graphic elements appeared (spikes and waves). The metabolic disturbances appeared in a high number of patients and included: high TGO, TGP level, higher than 100 UI/ml, gamma-GT >400 UI/ml, total bilirubin 1.7-2.3 mg%, low B12 vitamin value and abnormal alkaline phosphatase. The chronic pancreatitis was present in 49% of patients. The abnormal psychiatric pattern was expressed in 52% of patients by: psychotic episode, epilepsy, delirium tremens and a low cognitive capacity. Chronic alcoholism determinated an intense brain damage. Alcohol consumption stopping, dynamic evaluation by lab tests and EEG, therapy with neuro-trophic agents will keep under control the present status of these patients.

PP.139

The potential role of chronic otilonium bromide administration in preventing colonic dysmotility induced by repeated water avoidance stress in rats

Traini C[1], Garella R[2], Idrizaj E[2], Squecco R[2], Vannucchi MG[1], Baccari MC[2]

[1]Histology and Embryology Research Unit, Dept. of Experimental and Clinical Medicine, University of Florence, Italy; [2]Section of Physiological Sciences, Dept. of Experimental and Clinical Medicine, University of Florence, Italy

It has been shown that repeated water avoidance stress (rWAS) applied in rats induces changes in the colonic motility, similar to those described in Irritable Bowel Syndrome (IBS). Despite the wide use of otilonium bromide (OB) in the treatment of IBS, its mechanism of action has not been fully clarified yet. Aim of the present study was to investigate the effects of OB on colonic dysmotility induced by rWAS in Wistar rats. To this purpose, animals were distributed into four experimental groups: CTR (controls, untreated); OB-treated (orally treated with OB for 10 days); WAS (exposed to rWAS for 10 days); WAS+OB (exposed to rWAS and orally treated with OB for 10 days). Mechanical experiments were performed on full-thickness muscle strips from the distal colon, cut in the circular direction, mounted in organ baths for isometric recording of the spontaneous mechanical activity. The majority of strips from CTR, exhibited high amplitude contractions superimposed on smaller ones, similarly to OB-treated and WAS+OB rats. In WAS rats, the amplitude of the spontaneous motility pattern was greatly reduced and no high amplitude contractions were observed. In all groups and especially in the WAS one, tetrodotoxin (TTX) or L-NG-nitro arginine (L-NNA) increased the amplitude of the spontaneous contractions, thus indicating the removal of a nitrergic nervous control. Methacholine caused a similar contractile response in CTR and WAS but it was ineffective in OB-treated and WAS+OB rats. Immunohistochemical experiments are in progress to correlate morphological with functional data. From the present results it appears that OB, other than interacting with the muscular muscarinic receptors, prevents colonic dismotility induced by WAS, likely interfering with nitric oxide production/release.

PP.140

Wheat, gluten and gut inflammation: in vitro models to ascertain the pro-inflammatory proprieties of different wheat cultivars

De Fazio L[1], Truzzi F[2], Dinelli G[2], Valerii MC[3], Spisni E[1]

[1]Department of Biological, Geological and Environmental Sciences , University of Bologna, Italy; [2]Department of Agricultural Sciences , University of Bologna, Italy; [3]IBD Unit, S. Orsola-Malpighi Hospital and Department of Medical and Surgical Sciences, University of Bologna, Italy

More than. 50% of world daily caloric intake is derived directly from wheat consumption. Wheat is the most widely grown crop worldwide, with more than 25,000 different cultivars produced by plant breeders. The major protein complex contained in wheat is the gluten, composed of glutenins and prolamins. It is formed when the insoluble proteins (gliadins and glutenins) are mixed in the presence of water, becoming a viscoelastic mass. Gluten is only partially digested by enzymes in the gastro-intestinal tract. This could lead to the formation of immunogenic peptides as gliadin that has been demonstrated to increase intestinal permeability and gut inflammation in humans by altering the expression of junctional complex protein and by activating innate and the adaptive immune

responses. There have been cases of reactions to gluten-containing grains that involved neither allergic nor autoimmune mechanisms. These generally are termed non-celiac gluten sensitivity (NCGS) or simply gluten sensitivity. Patients suffering from NCGS show symptoms that usually occur within hours or days after ingestion of gluten-containing grains, and disappear rapidly when these grains are eliminated from the diet. NCGS most frequently produces a combination of intestinal and extraintestinal symptoms. In the gut, after wheat ingestion, NCGS patients show an increased intestinal permeability, the activation if innate immune responses and, in more than 50% of cases, an increased synthesis of anti-gluten antibody, such as anti-gliadin (AGA) IgG. To date, it is unclear whether only gluten proteins are involved in the etiopathogenesis of NCGS or whether other wheat proteins, such as those called amylase-trypsin inhibitors (ATI), can contribute to the onset of this disease. To characterize *in vitro* the inflammatory properties of wheat proteins we have developed two different models, one based on the use of sera from patients diagnosed with gluten sensitivity as sources of possible anti-wheat antibodies and the other based on cultured CaCO2 intestinal cells in the presence of wheat proteins. In this study, which examines different ancient and modern wheat cultivars, the results obtained using two methods are compared.

PP.141

Intake of table olives at two different doses: comparison between plasmatic concentrations of hydroxytyrosol and its metabolites in rat

Kundisovà I[1], Juan ME[1], Colom H[2], Planas JM[1]

[1]Department de Bioquímica i Fisiologia e Institut de Recerca en Nutrició i Seguretat Alimentària-UB, Universitat de Barcelona, Spain; [2]Departament de Farmàcia i Tecnologia Farmacèutica i Fisicoquímica, Universitat de Barcelona,Spain

The fruit of *Olea europaea* L., a typical food of Mediterranean diet, is an important source of minor components such as phenolic compounds. Hydroxytyrosol (HT), the most abundant polyphenol, stands out due to its strong antioxidant, anti-inflammatory and cardio protective activities. The aim of the present study was to evaluate the plasmatic concentrations of HT and its metabolites after the oral intake of table olives. Overnight fasted male Sprague-Dawley rats were orally administered by gavage with a homogeneous olive suspension containing HT at 3 and 6 mg/kg which are doses equivalent to the consumption of 30 and 60 olives by a 60-kg person. Blood was withdrawn from saphenous vein at 0, 30, 60, 90, 120, 240, 360 and 480 min. The plasmatic concentrations were determined by HPLC-ESI-MS/MS analysis after liquid-liquid extraction. HT underwent extensive II phase metabolism yielding 2 sulfates (M1-a and M1- b) and 2 glucuronides (M2-a and M2-b). HT represented only 8% of all. The maximum concentrations of 22.4 ± 4.0 nM (3 mg/kg) and 45.0 ± 6.7 nM (6 mg/kg) were achieved at 30 min. The major derivatives were sulfates and they accounted for more than 85%. M1-a was found at 53.5 ± 14.6 nM and 104.8 ± 15.0 nM, whereas M1-b at 351.7 ± 85.3 nM and 579.2 ± 74.7 nM, when doses of 3 mg/kg and 6 mg/kg were administered, respectively. Two glucuronides, found at very low concentrations represented less than 5%. The plasmatic concentrations of HT and its metabolites decreased with time and were still detected at 480 min. From this study can be concluded that the dose of 6 mg/kg resulted in almost 2-fold higher plasma level of the studied compounds in comparison with 3 mg/kg meaning that the different plasma concentrations strongly depend on the administered dose. Supported by grants AGL2013-41188 (MINECO) and 2017SGR945 (Generalitat de Catalunya).

PP.142

Blood pressure modifications in acordance with students habits

Gotia SL[1], Borza C[2], Mederle A[2], Toth E[2]

[1]Department of Physiology, University of Medicine and Pharmacy "Victor Babes", Romania, [2]Department of Pathophysiology, University of Medicine and Pharmacy "Victor Babes", Romania

The increasing obesity among young people, smoking, coffee, alcohol consumption associate with stress represent risk factors of high blood pressure. The aim was to study blood pressure variation at students correlated with their lifestyle. The students, 15 boys and 12 girls, 20.12± 2.44 years old, were asked about their lifestyle, coffee and alcohol consumption, smoking. Blood pressure was self-determined in the morning, afternoon, between November and February 2018-2019. During the session period determinations were performed before and after the exams. Coffee consumption was increased at girls (75%) comparative with boys (46.66%); alcohol consumption (41%); smoking was 53.33% in boys and 33.33% in girls. At the group of boys, during exams, a moderate increased systolic blood pressure 124.66±11.44 mmHg and diastolic blood pressure 74±8.42 mmHg was noticed, in the afternoon. At girls group before the exam systolic (113.33 ± 11.14 mmHg) and diastolic blood pressure (72.08 ± 5.82 mmHg) increased. Blood pressure modification showed a response from the activation of the sympathetic vegetative system induced from the

exam stress, associated with the effect of coffee consumption, which is higher at girls comparative with boys. We recommend periodical check of blood pressure at youths that have high values, informing the youth about the risk of the negative factors, and about the modification of their lifestyle to a healthy life.

PP.143

Nutritional status and body fat distribution in subjects with psychological traits typical of eating disorders

Leone A, Vignati L, De Amicis R, Ponissi V, Beggio V, Battezzati A, Bertoli S

International Center for the Assessment of Nutritional Status (ICANS), Department of Food Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

Eating disorders (EDs) are characterized by some typical psychological traits (PT) like drive for thickness (DT), bulimia (B) and body dissatisfaction (BD) that increase the risk of malnutrition. However, if they lead to a different body fat (BF) content and distribution independently from BMI is unknown. We aimed to investigate the total BF and its distribution in subjects with different PTs. We conducted a cross-sectional study on 5015 adults recruited among subjects seeking for a weight loss or maintenance program. Weight, height, BMI, waist circumference (WC) and BF by skinfolds were taken. PTs were investigated using the Eating Disorder Inventory 3 questionnaire. Subjects were categorized based on how many and which PTs typical of EDs were found of clinical interest. Subjects with different PTs typical of EDs were compared with subjects free of PTs (controls). 55.0% of subjects presented at least one of the PTs typical of EDs. Using a linear regression model adjusted for sex and age, we found that, with the only exception of subjects with only a DT trait, subjects with different PTs typical of EDs had an increased BF and WC than controls. In particular, subjects having both B and BD traits had 11.5 cm (CI95%: 9.9-13.2, $p<0.001$) and 3.3% (CI95%: 2.8-3.8, $p<0.001$) more of WC and BF, respectively, than controls. However, after inclusion of BMI in the model, such associations disappeared or were strongly mitigated. Definitively, psychological traits typical of eating disorders do not seem to be associated in a biologically meaningful manner with body fat content and its abdominal distribution after adjustment for BMI.

Poster Session II (5/7)

Physiology of Metabolism

PP.144

Uncoupling protein 3 affects lipid handling in mice

Gentile A[1], Busiello RA[1], De Matteis R[2], Delli Paoli G[3], Buonaiuto S[3], Lanni A[3], Goglia F[4], Lombardi A[1]

[1]Department of Biology, University of Naples "Federico II", Italy; [2]Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Italy; [3]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Italy; [4]Department of Science and Technology, University of Sannio, Italy

Uncoupling protein-3 (UCP3) is localized in the mitochondrial inner membrane and is expressed in skeletal muscle (SkM), heart, brown adipose tissue (BAT) and white adipose tissue (WAT). The physiological role of UCP3 and its involvement in energy homeostasis is still under investigation, and we give further insight into the role played by UCP3 in lipid handling. Wild type (WT) and UCP3 null mice (KO) housed at thermoneutrality were used as animal models. KO mice presented blunted ability to use fatty acids as a metabolic substrate, as revealed by an enhanced respiratory quotient compared to WT ones. In agreement with this, in KO mice, SkM and BAT mitochondria showed a reduced oxygen consumption when using palmitoyl carnitine (but not pyruvate) as respiratory substrate, thus indicating a lower ability of these mitochondria to oxidize fatty acids. The contribution of visceral WAT to the weight of the mice was lower in KO mice (-25% vs WT). Variations in visceral WAT mass were not associated with change in adipocytes size, while basal lipolysis was almost doubled in KO mice compared to WT, as revealed by glycerol release from the tissue. Interestingly, histological analysis of lean tissues (liver and SkM) indicated an ectopic accumulation of fat associated to the absence of UCP3. Indeed, H&E staining of KO SkM and liver sections showed many large intracellular lipid droplets. Numerous intramyocellular lipid droplets were present only in skeletal muscle of KO mice as well as a massive lipid accumulation was shown in the cytoplasm of all the hepatocytes. As a whole these data indicate a role of UCP3 in lipid homeostasis and in the protection lean tissue by lipid accumulation and the consequent lipotoxicity.

PP.145

Hepatic oxidative stress induced by "western diet" in middle-aged rats

Mazzoli A, Crescenzo R, Cigliano L, Gatto C, Cancelliere R, Iossa S

Department of Biology, University of Napoli "Federico II", Napoli, Italy

Middle age is an earlier stage of the aging process, during which gradual changes and some chronic illness may occur. Such changes would affect the outcomes at old ages. Many studies have emphasized the impact of diet composition on aging, but few data are available on the impact of dietary fats and sugars on physiological responses at middle ages. Therefore, we used a rat model that highly resembles dietary habits of western countries, i.e. we fed middle-aged (11 months old) rats with a high-saturated fat, high fructose (HF-F) diet for 4 weeks. The increased plasma levels of triglycerides and LDL-cholesterol confirmed the similarity with humans. HF-F diet was associated with increased hepatic lipid and cholesterol content, coupled with oxidative stress to lipids and proteins. The absence of variations in mitochondrial respiratory systems allow us to exclude increased ROS production by these organelles, while enhanced ROS production by NADPH oxidase was found. In addition, the antioxidant enzyme catalase was found decreased in face of increased activity of SOD. The HF-F diet also determined insulin resistance, with altered glucose tolerance test and expression of Akt in the liver of HF-F rats. Our present results point to the deleterious effect of a "western diet" on liver function, although the extent of hepatic impairment is very similar to that found in adult rats, thus suggesting that, at least in the liver, middle age does not determine a greater vulnerability to dietary stress.

PP.146

Anti-oxidant effects of melatonin on energy balance and oxidative stress in sepsis-induced rats

Ozkok E[1], Ates Ulucay G[2], Yorulmaz H[3], Aksu A[4], Balkis N[4], Tamer S[5]

[1]Department of Neuroscience, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey; [2]Department of Physiology, Faculty of Medicine, Istanbul Yeni Yuzyil University, Istanbul, Turkey; [3]School of Nursing, Halic University, Istanbul, Turkey; [4]Department of Chemical Oceanography, Institute of Marine Sciences and Management, Istanbul University, Turkey; [5]Department of Physiology, Istanbul Medical Faculty, Istanbul University, Turkey

Cytokine storm that occurs with the stimulation of the inflammatory cascade induces the oxidative stress, and results with mitochondrial dysfunction and energy imbalance in sepsis. Liver has an important role in the detoxification. Accumulation of the large number of toxic agents, reactive oxygen species and heavy metals causes severe damage in the organism due to liver damage that is stimulated with microbial threat in sepsis. Melatonin is a strong endogenous antioxidant produced by the pineal gland, and various organs. Its effects on lipid peroxidation, molecular degeneration and oxidative stress were reported in experimental studies. In our study, it was investigated the effects of melatonin on oxidative stress and energy balances in liver tissue in rats with lipopolysaccharide (LPS) induced sepsis. Adult Wistar albino rats were divided into 4 groups: Control, LPS (10 mg/kg i.p.), Melatonin (10 mg/kg i.p. x3), and Melatonin+LPS (Local Ethic Committe for Animal: 2016/08). Liver tissues were removed 6 hours after LPS injection. AMP, ADP, ATP, Creatine and Creatine phosphate levels were determined using the HPLC, and Glutathione reductase (GR), Glutathione peroxidase (GSH-Px), Superoxide dismutase (SOD) and YKL-40 levels were studied using ELISA. AMP, ATP, Creatine, Creatine Phosphate, GR, GSH-Px levels were found to be decreased in the LPS group compared with the other groups ($P<0.01$). In LPS group, YKL-40 levels decreased compared with the control group, ADP levels significantly increased compared with the other experimental groups (both; $P<0.05$). In conclusion, we suggested that melatonin may have a improving effect on energy metabolism and oxidative damage septic liver tissue.

PP.147

Reduced adipogenesis and improved glucose uptake induced by a black pepper extract

Geddo F[1], Scandiffio R[1], Antoniotti S[1], Cottone E[1], Querio G[1], Levi R[1], Maffei ME[1,2], Bovolin P[1], Gallo M[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Via Accademia Albertina 13, 10123, Turin, Italy; [2]Biosfered S.R.L., Corso Matteotti 42, 10121, Turin, Italy.

β-caryophyllene is a sesquiterpene with several important pharmacological activities, including antioxidant, anti-inflammatory, anticancer, cardioprotective, hepatoprotective, gastroprotective, nephroprotective, antimicrobial, and immunemodulatory activity. The biological properties of this natural product are due to its selective interaction with the peripherally expressed cannabinoid receptor 2 (CB2). Notably, activation of CB2 receptors is devoid of the typical psychotropic effect of cannabinoids mediated by the CB1 receptor. In addition β-caryophyllene activates peroxisome proliferated activator receptors (PPARs) and γ and inhibits pathways triggered by the activation of toll like receptor complex. Giving the growing scientific interest in this molecule, the aim of our study was to investigate the metabolic effects of a black pepper extract (PipeNig®), containing 80% βcaryophyllene. In particular our

interest was focused on its potential antiobesogenic and antidiabetic activities. Experiments were performed on 3T3-L1 preadipocytes and on C2C12 myotubes. Lipid accumulation during 3T3-L1 adipogenic differentiation was quantified with AdipoRed fluorescence staining. Glucose uptake in C2C12 myotubes was studied with the fluorescent glucose analog 2-NBDG. Our preliminary results show that PipeNig® reduces 3T3-L1 adipocyte differentiation and lipid accumulation. Moreover, acute exposure of C2C12 myotubes with different concentrations of PipeNig® improves glucose uptake activity. Conclusion Taken together, this initial analysis revealed interesting and novel properties of β-caryophyllene, suggesting potential applications in the prevention of metabolic syndrome.

PP.148

Involvement of TGR5 receptor in fat preference and obesity in mice

Bensalem A [1,2], **Murtaza B** [2], **Hichami A** [2], **Oulamara H** [1], **Khan A** [2], **Agli A** [1], **Khan N** [2]

[1]Laboratoire de Nutrition et Technologie Alimentaire (LNTA), Institut de la Nutrition, de l'Alimentation et des Technologies Agro-Alimentaires (INATAA) - Université Frères Mentouri Constantine 1, 25000, Alegria; [2]Physiologie de la Nutrition & Toxicologie, UMR U1231 INSERM/Université de Bourgogne-Franche Compté (UBFC), Dijon 21000, France. Email: benadel2005@yahoo.fr

The aim of this study is to investigate the involvement of TGR5 receptor in preference for dietary lipids and obesity. C57BL/6 wild type (WT) and TGR5 knock-out (TGR5- / -) mice were maintained on normal diet (ND) or high-fat diet (HFD). Food intake was calculated daily while body weight was measured weekly. After 20 weeks of feeding, body lean and fat mass were analyzed by EchoMRI 500. Metabolic monitoring was performed using a Comprehensive Laboratory Animal Monitoring System. Intra-peritoneal glucose tolerance test was carried out in mice. Spontaneous preference for fat solutions was investigated by means of the two-bottle preference test. At the end of study, mice were sacrificed and liver was removed to determinate total liver triglycerides and cholesterol concentrations. Venous blood was collected and blood parameters were determined. The TGR5- / - mice fed HFD consumed significantly more food and more energy, and exhibited lower energy expenditure. Consequently, TGR5- / - mice fed HFD were more obese and had higher body fat mass than WT mice fed HFD. Blood glucose, cholesterol and triglyceride were higher in TGR5- / - obese mice compared to WT obese animals. Accordingly, homeostasis model assessment-insulin resistance value was higher in TGR5- / - obese mice than WT obese animals. Interestingly, on HFD-fed, TGR5- / - mice exhibited high liver weight with high liver cholesterol and triglyceride than WT mice. Fat preference observed in ND-fed WT and TGR5- / - mice, was abolished in HFD-fed animals. However, in contrast to WT obese mice, TGR5- / - obese mice consumed fat containing solution similar to control solution. This leads us to conclude that TGR5 may modulate food preferences and may be a promising target for the management of obesity.

Poster Session II (6/7)

Renal Physiology

PP.149

Altered osmoregulation in kidney transplant recipients predict renal outcome

Bienaimé F, **Mazloum M**, **Jouffroy J**, **Neuraz A**, **Brazier F**, **Legendre C**, **Prié D**, **Anglicheau D**

Hôpital Necker-Enfants Malades, INSERM, France

Kidney transplant recipients (KTR) have impaired urine-diluting ability but seldom develop baseline hyponatremia before end stage renal disease. While hyponatremia is a risk factor for adverse events in chronic kidney disease (CKD) and KTR, the prevalence of subtler alterations in osmoregulation in KTR and their association with outcomes remain unassessed. Here, we studied a single center prospective cohort of 1258 kidney transplant recipients (KTR) who underwent a water-loading test 3 months after transplantation to determine osmoregulation performance. Glomerular filtration rate measurement (mGFR) measurement was performed at the same visit. A group of 164 healthy candidates for kidney donation served as controls. We further evaluated the association of osmoregulation performance with transplantation outcomes and subsequent kidney function. Our results indicate that, differing from controls, most KTR failed to maintain plasma sodium (PNa) during water loading (PNa slope of -0.6±0.4mmol/l/h in KTR *vs* -0.12±0.3 mmol/l/h in controls; P<0.0001). Steeper PNa reduction during the test independently associated with the composite outcome of all-cause mortality and allograft loss (HR=1.73 per 1 mmol/l/h decrease in PNa; 95% confidence interval: 1.23 to 2.45; P=0.002) and allograft loss alone (HR=2.04 per 1 mmol/l/h decrease in PNa; 95% confidence interval: 1.19 to 3.51; P=0.01). In addition, a steeper PNa slope 3 months after transplantation independently correlated with lower subsequent mGFR (b=1.93; 95% confidence interval: 0.46 to 3.41; P=0.01). We conclude that reduced osmoregulation performance is a frequent finding in KTR and predicts renal outcome.

PP.150

The vasopressin-regulated water channel aquaporin-2 as target of the green Olive Leaf Extract

Ranieri M[1], Di Mise A[1], Venneri M[1], Tingskov SJ[3], Centrone M[1], Pellegrino T, Difonzo G[2], Caponio F[2], Norregaard R[3], Valenti G[1], Tamma G[1]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari Aldo Moro, Italy; [2]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Italy [3]Department of Clinical Medicine, Aarhus University, Denmark

Water balance is mainly controlled by the hormone vasopressin (AVP). In the kidney, AVP binds the V2 vasopressin receptor (V2R) stimulating the cAMP-dependent signal transduction cascade leading to the translocation of the AQP2-vesicles to the apical plasma membrane, thereby increasing renal water reabsorption. Several disorders, including hypertension, usually occur as a result of abnormalities in water homeostasis. Over the last few years, the protective role of specific phytocompounds on human health has been proposed. Here, the functional effects of the olive leaf extract (OLE), isolated from the local *Coratina* cultivar, have been evaluated in renal collecting duct MCD4 cells expressing AQP2. Confocal analysis revealed that OLE prevents the vasopressin-induced AQP2 translocation to the plasma membrane. Functional studies showed that incubation with OLE decreased the vasopressin-dependent increase of the water permeability coefficient (Pf). To gain deeper insight into the molecular mechanism, FRET (Fluorescence Resonance Energy Transfer) experiments were applied to measure intracellular cAMP. Data revealed that treatment with OLE impaired the vasopressin-regulated increase of cAMP via phosphodiesterases activation. Accordingly, a relevant decrease in the vasopressin-induced phosphorylation of AQP2 at serine 256 was also observed. Indeed, we found that OLE exerts an antioxidant effect on vasopressin treated cells resulting in actin filament formation that may prevent AQP2 trafficking. Interestingly, *in vivo* studies demonstrated that OLE (250 mg/kg/day) reduced the apical localization of AQP2 in renal cortical ducts in rats treated with dDAVP. Together, our findings indicate that OLE behaves as a natural diuretic compound targeting its action on AQP2 trafficking.

PP.151

β3-AR as novel potential mediators of the cystogenetic process in Autosomal Dominant Polycystic Kidney Disease

Schena G[1], Gerbino A[2], Caplan MJ[1], Carmosino M[3]

[1]Department of Cellular and Molecular Physiology, Yale University, USA; [2]Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari, Italy; [3]Department of Science, University of Basilicata, Italy

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic condition caused by mutations in either Polycystin 1 (Pkd1) or Polycystin 2 (Pkd2) genes and characterized by disruption of the renal parenchima due to the formation of fluid filled cysts, with intracellular cAMP being a major driver of cyst growth. Interestingly, sympathetic system activity is elevated in ADPKD patients suggesting its involvement in the progression of the cystic phenotype. Given the expression of the β3-adrenoreceptor (β3-AR) in most of the nephron segments involved in the cysts formation, we wished to characterize its potential role in contributing to the pathogenesis of ADPKD. We found that the expression of β3-AR is significantly up-regulated in the kidneys of a ADPKD mice ($Pkd1^{fl/fl}$; $Pax8^{rtTA}$; TetO-Cre) compared to their wild type littermates. Moreover, we cloned the human β3-AR in a renal epithelial cell line derived from tubules of Pkd1-/- mice. Thanks to the ability of these cells of forming cysts when cultured in a 3D matrix we found that, upon treatment with beta agonists, $Pkd1^{-/-}$+ β3-AR cells form larger cysts as compared to $Pkd1^{-/-}$ cells suggesting that this is due to β3-AR presence. Fluorescence Resonance Energy Transfer (FRET) analysis was used to confirm that this effect is due to a significant increase in cAMP level elicited by β3-AR activation. Our results show β3-AR upregulation in an ADPKD mouse model and its involvement in cystogenesis *in vitro*. Our next step is to further test the potential of β3-AR as a therapeutic target both *in vitro* and *in vivo* by administration of selective antagonists in cell and mouse models respectively.

PP.152

The role of kisspeptin in myoglobinuric acute kidney injury in rats

Iskan NG[1], Soyleyici B[1], Kandemir N[2], Ali Aydin M[2], Aydogdu N[2]

[1]Trakya University School of Medicine, Turkey; [2]Department of Physiology, Trakya University School of Medicine, Turkey

Myoglobinuric acute kidney injury (MAKI) is a uremic syndrome caused by traumatically or non-traumatically injured striated muscles. Kisspeptin is suggested to

function as a vasoconstrictor in renal vascular muscle cells and to play an effective role in the regulation of renal tubular cells. Our aim is to examine the role of kisspeptin in the pathophysiology of MAKI. In this study, male Sprague-Dawley rats weighing 180-200 grams were divided into 2 groups (n=8) as Control and MAKI. MAKI was induced by intramuscular injection of glycerol (50%, 8 ml/kg). Urine samples were collected for 24 hours. The rats were euthanasised under anaesthesia for 48 hours after glycerol injection. The blood samples taken and kidneys tissues of the animals were removed. Mann-Whitney U test was used for statistical evaluation. Compared to the control group, urine microalbumin, fractional excretion of Na+ and K+ increased and creatinine clearance decreased significantly ($p<0.001$). Serum level of creatinine and urea increased whereas urine creatinine decreased ($p<0.001$). Renal level of kisspeptin decreased ($p<0.001$) whereas urine level increased ($p<0.05$). The renal level of aldosterone increased, but urine level decreased ($p<0.001$). The urine level of angiotensin II was decreased ($p<0.001$). Recent studies found that kisspeptin may play a role in the pathophysiology of chronic kidney injury. Our results indicated that kisspeptin may be related to aldosterone and the pathophysiology of MAKI. These findings suggest that kisspeptin might be used as a biomarker in the pathogenesis of MAKI. This study was supported by the project 1919B011700020 within the scope of TUBITAK- 2209A program.

PP.153

Determination of the protective role of spexin and adropin in chronic renal failure induced cardiovascular damage

Memi G[1], Yazgan B[2], Tastekin E[3]

[1]Kesan Hakkı Yoruk Health School, University of Trakya, Turkey; [2]Sabuncuoglu Serefeddin Health School, University of Amasya, Turkey; [3]Pathology Department of School of Medicine, University of Trakya, Turkey

Chronic renal failure (CRF) is a risk factor for cardiovascular diseases such as pericarditis, hypertension, cardiomyopathy. Spexin has many physiological effects in regulating renal/cardiovascular functions by playing important role in urine production but the mechanism has not been fully elucidated. Adropin is a newly identified peptide which has been shown to effects on endothelium and vasculature in previous studies. Adropin and spexin peptides both regulate energy metabolism. In this study; we investigated the possible protective effects of spexin and adropin peptides on cardiovascular damage caused by chronic renal failure induced by adenin in rats. Wistar albino rats both sexes (180-220 g) treated with adenine (600 mg/kg in %5 CMC, perioral, 10 days) for induction of CRF or vehicle (%5 CMC) for 10 days, then peptides injected with either saline or adropin (2.1 μg/kg/ml) or spexin (35 μg/kg/ml) peptides (i.p, 5 days/4 weeks). At the end of experimental procedure rats were sacrified under thiopenthal sodium anesthesia (40 mg/kg, i.p) to measure thoracic aorta contractility. Serum and urine samples taken at 1st , 2nd and 4th week of CRD induction for BUN, creatin, protein, CK, CK-MB, CRP levels. Values were compared by ANOVA. Adropin and spexin decreased CK ($p<0.01$) and CK-MB ($p<0.01$) in 2nd week but has no significantly effect on 4th week in CRD groups. In the other hand Spexin limited the increase of CK-MB levels on 2nd and 4th weeks ($p<0.05$). Adenine increased urine protein lost but it was abolished by adropin($p<0.05$) and spexin($p<0.05$). We investigated that both of two peptides improved cardiac injury parameters by aortic contractility measurements. Our work is ongoing and these preliminary datas suggest that adropin and spexin may have cardiac protective roles.

PP.154

Dandelion Root Extract reduces the activity of the renal ClC-Ka chloride channel through a Ca^{2+}/PKC-mediated mechanism

Gerbino A[1], De Zio R[1], Milano S[1], Russo D[2], Milella L[2], Procino G[1], Svelto M[1], Carmosino M[2]

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Italy; [2]Department of Sciences, University of Basilicata, Italy

Dandelion (*Taraxacum officinale*) has been used for centuries as an ethnomedical diuretic, although to date few scientific data empirically support this effect. We have recently shown that an ethanolic dandelion root extract (DRE) mobilized intracellular Ca^{2+} in renal cells and this, in turn, could potentially activate several downstream effectors. In this study, we evaluated at cellular level the effect induced by DRE on three transport mechanisms involved in urine concentration: Na-K-2Cl symporter (NKCC2) in the thick ascending limb; water channel aquaporin 2 (AQP2) of collecting duct cells, ClC-Ka chloride channel in the thin ascending limb. Here, NKCC2 phosphorylation was evaluated as index of its activation by Western blotting. The rate of AQP2 accumulation at the apical plasma membrane was analysed by confocal laser microscopy in MCD4 cells. Chloride flux through ClC-Ka was measured by patch clamp experiments in HEK293 cells expressing ClC-Ka and the accessory protein Barttin. We found that exposure to DRE 400μg/mL did not affect neither NKCC2 activity nor AQP2 trafficking. On the other hand, 20 min. pre-treatment with DRE time-

dependently reduced the Cl- currents through ClC-Ka in HEK293 cells. Interestingly, this effect was: (i) prevented by intracellular Ca^{2+} chelation induced by BAPTA-AM pre-treatment, (ii) blocked by calphostin C (a wide spectrum PKC antagonist) and (iii) mimicked by PMA (a PKC activator). In conclusion, we showed for the first time that DRE can inhibit the activity of ClC-Ka with a mechanism dependent by Ca^{2+} increases and PKC activation. In a physiological context, the reduced activity of ClC-Ka in the presence of DRE could reduce net salts and water reabsorption without affecting other mechanisms currently targeted by other diuretics.

PP.155

Renal function by developing population pharmacokinetics in Lithuania

Stankevicius E, **Radzeviciene A**, **Abramavicius S**, **Marcoux F**, **Kadusevicius E**

Lithuanian University of Health, Lithuania

Immunosuppressive drugs exhibit high variability in metabolism and pharmacokinetics that may result in drug toxicity or lack of efficacy. Low immunosuppressant drug exposure increases the risk of transplant rejection in the acute post-transplant period, while supratherapeutic drug concentration entails higher risk of adverse drug reactions1. These issues may be resolved by population pharmacokinetic modeling. Population pharmacokinetic modeling might be performed and researched by clinical pharmacologists, recently established clinical pharmacology residency in Lithuania may play a role in this area as well2. Anonymized medical records of kidney recipients receiving immunosuppressant tacrolimus and hospitalized at Limoges University Hospital (France) were included in the study. Tacrolimus analyses were performed using a liquid chromatography- tandem mass spectrometry method. A one-compartment model with first-order absorption was used as implemented in the NLMIXED procedure3, which fits nonlinear mixed models. Data analysis was performed by using SAS University Edition software. Model parameter estimated are provided with p-values and confidence limits computed by NLMIXED procedure. The p-values and confidence limits were computed from approximate standard errors (using the delta method) for the estimates. Anonymized medical records of 189 patients receiving immunosuppressant tacrolimus (2–20 mg/d BID regimen) were analyzed and a one-compartment model with first-order absorption was constructed. The population estimates in the final population model of tacrolimus were: clearance 14.64 L/h (CI 9.66; 19.62), p<0.0001, elimination rate 0.001657 min-1 (CI: 0.00098; 0.002336), p<0.0001 and absorption rate 2.7119 (CI: -45.7083; 51.1321), p=0.912. Mean value of concentration was 13.62 (SD: 7.5) μg/L, predicted concentration 10.83 (SD: 10.83) μg/L. Pearson correlation between measured and predicted concentrations was r=0.79 (p<0.0001). In conclusion, population pharmacokinetic models should be developed in-house. Kidney parameters still have a significant effect on the pharmacokinetics of medicines. This research was funded by a grant no. P-MIP-17-445 from the Research Council of Lithuania

PP.156

Direct physical interaction between Calcium Sensing Receptor and Polycystin-2: implication in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Di Mise A, **Venneri M**, **Ranieri M**, **Centrone M**, **Caroppo R**, **Tamma G**, **Valenti G**

Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy

ADPKD is caused by mutations in *PKD1* or *PKD2* genes, encoding polycystin-1 (PC1) and polycystin-2 (PC2) respectively, characterized by excessive cell proliferation and fluid secretion, resulting in renal cyst formation and growth. PC1 and PC2 form a complex localized to the primary cilium. PC2 is a non-selective cation channel which, in renal epithelial cells, contributes to calcium transport and signaling. It has been previously shown in renal cells, that high external calcium increases whole cell currents likely mediated by PC2. In this study, we explored the possibility that the Calcium Sensing Receptor (CaSR) is involved in the functional regulation of PC2. To this end, human conditionally immortalized Proximal Tubular Epithelial cells isolated from urine sediments (ciPTECwt) and with stably down-regulated PKD1 (ciPTEC-PC1KD) were used. Both ciPTECwt and ciPTEC-PC1KD express endogenous CaSR. Cellular fractionation showed a significant higher PC2 expression in the plasma membrane fraction of ciPTEC-PC1KD with respect to ciPTECwt, suggesting that PC1 silencing alters PC2 localization. By immunocytochemistry, PC2 was found to partially co-localize with CaSR, though to a higher extent in ciPTEC-PC1KD. Preliminary electrophysiological measurements demonstrated that, in ciPTECwt, the membrane potential is modulated by CaSR activation, consistent with modulation of cation channels. Worthy of note, co-immunoprecipitation experiments proved that CaSR and PC2 are interacting proteins in ciPTECwt. These studies underline the functional coupling of CaSR with PC2 in ciPTEC, which might be relevant to the amelioration of the principal cellular ADPKD dysregulations observed in our previous studies in ciPTEC-PC1KD exposed to calcimimetics as well as in animal model of ADPKD.

Poster Session II (7/7)

Reproductive Physiology

PP.157

HPV infection inhibits aquaporin-mediated hydrogen peroxide elimination and affects human sperm function

Pellavio G[1], Todaro F[2], Omes C[2], Alberizzi P[3], Scotti C[1], Gastaldi G[1,4], Caliogna L[4], Nappi R[2], Laforenza U[1]

[1]Department of Molecular Medicine, University of Pavia, Italy; [2]Center for Reproductive Medicine, Obstetrics and Ginecology Unit, Fondazione IRCCS Policlinico San Matteo, Italy; [3]IRCCS-Fondazione Policlinico San Matteo, Italy; [4]Center for Health Technologies, University of Pavia, Italy

Aquaporins (AQPs) 3, 7, 8, and 11 are expressed in human sperm cells and are localized in the plasma membrane and in intracellular structures. AQPs are involved in cell volume regulation, cytoplasm removal during sperm maturation and ROS (H_2O_2) elimination. Recently, AQP-mediated H_2O_2 permeability was found to be reduced by oxidative stress and related to a decrease in sperm number and motility. We studied the possible effect of Human Papillomavirus (HPV) on the expression and function of AQPs in human sperm cells of patients undergoing infertile couple evaluation. ELISA experiments showed that HPV infection is associated to an increased AQPs expression in normospermic patients and to a decreased one in sub-fertile patients. Functional experiments demonstrated that HPV infection heavily reduces water permeability of sperm cells of both normospermic and sub-fertile patients. Confocal IF experiments showed colocalization of HPV L1 protein with AQP8. Docking of HPV L1 and AQP8 atomic models suggested a distant effect of L1 on the pore NPA region. Present findings suggest that HPV infection affects AQPs expression and directly inhibits their function, probably by making sperm cells more sensitive to oxidative stress

PP.158

Evaluation of the association between dietary pattern and fat distribution in pregnant women

Ravella S, Spadafranca A, Lewandowski L, De Amicis R, Leone A, Foppiani A, Battezzati A, Bertoli S

International Center for the Assessment of Nutritional Status (ICANS), Department of Food Environmental and Nutritional Sciences (DeFENS), University of Milan, Italy

Pregnancy represents a very delicate physiological moment. An excessive increase in weight during pregnancy, as well as a condition of obesity or overweight before pregnancy, exposes the woman to a greater risk of pregnancy complications and affects the risk of developing atherosclerosis, hypertension and insulin resistance during child's life. We aimed to monitor the association of specific dietary changes and modification in fat distribution weight during gestation and after childbirth. The project includes 2 frontal and interactive group meetings focused on proper nutrition in pregnancy and how to turn food advice into a practical and fast culinary experience, and 3 individual meetings at the 36th gestational week, at 6 and 12 months after birth. Weight, skinfolds, circumferences and mediterranean food questionnaires (MedScore) were measured at every meeting. To date, 49 women have voluntarily joined the program (35.0±4.2 y). On average, the sample was in the second quarter (21.5±7.8 w). 73.5% of the sample was primiparous, 19.2% with a before-pregnancy condition of overweight or obesity. At baseline (T0), 20% were adherent to the Mediterranean Diet; at the 36th week 64.7% improved its MedScore compared to T0 and the weight trend was adequate in 91.8% of the sample without association with fat distribution. The 59.2% was reviewed 6 months after childbirth: 64.3% of this recovered the before-pregnancy weight. We analysed the relationship of specific changes in MedScore subcutaneous fat distribution but we could not find significant association with this preliminary dataset. However, education program seems to be an effective prevention strategy in the control of body weight during pregnancy as well as after child birth.

PP.159

Can irisin change partner preference of female rats?

Canpolat S[1], Ulker N[1], Bulmus O[2], Ozcan M[3]

[1]Department of Physiology, Firat University, Turkey; [2]Department of Physiotherapy and Rehabilitation, Firat University, Turkey; [3]Department of Biophysics, Firat University, Turkey

The partner preference and active investigation paradigms are used to investigate and quantify sexual motivation in female rats. Partner preference (sexually active male or incentive female) and active interest to sexual partner of females are measured as duration of time in these tests. Although it is known that moderate-intensity exercise has positive effects on sexual function, the effects of irisin, an exercise hormone, on sexual motivation is unknown. The purpose of the present study is to evaluate the effects of irisin on appetitive aspects of sexual behavior in female rats. Totally 24 female Sprague-Dawley rats (21 days old

and 35 ± 2 g weight) were used and randomly divided into two groups as control and irisin groups (n=12). The animals were administered daily intraperitoneally irisin (100 ng/kg) or saline from postnatal day 21 for about 10 weeks. From the 8th weeks of the treatments, partner preference and active investigation tests were performed for 10 min in all rats in the estrous phase. When compared to control group, irisin treatment significantly reduced time near the male ($p<0.05$) and male preference ratio ($p<0.001$) but increased time near the female ($p<0.001$). Time spent investigating the incentive female ($p<0.001$) and locomotor activity (the number of times the animal crossed the center of the testing apparatus; could influence investigation times and act as a confound) ($p<0.05$) were significantly higher in irisin group compared with control group. However, male investigation preference ratio was significantly decreased by irisin treatment ($p<0.05$). The findings suggest that irisin treatment reduced the sexual motivation of female rats against male rats. It can be thought that irisin may cause homosexuality in females. This study was supported by TUBITAK-118S519.

PP.160

The role of potassium channels and calcium in relaxing effect of minoxidil on the isolated rat uterus

Sokolović D[1], Blagojević D[2], Drakul D[1], Orečanin-Dusić Z[2], Kulić M[1], Pecelj M[3,4,5], Milovanović RS[1,2]

[1]Faculty of Medicine Foča, University of East Sarajevo, Republic of Srpska Bosnia and Herzegovina; [2]Department of Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia; [3]Faculty of Philosophy, University of East Sarajevo, Republic of Srpska, Bosnia and Herzegovina; [4]Geographical Institute "Jovan Cvijić" of the Serbian Academy of Sciences and Arts, Serbia; [5]Institute of Sports, Tourism and Service, South Ural State University, Russia

Minoxidil is a potassium channel opener that causes hyperpolarization of cell membrane and the relaxation of blood vessels and various smooth muscles. This study examined the effect of increasing minoxidil concentrations on the contractility of spontaneous, electrically or calcium (6 and 12 mM) stimulated isolated rat uteri and pretreated with potassium channel blockers: glibenclamide (GLB - 3, 10 and 30 μM), 4-aminopyridine (4AP - 0.3, 1, and 3 mM) and tetraethylammonium (TEA - 0.3, 1, and 3 mM). The uteri were isolated from virgin estrous Wistar rats (180-220 g) and suspended in an isolated organ bath chamber containing De Jalon's solution and aerated with 95% O_2 and 5% CO_2 at 37 C. Minoxidil inhibited uterine contractile activity in a dose dependent manner, and the inhibitory effect was significantly stronger for electrical stimulation comparing to spontaneous rhythmic activity. The relaxation effect of minoxidil was substantially reduced in the presence of higher Ca^{2+} concentration in medium (12 mM). GLB, 4AP and TEA antagonized to a certain extent the inhibitory effect of minoxidil on the spontaneous rhythmic activity of the isolated rat uterus. On an equimolar basis, GLB exhibited the strongest antagonistic effect. Obtained results suggest that the mechanism of action of minoxidil is related to the opening of KATP channels. However, the opening of BKCa and voltage-dependent Ca^{2+} channels also had a role, but to varying degrees that depends on the type of uterine activation. Our results also indicate involvement of Ca^{2+} and IP3 in the mechanism of minoxidil activity. Minoxidil could be a potential tocolytic drug.

PP.161

Irisin may have inhibitory effects on pubertal maturation in female rats

Ulker N[1], Bulmus O[2], Ozcan M[3], Canpolat S[1]

[1]Department of Physiology, Firat University, Turkey; [2]Department of Physiotherapy and Rehabilitation, Firat University, Turkey; [3]Department of Biophysics, Firat University, Turkey

The studies suggest that puberty onset is appeared to be more related to body weight than chronological age. When body reaches the critical fat and/or muscle mass, several metabolic signals (leptin, ghrelin, insulin, etc.) is activated and these signals regulate the onset of puberty. However, the effects of irisin, an important adipokine and myokine, are not fully defined on hypothalamus-pituitary-gonadal axis at the peripheral levels. The goal of this study is to carry out to reveal the effects of irisin on pubertal maturation and the day of the first estrus in female rats. For this purpose, totally 24 female Sprague-Dawley rats, 21 days old and 35 ± 2 g weight, were used and randomly divided into two groups as control and irisin groups (n=12). Irisin (100 ng/kg) was given intraperitoneally daily in the animals in irisin group from postnatal day 21 for about 10 weeks. Similarly, the control group received only saline. For determining the puberty onset, vaginal opening (complete canalization of the vagina, an external index of puberty onset) was monitored daily in rats. Vaginal smear was performed daily from the date of puberty onset to determine the day of the first estrus. When compared to control group, irisin treatment significantly delayed the onset of female puberty ($p<0.01$) and increased the pubertal weight ($p<0.001$). However, it was shown that irisin treatment did not affect the day of the first estrus when compared to control rats. In conclusion, our findings suggest that irisin may have

inhibitory effects on reproductive axis, delaying the puberty onset in female rats. One of the reasons of the development of menstrual dysfunction associated with exercise in female athletes may be irisin hormone. This study was supported by TUBITAK-118S519.

PP.162

Effects of irisin on pubertal maturation process in male rats

Ozcan M[1], Ulker N[2], Yardimci A[2], Bulmus O[3], Canpolat S[2]

[1]Department of Biophysics, Firat University, Turkey; [2]Department of Physiology, Firat University, Turkey; [3]Department of Physiotherapy and Rehabilitation, Firat University, Turkey.

Irisin, an exercise hormone, has been demonstrated to be effective on gonadotropin levels. However, the effects of irisin on pubertal maturation process are unknown. The aim of the present study was to determine the effects of exogenous irisin treatment on puberty onset, pubertal weight, formation time of first sperm and displaying time of first sexual behavior in male rats. In this study, totally 24 male Sprague-Dawley rats, 21 days old and 35 ± 2 g weight, were divided into two groups (n = 12), being control and irisin groups. The animals started to receive daily intraperitoneally irisin (100 ng/kg) from postnatal day 21 for about 10 weeks. The control groups received only saline. Puberty onset was monitored by examination of preputial separation in rats. All rats were daily mated to determine displaying time of first sexual behavior after determining the pubertal timing. When mating occurred, vaginal smear was conducted to detect formation time of first sperm. When compared to control group, irisin treatment did not affect the onset of puberty and pubertal weight. There was no any significant change in the displaying time of first sexual behavior in rats. However, in the irisin treated group, formation time of first sperm was significantly earlier compared to control rats ($p < 0.001$). Irisin hormone has an effect on the hypothalamus-pituitary-gonadal axis, stimulating spermatogenesis. This study was supported by TUBITAK-118S519.

PP.163

Effects of Irisin on Sperm Characteristics and Testicular Tissue in Rats

Bulmus O[1], Ulker N[2], Kaya N[3], Turk G[4], Canpolat S[2]

[1]Department of Physiotherapy and Rehabilitation, Firat University, Turkey; [2]Department of Physiology, Firat University, Turkey; [3]Department of Histology and Embryology, Firat University, Turkey; [4]Department of Reproduction, Firat University, Turkey

Recent studies suggest that irisin may participate in reproductive function. However, biological actions of irisin on sperm characteristics and gonads are exactly unknown in male. In this study, we aimed to determine the effects of irisin on sperm parameters and testicular tissue in rats. In this study, 24 male Sprague-Dawley rats were used and randomly separated into two groups as control and irisin (n=12). The rats were injected intraperitoneally daily with irisin (100ng/kg) or saline for about 10 weeks from postnatal day 21. At the end of the study, rats were killed by decapitation and sperm parameters assessed. Testis tissues were prepared for histological and histometric studies. Histopathological evaluations were performed, and histopathological findings were scored semi-quantitatively; non-existent (0), mild (1), moderate (2) or severe (3). When compared with control rats, epididymal sperm concentration was found significantly higher in irisin group ($p<0.05$), but no significant differences were found in sperm motility and the head, tail and total abnormality rates of spermatozoa. Irisin treatment significantly increased germ cells spilled into the lumen of the seminiferous tubules ($p<0.01$), detachment of the basement membrane of seminiferous tubules ($p<0.05$) and seminiferous tubule diameter ($p<0.001$) compared to control group. However, thickness of germinal epithelium was significantly lower in irisin group compared with control group ($p<0.05$). Our results showed that irisin treatment increased epididymal sperm concentration and also caused histopathological changes in testicular tissue in male rats. All these results suggest that irisin hormone may have peripheral effects on the reproductive system in male. This study was supported by TUBITAK-118S519.

PP.164

Remodeling extracellular matrix of seminal vesicles by hyperhomocysteinemia in Sand rat (*Psammomys obesus*)

Ghoul A[1], Moudilou E[2], Zerrouk F[1], Chaouad B[1,3], Exbrayat J-M[2], Benazzoug Y[1]

[1]Faculty of Biological Sciences (FSB), USTHB, Algeria; [2]Laboratory of General Biology, Catholic University of Lyon, France; [3]University Khemis Miliana, Algeria

The main objective of this work is to analyze the impact of hyperhomocysteinemia on the seminal vesicles (SV) of a rodent from southern Algeria, the sand rat *Psammomys obesus*. Control group (n=10), received natural halophile diet and experimental group (n=9) received the same diet supplied with methionine at

dose of 200mg/Kg of B.W/day during 3 months. Plasma homocysteine was measured in all the groups. Histological and histochemical staining are used to detect collagen and mucin. The metalloproteinases (MMP-2, -3,-7,-9) in SV were examined using immunohistochemical and Western blotting methods. The mean plasma levels of homocysteine at the end of experiment were 23.42± 4.46 μmol/L and 8.55 ± 2.53 μmol/L in experimental and control rats, respectively. On the tissue level, the Hhcy induced seminal vesicles fibrosis. Therefore, we observed an increase of ECM proteins like collagen and MMP activation. These results may suggest that homocysteine causes SV remodeling by activating MMP.

Poster Session III (1/3)

Neurophysiology – Synaptic Transmission

PP.165

Moderate extracellular acidification affects presynaptic endings via activation of phospholipase C pathway and calcium transport from SERCA-linked stores

Dubouskaya T[1], Fedorovich S[1,2]

[1]Laboratory of Immunology and Cell Biophysics, Institute of Biophysics and Cell Engineering National Academy of Sciences of Belarus, Belarus. [2]Department of Biochemistry, Belarusian State University, Belarus

Brain ischemia is accompanied by lowering of intra- and extracellular pH and often leads to irreversible damage of synaptic transmission. Influence of ischemic acidosis on presynaptic endings is still not well understood. We investigated an influence of pHi and pHo lowering on the basic physiological parameters of rat brain synaptosomes. We have shown that extracellular, but not intracellular, acidification led to depolarization of synaptosomal mitochondria and increase reactive oxygen species (ROS) formation in synaptosomes. This suggests the presence of receptor for protons on the plasma membrane of presynaptic terminals. We confirmed, that mitochondria are the main source of ROS in synaptosomes at moderate extracellular acidification. Acid sensitive ion channels (ASICs) are widely considered neuronal H+-sensors. But Amiloride, that inhibit these channels, did not affect mitochondrial potential changes at moderate extracellular acidification. Depolarization of synaptosomal mitochondria and synaptosomal ROS formation at moderate extracellular acidification are partially blocked by phospholipase C (PLC) inhibitor U-73122 and by calcium ion pumps of endoplasmic reticulum inhibitor Thapsigargin. We have shown that lowering of pHo down to 7.0 led to calcium rise in cytosol and to a small, but significant increase in mitochondrial calcium concentration. Our results suggest that activation of PLC-linked proton receptor on presynaptic plasma membrane leads to calcium release from endoplasmic reticulum followed by accumulation in mitochondria, that probably causes a decrease in mitochondrial membrane potential and increase in mitochondrial ROS formation.

PP.166

Different nicotinic receptor (nAChR) subtypes regulate glutamate and GABA release onto regular spiking non-pyramidal cells in prefrontal (Fr2) layer V

Meneghini S[1], Di Girolamo S, Modena D[2], Amadeo A[2], Becchetti A[1]

[1]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy; [2]Department of Biosciences, University of Milano, Italy

In neocortex, pyramidal cell activity is controlled by different populations of GABAergic neurons, whose physiological role is debated. In layer V of the premotor Fr2 region, the prevalent GABAergic neurons are PV+ fast-spiking cells, and SOM+ regular-spiking non pyramidal (RSNP) cells, not expressing ionotropic 5HT3aRs. In Fr2 brain slices from adult mice, we studied how nAChRs regulate RSNP cells. Nicotine (10 μM) caused a 10-fold and persistent increase of the frequency and amplitude of spontaneous excitatory postsynaptic currents. The effect was blocked by 30 μM dihydro-β-erythroidine (DHβE), but not by 1 μM DHβE or 10 nM methyllicaconitine, suggesting the implication of 2* heteromeric nAChRs. Nicotine also increased (~4 times) the frequency of spontaneous inhibitory postsynaptic currents, and the effect was blocked by 1 μM DHβE, pointing to the implication of 4* nAChRs. These effects were similar in mice expressing β2-V287L, a mutant nAChR subunit linked to sleep-related frontal epilepsy. RSNP cells also displayed whole-cell nicotinic currents, indicating somatic expression of nAChRs, which presented a mixed subunit composition. The somatic currents had higher average amplitude in mice expressing β2-V287L, which could contribute to local hyperexcitability in epileptic frontal areas. Conclusions: 1) RSNP cells are very sensitive targets of ACh, in mature associative neocortex, and could be critical regulators of cortical output during attentive tasks; 2) different nAChR subtypes regulate glutamate and GABA release onto these cells, which could help interpret the apparently contradictory functional features displayed in vitro by 4β2 (gain-of-

function) and 2* (loss-of-function) mutant nAChRs linked to sleep-related epilepsy.

PP.167

Influence of ketoacidosis on rat brain synaptosomes

Voronina P[1], Adamovich K[1], Adamovich T[1], Hrynevich S[1], Waseem T[1], Fedorovich S[1,2]

[1]Institute of Biophysics and Cell Engineering NASB, Minsk, Belarus; [2]Department of Biochemistry, Belarusian State University, Minsk, Belarus

It is shown that ketogenic diet which leads to a moderate increase of plasma ketone body concentration up to 4-5 mM can be used for treatment of different brain diseases. Otherwise, very high concentration of ketone bodies up to 25-50 mM which occurred at ketoacidosis can damage neurons followed by coma and even death. Ketoacidosis is very dangerous complication of diabetes mellitus or alcohol poisoning. It is unknown why different concentrations of ketone bodies have different effects on neurons.

In the present paper we investigate the influence of 25 mM of main ketone body DL-β-hydroxybutirate (BHB) on intracellular pH with fluorescent dye BCECF-AM, synaptic vesicle recycling with fluorescent dye acridine orange and plasma membrane potential with fluorescent dye DiSC3(5) in rat brain synaptosomes. We compare data with our previously published results on influence of neuroprotective 8 mM of BHB. We have shown that BHB even at concentration of 25 mM does not influence pHi in synaptosomes. Replacing glucose for 25 mM BHB inhibits endocytosis. This effect was significantly more pronounced compared to similar effect of 8 mM BHB. 25 mM HEPES or 25 mM sucrose were not effective. It is suggested that inhibiting endocytosis deals with rather specific influence of BHB than non-specific influence of osmolarity or decreasing of synaptic vesicle reacidification due to high buffer capacity. We have found that replacing of glucose by 25 mM BHB in contrast to 8 mM BHB depolarizes plasma membrane. Addition of 25 mM BHB to glucose-containing incubation medium also decreases plasma membrane potential. Our results suggest that the key difference between neurotoxic and neuroprotective influence of different concentration of BHB is depolarization of plasma membrane.

PP.168

Pulsed electromagnetic field exposure disrupts post-ischemic long-term potentiation of synaptic strength in rat perirhinal cortex slices

Roncacè V[1], Burattini C[1], Camera F[2], Apollonio F[2], Liberti M[2], Capone F[3], Di Lazzaro V[3], Aicardi G[1,4]

[1]Department for Life Quality Studies, University of Bologna, Italy; [2]Department of Information Engineering, Electronics and Telecommunication, University of Rome "La Sapienza", Italy; [3]Neurology Unit, Campus Bio-Medico University of Rome, Italy; [4]Interdepartmental Center "Luigi Galvani" for the Integrated Study of Biophysics, Bioinformatics and Biocomplexity, University of Bologna, Italy.

Previous studies have shown that exposure to a pulsed electromagnetic field (PEMF; 75 Hz) reduces ischemic neuronal damage in a rabbit model of transient focal ischemia, counteracts hypoxia damage in cultured neuron-like cells, and upregulates A2A and A3 adenosine receptors (known to play a major role in post-ischemia recovery) in the rat cerebral cortex. In the present study, we investigated the mechanism of the neuroprotective action of this PEMF, by examining its effect on synaptic transmission in rat perirhinal cortex slices made ischemic in vitro. Synaptic transmission was studied by extracellular field potential recording. Ischemia was induced by oxygen/glucose deprivation (OGD). Single slices were exposed to 1.5 mT PEMF after OGD or in control conditions. A careful dosimetric analysis was performed. Four min of OGD induced a strong post-ischemic long-term potentiation of synaptic strength (iLTP). The exposure to 1.5 mT PEMF for 120 min, which did not affect synaptic transmission in control conditions, disrupted OGD-induced iLTP. These results provide the first evidence that PEMF exposure disrupts this pathological form of synaptic plasticity, that increases excitotoxicity in the ischemic brain. This strengthens the expectation of a positive impact of PEMF exposure on stroke patient's recovery.

PP.169

Analysis of dopamine release from GABAergic periglomerular cells in the olfactory bulb using false fluorescent neurotransmitters and brain-on-chip systems

Viggiano D[1], Bellenchi G[2]

[1]Dept. Medicine and Health Sciences, Univ. Molise, Campobasso, Italy; [2]IGB, CNR, Naples, Italy

The periglomerular (PG) cells of the olfactory bulb are a puzzling example of neurons escaping Dale's principle: they co-release dopamine (DA) and GABA. Using acute olfactory-bulb slice, we have characterized DA release using optical methods (false fluorescent

neurotransmitters, FFN) and dopamine-sensitive multi-electrodes (brain-on-chip); DA cells have been identified in vivo using tyrosine-hydroxylase (TH)-EGFP expressing animals. PG cells TH-EGFP+ can uptake FFN511 (an FFN similar to DA) and release it in time-lapse confocal imaging upon KCl depolarization. This corresponded to true release of native DA as tested by multielectrodes for electrochemical detection of DA. The release is calcium-dependent as it is inhibited by Ca-free medium. These cells contain GABA vesicles as shown by expression of VIAAT, whereas do not express VGLU3. These terminals do not express VMAT2 immunofluorescence, however reserpine can block FFN511 uptake by VMAT, suggesting that another isoform of VMAT may exist. Furthermore, the internalization of FFN511 in periglomerular cells occurs in vesicles that are sensitive to tetrabenazine, as this blocker avoid FFN511 concentration. The olfactory deprivation using ZnSO4 determines a reduction of TH expression; however, VMAT-like activity is still present and vesicle machinery release is intact. Finally, local glutamate release represents one of the physiological stimuli that induce dopamine degranulation, as demonstrated by time-lapse studies with caged-glutamate. In conclusion, these new technological advancements have confirmed some of the previous observations about dopamine release in these neurons and add further observations that await full characterization.

PP.170

Canonical AKT pathway may serve as compensatory mechanism for AKT-induced eLTP abolishment

Gorobetc D[1,2], Sheinin A[3], Pinhasov A[1,2], Michaelevski I[1,2]

[1]Dept. of Molecular Biology, Ariel University, Ariel, Israel; [2]Integrated Brain Science Center – Ariel (IBSCA), Ariel University, Ariel, Israel; [3]Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel

AKT is a serine/threonine kinase playing a substantial role in the multiple processes in the neural system e.g. neurons development and survival, spinogenesis, and long-term synaptic plasticity. Our recent findings showed that AKT activation is essential for expression of early phase long-term potentiation (eLTP). This effect on eLTP expression requires AKT membrane tethering. Moreover, eLTP expression was found not necessarily depends upon AKT downstream canonical pathways. We hypothesized that AKT mediates eLTP expression via a novel non-canonical downstream pathway, while the canonical signaling involving mTORC1 and/or GSK3β may serve either compensatory or synergistic mechanisms of LTP. Here we report about our preliminary findings of the interplay between AKT non-canonical and mTORC1, GSK3β-dependent signaling pathways. We found that: a) separate or concomitant pharmacological activation of mTORC1 as well as inhibition of GSK3β failed to show shift of eLTP expression level; b) parallel inhibition of GSK3β by TWS119 and AKT by A6730 rescued eLTP expression attenuation; c) likewise, concomitant activation of mTORC1 by dorsomorphin and inhibition of AKT restored eLTP expression. Summarizing, we suppose that the canonical and non-canonical pathways of AKT-driven regulation of eLTP may exist independently and as a fail-safe mechanism for eLTP expression.

PP.171

A somatosensory cortex microcircuit involving layer 2/3 cortical pyramidal cells and somatostatin-expressing interneurons is altered in a genetic mouse model of migraine

Marchionni I[1,2], Pilati N[1], Forli A[1], Sessolo M[1], Pietrobon D[1,2,3]

[1]Department of Biomedical Sciences and [2]Padova Neuroscience Center, University of Padova; [3]CNR Institute of Neuroscience, Padova, Italy.

Familial hemiplegic migraine type 1 (FHM1) is a rare form of migraine with aura caused by gain-of-function mutations in the gene encoding the neuronal Cav2.1 calcium channel. In mice carrying a FHM1 mutation, we have previously shown enhanced excitatory synaptic transmission at unitary synapses between pyramidal cells (PCs) and fast-spiking interneurons, but unaltered inhibitory neurotransmission at the reciprocal inhibitory synapses. Here we show that, in FHM1 mice, excitatory transmission is enhanced also at unitary synapses between L2/3 pyramidal cells and somatostatin-expressing interneurons (SST-INs), and that inhibitory transmission is unaltered also at the synapses between SST-INs and pyramidal cells. Short-term plasticity at synapses between PCs and SST-INs was not affected during trains of action potentials (APs) at 25 Hz but significantly reduced during trains at 100 Hz. Using paired whole-cell patch clamp recordings between neighboring L2/3 PCs, we investigated the consequences of these synaptic alterations on the cortical microcircuit underlying the frequency dependent disynaptic inhibition (FDDI) of PCs. We induced high frequency (100 Hz) train of APs (10) in one individual PC and recorded the disynaptic inhibitory response in a nearby PC, due to activation of SST-INs receiving facilitating synapses from PCs. We found a significant shortening in the latency of the onset of FDDI in FHM1 KI mice when compared to WT mice. This could influence the integration of the information

within S1 arising from other cortical areas and the output signal of cortical circuits.

PP.172

Influence of glucose deprivation on membrane potentials of different membranes in rat brain synaptosomes.

Hrynevich SV[1], Fedorovich SV[1]

[1]Laboratory of Biophysics and Engineering of Cell, Institute of Biophysics and Cell Engineering National Belarus Academy of Sciences, Belarus

Hypoglycemia can cause neuronal cell death similar to that of glutamate-induced cell death. We investigated the effect of glucose removal from incubation medium on changes of mitochondrial and plasma membrane potentials in rat brain synaptosomes using the fluorescent dyes DiSC3(5) and JC-1. We also monitored pH gradients in synaptic vesicles and their recycling by the fluorescent dye acridine orange. Glucose deprivation was found to cause an inhibition of K+-induced Ca2+-dependent exocytosis and a shift of mitochondrial and plasma membrane potentials to more positive values. The sensitivity of these parameters to the energy deficit caused by the removal of glucose showed the following order: mitochondrial membrane potential > plasma membrane potential > pH gradient in synaptic vesicles. The pH-dependent dye acridine orange (AO) was used to investigate synaptic vesicle recycling. However, the compound's fluorescence was shown to be enhanced also by the mixture of mitochondrial toxins rotenone (10 μM) and oligomycin (5 μg/mL). This means that AO can presumably be partially distributed in the intermembrane space of mitochondria. Glucose removal from the incubation medium resulted in a 3.7-fold raise of AO response to toxins suggesting a dramatic increase in the mitochondrial pH gradient. Our results suggest that the biophysical characteristics of neuronal presynaptic endings do not favor excessive non-controlled neurotransmitter release in case of hypoglycemia. The inhibition of exocytosis and the increase of the mitochondrial pH gradient, while preserving the vesicular pH gradient, are proposed as compensatory mechanisms.

PP.173

Long-lasting response changes in deep cerebellar nuclei in vivo correlate with low-frequency oscillations

Moscato L[1*], Montagna I[1*], De Propris L[2], Di Domenico D[1], Tritto S[1], Mapelli L[1], D'Angelo E[1,2**]**

[1]Dept of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy; [2] Brain Connectivity Center, C. Mondino National Neurological Institute, Pavia, Italy; * co-first authors; ** co-last authors

The deep cerebellar nuclei (DCN) have been suggested to play a critical role in sensorimotor learning and some forms of long-term synaptic plasticity observed in vitro have been proposed as a possible substrate. However, till now it was not clear whether and how DCN neuron responses manifest long-lasting changes in vivo. Here, we have characterized DCN unit responses to tactile stimulation of the facial area in anesthetized mice and evaluated the changes induced by theta-sensory stimulation (TSS), that is known to induce plasticity in the cerebellar cortex in vivo. DCN units responded to tactile stimulation generating bursts and pauses, which reflected combinations of excitatory inputs most likely relayed by mossy fiber collaterals, inhibitory inputs relayed by Purkinje cells, and intrinsic rebound firing. Interestingly, initial bursts and pauses were often followed by stimulus-induced oscillations in the peri-stimulus time histograms (PSTH). TSS induced long-lasting changes in DCN unit responses. Spike-related potentiation and suppression (SR-P and SR-S) were correlated with stimulus-induced oscillations. Fitting with resonant functions suggested the existence of peaks in the theta-band. Optogenetic stimulation of the cerebellar cortex altered stimulus-induced oscillations suggesting that Purkinje cells play a critical role in controlling DCN oscillations and plasticity. This observation complements those reported before on the cerebellar cortex supporting the generation of multiple distributed plasticities in the cerebellum following naturally patterned sensory entrainment. The unique dependency of DCN plasticity on circuit oscillations discloses a potential relationship between cerebellar learning and activity patterns generated in the cerebellar network.

Poster Session III (2/3)

Neurophysiology – Memory formation, storage and recall

PP.174

The Parkinson-related $E_{193}K$ $LRRK_2$ variant impacts neuronal vesicles dynamics through perturbed protein interactions.

Marku A[1], Casiraghi Z[1], Galli A[1], Ghislanzoni S[1], Marciani P[1], Piccoli G[2], Perego C[1]

[1]DISFEB Dept of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; [2]CIBIO Dept of Cellular, Computational and Integrative Biology, Università degli Studi di Trento,Trento, Italy

The Leucine-Rich Repeat Kinase 2 (LRRK2) is a complex protein, expressed in neurons and implicated in Parkinson disease (PD). LRRK2 contains a dual enzymatic activity and several structural domains that constitute a versatile platform for multiple protein interactions at the synapses. In this study, we characterize the functional role of the N-terminal Armadillo repeats domain of LRRK2 and the impact on synaptic vesicle (SV) dynamics of a novel variant, E193K, harboured within this domain and identified in an Italian family affected by PD. Using a genetically encoded sensor of recycling, synaptopHluorine, and total internal reflection fluorescence microscopy, we visualized SV trafficking in the N2A neuroblastoma cells expressing the wild type LRRK2 protein, a mutant lacking the Armadillo domain (ΔN LRRK2) or the E193K variant. We found that expression of the ΔN construct increased the frequency and the amplitude of spontaneous synaptic events. A similar phenotype was detected in the presence of the E193K variant, suggesting that this mutation behaves as a loss-of-function mutation. A domain-based pulldown approach demonstrated that the LRRK2 N-terminus binds to cytoskeletal (β-actin and -tubulin) and SV (synapsin I) proteins and the E193K substitution alters strength and quality of LRRK2 interactions. The results support a role of the Armadillo domain in interaction with synaptic proteins and suggest that the E193K mutation affects LRRK2 function via perturbation of its physiological network of interactors, resulting in impaired vesicular trafficking. These findings may have important implications for understanding the role of LRRK2 at the synapses and the pathophysiological mechanism for LRRK2-linked diseases.

PP.175

Characterization of the potentiated synapse-specific PSD-95 interactome via activity-dependent in vivo expression of a proteomic probe

Mainardi M[1], Gobbo F[1], Jacob A[1], Faraone A[1], Sorokina O[2], Zentilin L[3], Marrone MC[5], Marinelli S[5], Cellerino A[1,4], Armstrong JD[2], Ori A[4], Cattaneo A[1,5]

[1]Laboratory of Biology "Bio@SNS", Scuola Normale Superiore, Pisa, ITALY; [2]School of Informatics, University of Edinburgh, Edinburgh, UK; [3]International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, ITALY; [4]Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), GERMANY; [5]European Brain Research Institute "Rita Levi-Montalcini" (EBRI), Rome, ITALY

The acquisition of new memories relies on synaptic plasticity. Changes in the quality and quantity of the protein content of dendritic spines, also via local translation, play a paramount role in this process (Nakahata & Yasuda, Front Syn Neurosci 2018; Aug 29). However, current approaches are inadequate for a systematic analysis of the in vivo potentiation-specific synaptic proteome (Dietrich & Kreutz, Mol Cell Proteomics 2016;15, 368). Recently, we developed the "SynActive" strategy to express any reporter protein specifically at potentiated synapses (Gobbo et al., Nat Comm 2017;8, 1629). Here, we exploited SynActive to express proteomic reporter baits to study the PSD-95 interactome – a hub for dendritic spine plasticity - of in vivo potentiated hippocampal synapses. We constructed a SynActive-controlled, FLAG-tagged PSD-95, which was expressed in the mouse hippocampus via AAV. Exposing mice to contextual fear conditioning triggered structural plasticity and concurrent production of SynActive-FLAGged PSD-95 at potentiated spines. This allowed immunoprecipitation of the potentiation-specific PSD-95 interactome, which was characterized by mass spectrometry. In a parallel control experiment we used constitutively expressed, FLAGged PSD-95. Comparative bioinformatics analysis of the two datasets allowed to isolate the molecular fingerprint of post-synaptic plasticity serving learning of a new behaviour, the first example of potentiation-specific interactome.Our results provide the proof of concept of a new approach to characterize the structural features of synaptic plasticity, with promising implications for advancing our knowledge not only on the physiology of learning and memory, but also on activity-dependent synaptic alterations at early stages of neurodegeneration.

PP.176

Quantifying barcode information content in dendritic spines of the rodent brain

Viggiano D[1], Perrone Capano C[2]

[1]Dept. Medicine and Health Sciences, Univ. Molise, Campobasso, Italy; [2]Department of Pharmacy, University of Naples "Federico II", Naples, Italy.

The study of the relationship between dendritic spine plasticity and memory employs spine counting methods or dynamic spine motility analysis. There are theoretical and practical limitations in this approach, mainly linked to the lack of information on the spine's distribution along the dendrite, that in turn dictates the pattern of connections between neurons. This protocol describes the application of non-linear techniques to extract the information content of dendritic spines from still images: the resulting new morphological parameter, entropy

and maximum Lyapunov exponent, are linked to adaptive changes of the brain, such as memory processes. Indeed, dendritic spine organization displays greater complexity after motor learning. Two approaches are here described, one based on entropy estimate of the spatial distribution of dendritic spines, which correlates with spine motility; the second is based on maximum Lyapunov exponent as an index of spine distribution complexity, which correlates with memory maintenance. These methods can be used for in vivo or ex-vivo preparations. Advantages of this technique are the possibility to identify regions of memory storage (the entire brain can be scanned using the ex-vivo preparation) and the higher sensitivity over spine-counting methods.On the mechanisms of GFR adaptation after protein load.

PP.177

The relevance of a standardized experimental context to assess recognition memory: realization of a novel modular behavioral apparatus

Gulisano W[1], **Tropea MR**[1], **Puzzo D**[1,2]

[1]Department of Biomedical and Biotechnological Sciences, Section of Physiology, University of Catania, Catania, Italy; [2]Oasi Research Institute (IRCCS), Troina, Italy

The object recognition test is a widely used behavioral test to asses learning and memory in mouse models, due to easy technical execution, the lack of aversive stressful stimuli and the possibility to repeat the test on the same mice. However, mice exploration is strongly influenced by the environment and the high variability of protocols used might critically affects the reliability of the results. To standardize the experimental context, we designed a customized behavioral apparatus by using the SolidWorks software. The apparatus consisted in a cage made by assembling aluminum profiles equipped with 3D-printed components to insert the Plexiglas panels constituting the arena. Lights and a webcam to record mouse behavior were allocated on the lid of the cage. We first tested different light intensities to assess the optimal environmental conditions. Next, we investigated the preference and/or discrimination capability for objects characterized by different colors, materials, shapes or dimensions. All experiments were compared with results obtained by using the classical arena. We found that mice tested by our customized behavioral apparatus presented a basal improvement of exploration time and a less variability in latency, i.e. the time needed to explore the object for the first time, and exploration time, suggesting a high repeatability of the experimental setting and an improvement of the context. Thus, our customized apparatus might be useful when designing a behavioral experiment in order to improve the scientific outcomes and minimize possible biases. Moreover, besides its low cost, the modular design can be easily applied to perform different behavioral tests when the exploration of an arena is required (e.g. open field test, novel object location test).

PP.178

Cellular mechanisms of time-dependent fear generalization

Concina G, **Renna A**, **Sacchetti B**

Department of Neuroscience, University of Turin, Italy

Fear experiences leave deep traces in our brain, forming a long lasting emotional memory. Inferring the most appropriate behavior in the presence of new situations, through their appropriate evaluation as safe or dangerous, represents a major challenge for animals, including humans. Fear generalization is a process in which defensive responses are displayed towards similar yet distinct stimuli with respect to those associated with an aversive experience. Recent works have demonstrated a time-dependent nature of fear generalization, consisting in a growth of fear responses as the time from the negative experience increases. In the present study we showed that fear generalization does not vary uniformly with the passage of time but rather that the level of precision of recent memories influences the growth of fear reactions. Given a recognized role of insulin-growth factor 2 in memory consolidation and enhancement, we reported a strong decrease in fear generalization after administering this protein in vivo. Critically, by analyzing the expression of the insulin-growth factor 2 receptors and the extracellular matrix structure of perineuronal nets, we defined possible cellular mechanisms accounting for both recent memory precision and over time fear generalization. Combined, our results provide new information on the neural mechanisms that underlie the complexity of fear generalization process. In particular we provided a link between memory precision at recent time interval and memory stability over time. Critically, the time-dependent growth of fear generalization characterizes fear disorders such as anxiety and phobias and from this perspective our project may offer new insights on the contribution of these processes to the development of such diseases.

PP.179

An Examination on the Possible Effects of Exogenous BDNF on AMPA Receptor GluR1 Subunit in Rat Hippocampus

Sırmatel B[1], Özer Ç[1], Seymen CM[2]

[1]Department of Physiology, Gazi University, Turkey; [2]Department of Histology and Embryology, Gazi University, Turkey

It is known that hippocampus plays a crucial role particularly in long-term memory and spatial perception processes and BDNF has an effect on the synaptic plasticity mechanism – the molecular process of learning and memory. Although previous studies have demonstrated the in vitro effects of BDNF on AMPA receptor, which plays a key role in hippocampal LTP, they have not examined its in vivo effects throughly. In this study, we investigated the long-term effect of exogenously administered BDNF on hippocampal AMPA receptors of female rats and their Morris Water Maze task performances. For this purpose, we infused BDNF (4 μg/day) on right Dentate Gyrus via osmotic mini pumps for 7 days and used MWM task to measure spatial learning and memory. Then we examined both right and left hippocampal AMPA receptor levels using IHC method and evaluated distribution and intensity pattern of AMPA receptor GluR1 subunit immunoreactivity using H-score. Compared to those in the control group, the BDNF infused rats spent significantly more time in the area of the pool where they were supposed to be during MWM probe trials. We also found out that BDNF protein administration significantly increased the GluR1 immunoreactivity in the right hippocampus. This increase was observed in the neuroplasm in addition to the neurolemma. Moreover we noted moderately increased immunoreactivity in the left hippocampus of these rats. This study demonstrates that long-term administration of BDNF increases the AMPA membrane levels and production of AMPA receptors in female rats. It also suggests that this may have a positive effect on spatial learning and memory formation. Our findings corroborate previously published data on hippocampal neuron culture.

PP.180

The relationship between the locomotor and cognitive decline during physiological aging in wild type mice

Ratto D, Occhinegro A, Di Iorio C, Bottone MG, Rossi P

Department of Biology and Biotechnology "L. Spallanzani", University of Pavia, Italy

Frailty is a geriatric syndrome associated with poor quality of life and negative health outcomes. To assess whether phenotypic frailty is associated with cognitive impairment, we studied, through spontaneous behavioral tests, locomotor and cognitive performances in wild-type mice during aging. To monitor the physiological aging, we chose five experimental times: T0 and T1 in adulthood phase, T2 in reproductive senescence, and T3 and T4 in senescence phase. To achieve a translational approach, we monitored locomotor and cognitive indicators comparable to phenotypic and cognitive frailty indicators in human. As cognitive performance, we studied recognition memory that is a form of declarative memory. Two functional distinct processes (knowledge and recollection), which are mediated by different structures in the medial temporal lobe, support recognition memory. Perirhinal cortex is involved in novelty recognition memory (knowledge), whereas hippocampus is involved in spatial one.Both locomotor and cognitive performances decline gradually during aging. In 3 months locomotor performances worsened by 38%, spatial recognition memory by 11% and novelty recognition memory by 37%. These data indicate that locomotor frailty is paralleled with lower performance in novelty recognition memory and therefore, we integrated the two frailty aspects in the Phenotypic and Cognitive Frailty Index. In conclusion, we suggest that when mice meet phenotypic frailty criteria they should be considered as mice at risk of cognitive decline.It would be interesting to correlate, through immunohistochemical analysis, these data with the expression of SIRT1, which plays an important role in cognitive functions and has function of protection against aging, neuronal degeneration and cognitive decline.

PP.181

Tau paired helical filaments and neuronal loss in an experimental model of metabolic syndrome

Lyutfi E[1], Stoyanov GS[2], Tonchev AB[3], Bratoeva K[4]

[1]Student, Faculty of Medicine, Medical University of Varna, Bulgaria; [2]Department of General and Clinical Pathology, Faculty of Medicine, Medical University of Varna, Bulgaria; [3] Department of Anatomy and Cell Biology, Faculty of Medicine, Medical University of Varna, Bulgaria; [4]Department of Physiology and Pathophysiology, Division of Pathophysiology, Faculty of Medicine, Medical University of Varna, Bulgaria

Recent studies have shown a tight relationship between tau pathology and neurodegeneration in cognitive impairment across the Alzheimer's disease

spectrum. The connection between the mechanisms of injury and neurodegeneration, leading to cognitive deficits in metabolic syndrome (MS) patients is still poorly understood. We evaluated the degree of neuronal damage and their relationship with the presence of Tau paired helical filaments (PHF) in an experimental model of fructose-induced MS. The study was performed on male Wistar rats (n=12) divided into a control (C) and fructose-fed (35%, 16 weeks) groups. Central nervous system (CNS) tissue specimens (paraformaldehyde fixed paraffin embedded) were cut into 7μm slides (ventrodorsal section) and fixed on glass slides for immunohistochemistry with Hexaribonucleotide Binding Protein-3 (NeuN) and anti-PHF-Tau. Digital slide cell counting analysis was performed automatically on the QuPath opensource software, with whole hippocampus cell counting and frontal, temporal and occipital cell counting over an area of 6 square millimeters each, while the PHF-Tau positive cells in the respective areas were quantified as an h-score. All fructose-fed rats developed obesity, hyperglycemia, hypertriglyceridemia and imbalance in anti-oxidative defense (defined by serum malondialdehyde and sulfhydryl groups), decreased neuronal count in all areas, most significantly in the temporal lobe ($p<0,05$) and hippocampal CA1 region ($p<0,05$), whereas PHF-Tau deposits were elevated in the temporal lobe ($p<0,05$) and the hippocampal CA1 ($p<0,05$) relative to the C group. The results indicate that the temporal lobe and hippocampal CA1 region might be the main affected regions of the CNS in fructose-induced MS. Acknowledgements: Bulgarian National Research Fund support, Project КП-06-ОПР03/11 from 18/12/2018

PP.182

Cytotoxicity, genotoxicity and apopitozis changes elicited by exposure of hippocampal cell line HT-22 cells to Cysteine-S-sulfate

Tekin V, Altintas F, Oymak B, Tunc Ata M, Burcu Tuzcu E, Kucukatay V

Department of Physiology, University of Pamukkale Denizli, Turkey

Sulfite is a toxic molecule by generated during the metabolism of sulfur-containing amino acidsin the body. Cysteine-S-sulfate, which is a metabolite of sulfite that is structurally similar to glutamate and other excitotoxic aminoacids, may also be responsible for the observed detrimental effects of sulfite. Since the precise mechanism of its action, the main purpose of this study was to investigate the in vitro effects of Cysteine-S-sulfate on cytotoxicity, Caspase 3 activity, and genotoxicitiy in HT_22 cell line.HT-22 cells were cultivated in DMEM with high sugar supplemented with 10% FCS and 2 mM L-glutamine at 37 C and 5% CO2. Toxicity studies using the WST-1 assay were conducted in 96-well plates at a density of 10,000 cells/well for 24 hours in the presence of 125 μM of SSC. The genotoxicity and apoptosis were measured by using the comet assay, caspase 3 activity, respectively. the tail intensity and tail length of the comets were used as the parameter for evaluation of DNA damage. The results of cytotoxicity assay clearly demonstrated significant concentration and time-dependent effects of SSC on HT-22 cell line. In terms of values of comet tail length and tail intesitiy, there was no difference between groups. We also found no difference in caspase 3 activity between groups. In point of these data, it can be speculated that SSC in the neuron's external milieu may cause excitotoxic cell death in the brain. However, this effect is not mediated by apoptosis and genototoxicity.

PP.183

Effects of Optogenetic and Chemogenetic Manipulations of Hypothalamic Kisspeptin Neurons on Hippocampal Astrocytes in Kiss-Cre Mice

Aguş S[1], Civas CC[1], Eyüboglu S[1], Yavuz Y[1], Baser O[1], Topcu IC[1], Bilgin VA[1], Atasoy D[3], Yilmaz B[1]

[1]Yeditepe University, Faculty of Medicine, Departments of Physiology, İstanbul, Turkey; [2]University of Iowa, Carver College of Medicine, Department of Physiology, Iowa City, USA

Objectives: We have investigated effects of acute (optogenetic) and chronic (pharmacogenetic) manipulations of hypothalamic arcuate nucleus (ARC) kisspeptin neurons on hippocampal astrocytes in female kiss-cre transgenic Alzheimer's Disease (AD) model mice. Methods: Female kiss-cre transgenic mice were used. AD model was induced by bilateral of infusion of amyloid-β in hippocampal dentate gyrus area. ChR2, iChloC2, hM3D receptor and hM4D receptor genes were injected intracranially into the hypothalamus by using adeno associated virus (AAV) for optogenetic (acute) and chemogenetic (chronic) manipulation of kisspeptin neurons. Clozopine-N-Oxide (CNO) was intraperitoneally administered to mice for one month to chronically activate and inhibit kisspeptin neurons. TdTomato AAV virus injected animals and non-virus injected wild type animals served as control groups. For acute optogenetic experiments, ferrule implantations were done. At the end, all mice were cardiac perfused, brains removed and sliced by vibrotome. Injection coordinates, presence of amyloid-β and glial fibrillary acidic protein (GFAP) were determined using immunofluorescent staining and confocal microscopy.Results: GFAP was increased in dentate gyrus, CA1, CA3 and cortex area in AD mice.

GFAP positive cells were significantly increased in chronic inhibition group animals compared to controls ($p<0.01$). In the optogenetic experiments, GFAP positive staining showed an increasing trend, but changes were not statistically significant. Conclusion: Our findings demonstrate that reactive astrocytosis was seenin AD mice and also pharmacogenetic inhibition of kisspeptin neurons in the kiss-cre transgenic mice.

Poster Session III (3/3)

Cellular Physiology

PP.184

Revealing regulation patterns of the coupled apical SLC15A1/PEPT1 and endosomal SLC15A4/PHT1 oligopeptide transport systems in enterocyte-like cell monolayers under insulin stimulation

Barca A[1], Mazzei A[1], Croce F[1], Del Vecchio G[1], Verri T[1]

[1]Department of Biological and Environmental Sciences and Technologies (DeBEST), University of Salento, Lecce, Italy

Insulin actions are intertwined with nutrient uptake in many tissues. Insulin sensitivity occurs, also, in enterocytes, through both apical and basolateral receptors. On GI epithelial apical membranes, insulin is able to stimulate expression of the oligopeptide transporter SLC15A1/PEPT1 which is a prototypical marker at the same time of the enterocyte-like differentiation and of the insulin-dependent effects on uptake dynamics and enterocyte maturation rearrangements. However, insulin effects on PEPT1 and GI are poorly investigated. Here, we evaluate the action of insulin on the expression of PEPT1 and on the SLC15A4/PHT1 endosomal counterpart, in Caco-2 intestinal cell monolayers at two different stages of spontaneous differentiation i.e. pre-differentiated (14 dps, days post seeding) and differentiated enterocyte-like cells (21 dps). We found differential regulation by insulin of the PEPT1 gene products depending on the differentiation stage, i.e. significant down-regulation in pre-differentiated monolayers vs. stabilized expression in differentiated monolayers. Regarding PHT1, its gene products were found up-regulated in differentiated cells, with insulin not exerting expression variations both in pre-differentiated both in enterocyte-like cells. But, remarkably, in the pre-differentiated insulin-treated monolayers we found that PHT1 expression levels are mainly due to an unproductive mRNA splice variant which in turn is not induced by insulin, totally, in mature monolayers. Overall, results suggest a coupled, splicing-mediated regulation of SLC15A1/PEPT1 and SLC15A4/PHT1 depending on insulin and differentiation stage, hinting a dual marker system for sensing the route of peptide absorption/turn-over and on-target/off-target effects of insulin on enterocytes. Key words: (3) enterocyte monolayer, insulin, SLC15 oligopeptide transporters

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A cell culture model of human mesothelium to study the physiology of water transport and biocompatibility of innovative glucose-sparing solutions for peritoneal dialysis.

Piccapane F[1], Arduini A[2], Corciulo R[3], Svelto M[1], Caroppo R[1], Procino G[1].

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Italy; [2]Department of R&D SAGL Corequest, Manno, Switzerland, [3]Department of Emergency and Organ Transplantation, University of Bari, Italy.

The three pore model postulates that the endothelium of peritoneal capillaries is the major limiting barrier for the transport of water during peritoneal dialysis (PD). We hypothesize that the mesothelium may represent an additional selective barrier to water diffusion in PD. We characterized an immortalized cell line of human mesothelium (HMC) to study the functional role of the water channel AQP1 expressed in vivo by mesothelial cells and also to test the biocompatibility of glucose-sparing PD solutions containing xylitol and carnitine as the main osmotic ingredients. Transepithelial water transport was measured by TEA^+-sensitive microelectrodes. The biocompatibility of conventional versus innovative PD solutions was evaluated by MTTtest, measurement of transepithelial electrical resistance (TEER) and production of proinflammatory cytokines. HMCs showed polarized expression of Na^+/K^+-ATPase and tight junctions markers but no endogenous expression of AQP1. HMC showed a low TEER compared to renal cells not expressing AQP1. However, the transepithelial water transport was comparable between the two cell types. Experiments in HMCs transfected with AQP1 cDNA, suggest that the water permeability of HMC is strictly dependent of the presence of AQP1. Biocompatibility assays indicate that conventional dialysis solutions with high glucose significantly reduce both cell viability and TEER and increase TNF-α and IL-6 production. Interestingly, innovative PD solutions minimize these effects. These results suggest that the mesothelium may represent an

additional selective barrier regulating water transport in PD. Importantly, we also demonstrated that the formulation of glucose-sparing PD solutions containing xylitol and carnitine better preserve mesothelial cells viability.

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The ERα L_{370} and E_{471} residues control receptor stability and E_2-induced gene transcription.

Bartoloni S, Leone S, Busonero C, Cipolletti M, Acconcia F

Department of Sciences, Section Biomedical Sciences and Technology, University Roma Tre, Rome, Italy

The hormone 17β-estradiol (E2) exerts its effects through the binding to the ligandactivated transcription factor estrogen receptor (ER). The E2:ER complex regulates several physiological processes including cell proliferation through transcriptional [i.e., estrogen responsive element (ERE)-based gene transcription] and non-transcriptional membrane-initiated effects. These ER activities relate to specific receptor structural determinants that allow E2-dependent effects. Through the inspection of cbioportal.org, we identified two natural ER point mutations (i.e., L370F and E471D) found in metastatic breast cancer (BC), which are located within the ER -E2 binding domain. Interestingly, L370 and E417 amino acids face each-other in the receptor 3D structure, thus suggesting their involvement in ER function. Thus, we generated these two ER point mutations and studied them in transient transfection experiments. Results demonstrated that ER point mutations L370F and E471D are less active in inducing E2-induced ERE-based gene transcription as they become less phosphorylated in response to E2. Interestingly, the E2induced transcriptional activity of the ER point mutant L370F and E471D cannot be prevented by classic ER inhibitors (i.e., 4OH-Tamoxifen and fulvestrant). Remarkably, turnover studies indicated that L370F and E471D ER point mutants appear more stable than the wild type ER . Thus, our results suggest that the ER amino acids L370 and E471 are involved in the structural control of receptor turnover and, consequently, E2induced transcriptional activity. Moreover, the L370F and E471D ER mutations are resistant to anti-estrogens, thus highlighting the necessity to better characterize them to identify novel anti-ER based BC drugs.

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Changes in the expression of autophagy markers in a rat model of retinopathy of prematurity

Pesce NA[1], Locri F[1], André H[2], Dal Monte M[1]

[1]Department of Biology, University of Pisa, Italy
[2]Department of Clinical Neuroscience, St Erik Eye Hospital, Karolinska Institute, Sweden

Autophagy is an essential process in maintaining the normal cellular homeostasis and energy balance under physiological conditions. In retinal diseases, autophagy helps retinal cells defend themselves against harmful stress; however, excessive autophagy may result in retinal deterioration. Therefore, modulating autophagy may provide an alternative therapeutic strategy to treat retinal diseases. Retinopathy of prematurity (ROP) is a human vascular disease characterized by the formation of neovascularization in the premature newborn retina. There is only limited information on the role of autophagy in ROP. Here, we used a rat model of oxygen-induced retinopathy (OIR), an acknowledged model of ROP, to evaluate changes in the expression of key mediators of autophagy induced by the disease. In this model, rat pups are exposed to alternating cycles of 50% and 10% oxygen for 24-h for the first 14 days followed by exposure to the room air until post-natal day 18. The expression of autophagy markers during retinal development from birth to post-natal day 18 was assessed at transcript and protein levels by real-time PCR and Western blot, respectively. In addition, the phosphorylation status of signaling molecules known to activate or to repress autophagy was investigated. Overall, our results showed that, in control rats, the autophagic flux decreased over time during post-natal development. In contrast, in OIR rats the levels of autophagic markers transiently increased at post-natal day 7 to then gradually decrease until post-natal day 18. The present findings sustain the role of dysregulated autophagic processes in the pathogenesis of ROP and point to the targeting of autophagy as a potential treatment for neovascular eye diseases.

PP.188

Effects of thyroid hormones and analogues in the immune system cells

Gionfra F[1], Pallottini V[1], Incerpi S[1]

[1]Department of Sciences, University Roma Tre, Rome, Italy

Accumulating evidence suggests a close bidirectional communication and regulation between the neuroendocrine and the immune system. Thyroid hormones (THs) are important regulators of metabolism, differentiation and cell proliferation but can also modulate several inflammation-related processes such as chemotaxis, reactive oxygen species generation and cytokines production in immune cells.

These effects involve genomic mechanisms, mediated by specific nuclear receptors (TR) as well as nongenomic mechanisms, through the membrane receptor integrin Vβ3. Integrin Vβ3 has a large number of extracellular protein ligands, including growth factors and extracellular matrix proteins, and upon thyroid hormone binding can activate both MAPK and PI3K signaling cascades. We carried out experiments of cell migration by transwells on THP-1 leukemic monocytes and scratch test on BV-2 mouse microglia together with proliferation curves, cytotoxicity assay by MTT and ROS determination by the intracellular fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA). Our data show that THs through integrin Vβ3 modulate responses typical of immune cells in THP-1 and BV-2 cells, in the presence of inflammatory mediators such as MCP-1 and LPS. Moreover bisphenol A (BPA), a monomer of plastic materials, binds integrin Vβ3 interfering with the actions of THs as a hormone antagonist. A more assessed knowledge on the role of THs and their interaction with immune system cells through integrin Vβ3 could be useful to find possible therapeutic tools as anti-inflammatory agents.

PP.189

Large-scale analysis of protein networks involved in oligodendrocytes differentiation reveals novel key regulators of the differentiation program

Vergara D[1,2], **Damato M**[1,2,3], **Musarò D**[1,2], **Fournier I**[3], **Salzet M**[3], **Maffia M**[1,2]

[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy [2]Laboratory of Clinical Proteomic, '"Giovanni Paolo II"' Hospital, ASL-Lecce, Italy [3]Laboratoire PRISM: Protéomique, Réponse Inflammatoire, Spectrométrie de Masse, - U1192 INSERM, Bât SN3, , Université de Lille 1, Villeneuve d'Ascq, France

Multiple biological cues collaborate at the cellular level to direct cell fate during differentiation. Cells actively respond to these extracellular and intracellular stimuli by the activation of a plethora of biochemical networks. In this scenario, systems-level approaches have emerged as essential tools for identifying new molecular pathways and underlying protein members. Here, we applied a label-free mass-spectrometry technique to profile the proteome modifications associated with differentiation in a human oligodendrocyte precursor model. In response to PKC activation, we identified significant changes of modules enriched with proteins related to neurogenesis, cell differentiation, ion channels, metabolism and cytoskeletal remodelling, indicating that precise regulatory mechanisms govern the process of differentiation. Major pathways critical for oligodendrocytes differentiation were identified, as the functional perturbation of specific protein kinases involved in the regulation of cytoskeletal dynamics, abolished the PKC-induced differentiation program. Overall, we delineated a proteome regulatory network signature of the physiological response to PKC activation, and provided a mechanistic insight into the functional role and interactions of multiple regulatory networks during oligodendrocytes differentiation.

PP.190

Novel 17β-estradiol pathways necessary for neuroglobin-induced cell survival.

Fernandez VS, **Pescatori S**, **Fiocchetti M**, **Marino M**

Department of Science, Roma Tre University, Rome, Italy

We recently demonstrated that, neuroglobin (NGB), a heme protein, functions as a compensatory protein of the steroid hormone 17β-estradiol (E2) protecting cells, including cancer cells, against the apoptotic death induced by oxidative stress. However, the E2-induced signaling pathways at the root of NGB over-expression and its mitochondrial re-localization are still elusive. By using a kinase screening library, here, we analyzed the possible pathway activated upon E2:ER bond. The screening performed with 87 inhibitors of 22 kinase demonstrated the implication of several proteins involved in the E2effect. Among other kinases, we demonstrated that PI3K/AKT and the transient activation of p38β kinase proved to be essential for the upregulation of the NGB levels. Moreover, both kinases were involved in E2-induced NGB accumulation to the mitochondria. The results demonstrated that p38 activation was down-stream AKT activation. High level of NGB into mitochondria are necessary for the pro-survival and anti-apoptotic effect of this globin in cancer cells. As a whole, these results underline the E2 triggered pathways in E2-responsive cells that involve NGB as a compensatory protein devoted to cell survival.

PP.191

PKA signaling cascade regulation in cancer

Grisan F.[1,2], **Iannucci L.F.**[3,2], **Di Benedetto G.**[1,2] **Lefkimmiatis K.**[1,2]

[1]Institute of Neuroscience, CNR , Padova ,Italy; [2]Veneto Institute of Molecular Medicine, Padova, Italy; [3] Department of Biology, University of Padova, Italy

Protein Kinase A (PKA), the main effector of the second messenger cyclic AMP (cAMP), is involved in several cellular processes from migration to cell metabolism and transcription. It is not surprising that PKA dysfunction is involved in various human pathologies including cancer, where a number of mutations that result in aberrant PKA activation have been identified. However, activating PKA mutations are present only in a limited array of cancers, opening the possibility that PKA may be differentially regulated in tumors that express its wild type form. To address this question we characterized cAMP/PKA signaling in a human colon adenocarcinoma cell line (HT29) often used as model studying cancer. We used a comprehensive approach combining single cell imaging and biochemistry. Western Blotting (WB) confirmed normal expression levels of all PKA subunits. However, PKA-dependent phosphorylation measured using FRET-based sensors, was nearly undetectable in the cytosol of HT29 cells, albeit normal cAMP levels. Unexpectedly, WB detected PKA activity in total cell lysates suggesting that PKA-driven events may be compartmentalized. FRET sensors targeted on different cell compartments confirmed that PKA-dependent phosphorylation was higher at the endoplasmic reticulum, outer mitochondrial membrane and plasma membrane than in the cytosol. Further experiments demonstrated that PKA activity in the soluble fractions of HT29 is kept low by the actions of resident phosphatases that are less able to dephosphorylate PKA targets tethered on membranous organelles due to stereoscopic impediments. We suggest that in cancer cells lacking PKA mutations this protein may be regulated at the functional level by modulators of the cAMP signaling cascade such as phosphatases and phosphodiesterases.

PP.192

Two novel SCN5A loss-of-function mutations affect patients with severe arrhytmogenic syndromes.

Murano C[1], Binda A[1], Lucano D[1], Micaglio E[2], Ciconte G[2], Ghiroldi A[3], Anastasia L[3,4], Pappone C[2], Rivolta I[1].

[1]School of Medicine and Surgery, University of Milano Bicocca, Monza [2]Arrhythmology Department, Scientific Institute for Research, Hospitalization, and Health Care (IRCCS) Policlinico San Donato, San Donato Milanese [3]Laboratory of Stem Cells for Tissue Engineering, Scientific Institute for Research, Hospitalization, and Health Care (IRCCS) Policlinico San Donato, San Donato [4]Department of Biomedical Sciences for Health (L.I.T.A.), University of Milano

SCN5A gene encodes for the subunit of the cardiac isoform of the voltage gated sodium channel. Mutations in this gene have been correlated with several arrhytmogenic syndromes among which Long QT Syndrome (LQTS) type 3 and Brugada Syndrome (BrS) are the most studied. Here we describe two novel mutations related to these phenotypes. The c.86_87delinsTG causes the substitution of Ala 29 in the N-terminus of the protein with a Val and was found in a 36 years old man diagnosed with BrS. The mutation c.5089T>C substitute the Phe 1697 with a Ser in the intramembrane pore forming region and was found in a 45 years old woman diagnosed with LQTS. Whole cell patch-clamp studies on HEK293 cells revealed that both the mutations are responsible for a loss-of-function of the protein. In particular the A29V completely abolished the inward current. The F1697S mutation reduced the current density (-183.1±37.1 pA/pF, n=37 in WT vs -107.3±33.7 pA/pF, n=29 in F1697S; $p<0.01$) and caused a significant negative shift in the half-maximal voltage (V1/2) of steadystate inactivation curve and a positive one in the V1/2 of the activation curve (-8 and +6.4 mV, respectively). These gating changes caused a shift and a reduction in the window current that may markedly modify the action potential duration. Moreover, F1697S substitution slowed down the recovery from inactivation, being 8.1±0.2 ms (n=27) in WT and 12±0.4 ms (n=17) in F1697S the time necessary for the recovery of the 50% of the channels. These findings suggest that both the mutations strongly reduce the sodium inward current anticipating a higher risk of arrhythmogenesis for the patients. Still more has to be done in order to explain the clinical phenotype of LQTS in light of a loss-of-function of the F1697S Nav1.5 channel.

PP.193

Aquaporin-9 (AQP-9) is involved in the lipid-lowering activity of the nutraceutical silybin on hepatocytes through modulation of autophagy

Vergani L[1], Baldini F[1], Grasselli E[1], Voci A[1], Khalil M[1], Portincasa P[3], Gena P[4], Calamita G[4]

[1]Dept. of Earth, Environment and Life Sciences, and [2]Dept. of Experimental Medicine, University of Genova, Italy; [3]Dept. of Biomedical Sciences and Human Oncology, [4]Dept of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Italy.

Hepatic steatosis and non-alcoholic fatty liver disease (NAFLD) originate from increased uptake of circulating long chain fatty acids (FA) in the hepatocyte. In the liver, excess FAs are then esterified to triglycerides (TG) and stored as lipid droplets (LD). NAFLD prevalence is increasing worldwide, and can progress to non-alcoholic steatohepatitis (NASH), cirrhosis, and

hepatocellular carcinoma. TNF is a key mediator of this progression. In clinical studies, the nutraceutic silybin was beneficial in histologically proven NAFLD. Here, we studied the molecular mechanisms through which silybin (Istituto Biochimico Italiano, Italy) improves hepatic lipid dyshomeostasis in an in vitro model of NAFLD progression induced by sequential exposure of FaO hepatoma cells to FA and TNF . Interestingly, in both models the lipid lowering activity of silybin was found to be associated to: (i) upregulation of AQP9, the aquaporin glycerol channel that mediates the uptake of glycerol by hepatocytes; (ii) increase in glycerol permeability; (iii) reduction in the fat-stimulated autophagy through reduction of LC3II and Atg7 levels; (iv) stimulation of mitochondrial FA oxidation through upregulation of very long-chain acyl-CoA dehydrogenase (VLCAD) and uncoupling protein 2 (UCP2) expression, and stimulation of Cytochrome C oxidase (COX) activity. Silybin also modified the profile of FA stored in lipid droplets by upregulating the stearoyl CoA desaturase (SCD1) mRNA expression resulting in increased levels of short/medium chain FA and decreased saturated/monounsaturated FA ratio. The hepatoprotective effects of silybin point to involvement of signalling pathways intersecting lipid metabolism, autophagy, and AQP9 function.

PP.194

Physio/mechanical challenges regulate Schwann cell changes by Nf2/merlin signaling and DNA methylation

Colciago A.[1], Bonalume V.[1], Castelnovo LF.[1], Ballabio M.[1], Caffino L.[1], Fumagalli F.[1], Magnaghi V.[1]

[1]Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy

Physio/mechanical challenges, such as environmental epigenetic cues, may be pathogenically relevant for several mammal cells and tissues. Schwann cell (SC), the main glial cells forming myelin in the peripheral nervous system, express the Neurofibromin type 2 gene (Nf2). It encodes the tumor suppressor protein merlin, a cytoskeleton-associated protein regulating cell proliferation and survival. Nf2/merlin inactivation causes protein loss and leads to SC transformation into a form of benign tumor called schwannoma. Moreover, Nf2/merlin is mutated in an autosomal dominant multiple syndrome, called neurofibromatosis type 2. We recently showed that the exposure to electromagnetic fields (EMFs) causes SC oncotransformation. EMFs induce changes in SC Nf2/merlin expression, cell migration, chemotactic responsivity and cytoskeleton reorganization. We showed a downstream MAPK/Erk activation, involved in SC proliferation, as well as activation of Hippo/YAP signalling commonly altered during tumorigenesis. We also showed that some genes, known to be upstream or downstream mediators of Hippo (Amotl2, Dchs, Fat, Wnt1) were changed. Further studies on rat SC oncotransformation following acute EMF exposure (0.1 T, 50 Hz, 10 min) demonstrated that the number of cells in G1 phase was increased. Focus forming analysis, after repeated exposures, showed an increase in 3D SC growth. EMF affects also the SC epigenome, as total DNA methylation, de novo DNMT and HDAC were reduced. Furthermore, RT2-profile assay evidenced that genes crucial for SCs are upregulated in EMF exposed cells. Overall, we identified some mechanisms responsible of environmental-induced SC changes toward a proliferative/migrating state, which may be pathologically relevant for nerve neoplastic development.

PP.195

Gain of function of TTX-sensible voltage dependent sodium currents promoted by Vitamin D3 induced hippocampal embryonic neuronal differentiation.

Monarca L[1], Ragonese F[2], Bastioli F[1], Cataldi S[1,3], Arcuri C[2], Mancinelli L[1], Iannitti RG[1], Albi E[3], Fioretti B[1]

[1]Department of Chemistry, Biology and Biotechnologies, University of Perugia, Italy; [2]Department of Experimental Medicine, University of Perugia, Italy; [3]Department of Farmaceutical Sciences, University of Perugia, Italy

Our previous data show that chronic application (100 nM, 72 h) of Vitamin D3 (VD3, Cholecalciferol) induced morphological and mitochondrial activity associated with neuronal hippocampal differentiation in HN9.10e cell line. Electrophysiological recording in wholecell dialyzed configuration shows that VD3 treatment induced a gain of function of the tetrodotoxin-sensible voltage dependent sodium (NaV-TTXs) current with a shift of half voltage activation to more negative potential (gain on function) about 10 mV. Mitochondrial activity of the cell body, estimated at the single cells levels by using nernstian dye TMRM (Tetramethylrhodamine, Methyl Ester), was reduced in neuronal cell treated with VD3. Similar results were obtained by estimating the formation of formazan crystals, due to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) metabolism, at the single cell level. Mitochondrial activity was observed along with neurite-like structures in VD3 treated cells. The reduction of the cell body mitochondrial activity could be partially explained by a redistribution of the mitochondria, by a reduction of their membrane potential and by a modulation of their biogenesis. TTX (tetrodotoxin) 1 M abolished morphological changes and somatic mitochondrial activity reduction induced by VD3 treatment. Altogether, these data suggest that the gain on function

of NaV-TTXs current is required for metabolic and morphological changes promoted by VD3 in embryonic neuronal differentiation. GRANT: SIR 2014 to Fioretti Bernard. KEY WORDS: Voltage dependent sodium current, hippocampal cells, vitamin D3

PP.196

Gaseous mediators in gastrointestinal physiology, pathophysiology and pharmacology

Bakalarz D[1,2], Wójcik D[1], Magierowska K[1,3], Korbut E[1], Danielak A[1], Janmaat V[3], Ginter G[1], Mateusz Wierdak M[1], Kwiecień S[1], Śliwowski Z[1], Brzozowski T[1], Magierowski M[1,3]*

[1]Department of Physiology, Jagiellonian University Medical College, Krakow, Poland [2]Department of Forensic Toxicology, Krakow, Poland [3]Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, The Netherlands

Hydrogen sulfide (H2S) and carbon monoxide (CO), produced by CTH/CBS/MPST or HMOX enzymatic activity and released from pharmacological donors prevent gastrointestinal (GI) mucosa against erosions induced by various damaging factors such as nonsteroidal anti-inflammatory drugs (NSAIDs), alendronate, ethanol or stress. H2S and CO exert anti-inflammatory and anti-oxidative activity modulating NFkB, Nrf-2 or HIF-1 -dependent pathways. We have reported that CO and H2S accelerate gastric ulcers healing and that the beneficial effects of H2S depend on the endogenous CO production. These gaseous mediators were shown to regulate gastric blood flow and to affect the expression of enzymes involved in endogenous prostaglandins biosynthesis. Importantly, the gastroprotective effect of these molecules has been already implemented in clinical pharmacology since new derivatives of H2S-releasing NSAIDs have been reported to exert better antiinflammatory action and reduced gastrotoxicity. Therefore, endogenous H2S and CO are crucial components of GI barrier physiology with possible implementation to GI pharmacology. However, it still remains unknown if H2S is involved in the pathophysiology of Barrett's esophagus (BE), a premalignant condition of esophageal epithelium caused by gastroesophageal reflux disease. Therefore, we have investigated therapeutic potential of H2S in the development of esophageal metaplasia based on in vivo animal and in vitro models with human-derived esophageal cell lines. We have used novel H2S releasing prodrugs and the genome editing within CTH and CBS genes by CRISPR/Cas9 technique. Funding sources: The National Centre for Research and Development(LIDER/9/0055/L8/16/NCBR/2017),National Science Centre (UMO-2016/23/D/NZ4/01913)

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Efficacy of Echinomycin in hypoxia-inducible factor-mediated ocular angiogenesis

Plastino F[1], Santana Garrido Á[1,2], Aronsson M[1], Mate Barrero A[2], Kvanta A[1], Vázquez Cueto CM[2], André H[1]

[1]Department of Clinical Neuroscience, St. Erik Eye Hospital, Karolinska Institutet, Stockholm, Sweden; [2]Department of Physiology, University of Seville, Spain

Echinomycin (EKN), an inhibitor of hypoxia-inducible factor (HIF)-1 DNA-binding activity, has been implied as a possible therapeutic agent in ischemic diseases. Here, we assess EKN in hypoxia-driven responses in vitro using human primary adult retinal pigment epithelium cells (aRPE), and in vivo using the laser-induced mouse choroidal neovascularization (CNV) model. aRPE were kept at normoxia (20% O2) or exposed to hypoxia (1% O2), in the presence or absence of EKN. Epithelial wound-healing was used to determine the dose of EKN for our experiments. Effects of EKN on hypoxia-mediated pathways were analyzed by western blot for HIF-1 protein, quantitative PCR of HIF-target genes, and proteome array for soluble angiogenic factors. aRPE treated with 5pM of EKN showed hypoxia-dependent significantly decreased cell proliferation in the wound-healing assay. A lower protein expression of HIF-1 concomitant with lower HIF-mediated transcripts were detected in hypoxic aRPE cells treated with EKN compared with nontreated controls. These results were confirmed by proteome profiler for angiogenic factors. 8-week-old C57BL/6J mice underwent laser-induced CNV, as a model of HIF-associated ocular neovascularization. Animals were treated on days 3 and 6 post-laser with recombinant mouse VEGFR1-Fc chimera protein or EKN, and compared to vehicle-treated controls. CNV lesion area was determined on day 9, by fluorescein angiography. Laserinduced mice treated with 1 g of intravitreally injected EKN showed significantly decreased vascular lesion area. Our data suggest EKN as a potent inhibitor of HIF-mediated angiogenesis in retinal cells and in the mouse model of CNV, which could have future implications in the treatment of patients with age related macular degeneration (AMD).

PP.198

Ghrelin influence on neural differentiation of Adipose-derived mesenchymal Stem Cells

Lo Furno D

Department of Biomedical and biotechnological sciences, University of Catania, Italy

Previous studies indicate that a conditioned medium (CM) from glial cells, such as Schwann cells (SCs) and Olfactory Ensheathing Cells (OECs) can promote a neural differentiation of Adipose-derived mesenchymal Stem Cells (ASCs). In the present study, the effects of Ghrelin (Ghre) were tested on ASCs, as this hormone is able to improve the growth and differentiation of cultured cells. In the central nervous system, Ghre has been mainly observed in specific populations of hypothalamic neurons. However, its effects involve other nervous structures, such as amygdala and hippocampus. ASCs were isolated and expanded after collagenase digestion and centrifugation of raw lipoaspirates. Different culture conditions were tested. ASCs grown in the basal medium and two samples, grown in OEC-CM or SC-CM were considered as controls. In three other corresponding ASC samples, Ghre (2 M) was added. At day 1, 3 and 6 of growth, cells were tested by immunocytochemistry to detect the expression of some neural markers, such as PGP 9.5, MAP2 and GFAP. Results showed that all these markers were overexpressed when ASCs were cultured in OEC- or SC-CM. The combination of CM and Ghre further improved these increases. On the other hand, the addition of Ghre alone produced weaker effects. In general, these effects were clearly visible already at day 1 and were more evident at day 6. Overall, it is confirmed that the use of a glial CM may induce a neural differentiation of ASCs. Although Ghre alone was not capable of pronounced effects, its addition facilitated neural ASC differentiation. This study provides information on the action of Ghre, being able to modulate cell growth and differentiation.

PP.199

Spatiotemporal coordination of nuclear cAMP signals via EPAC1

Iannucci LF[1,3], Grisan F[1,2], Quotti Tubi L[1], Scorrano L[1,3], Di Benedetto G[1,2], Piazza F[1], Lefkimmiatis K[1,2]

[1]Foundation of Advanced Biomedical Research, Veneto Institute of Molecular Medicine,Padua, Italy, [2]Neuroscience Institute of Italian National Research Council, Padua, Italy, [3] Department of Biology, University of Padua, Italy

Membrane-less organelles arise from the condensation of cellular material, mainly proteins and/or nucleic acids, throughout a liquid-liquid phase separation process. These structures act as organizational centers that favor biochemical reactions exploiting the spatial proximity of the implicated molecules. Considering their functional importance, it is not surprising that they have been found to be implicated in many diseases, ranging from cancer to neurodegeneration. However, the triggers as well as, the molecular components of membrane-less organelles remain poorly understood. Here we present strong evidence that the exchange protein directly activated by cAMP (EPAC1) can generate membrane-less organelles within the nucleus in response to cAMP-generating stimuli. While the cytosolic moieties of EPAC1 responded to cAMP as expected, by moving to the plasma membrane, we noted that this relocation of the cytosolic pool did not affect the nuclear EPAC1 moiety which responded to cAMP by forming reversible nuclear body-like structures (EPAC1-NBs). Immunofluorescence and biochemical approaches strongly suggested that EPAC1-NBs interact with the promyelocytic leukemia protein (PML), a well-known tumor suppressor and the main component of the PML-nuclear bodies. In view of this finding, screening of leukemia cell lines revealed that EPAC1 is overexpressed in a diffuse large B-cell Lymphoma model. Thus, our data uncover a possible EPAC1 role in lymphoma and a yet undescribed phenomenon able to relay cAMP-encoded information to the nucleus.

PP.200

p53 system at the molecular crossroad regulating apoptosis/autophagy balance: natural bioactive compounds as small molecule killing tumours and dual targeting prodrug strategy

Catalani E[1], Buonanno F[2], Zecchini S[3], Coazzoli M[4], Giovarelli M[4], Perrotta C[4], De Palma C[3], Clementi E[3,4], Marcantoni E[5], Ortenzi C[2], Fausto AM[1], Picchietti S[1], Cervia D[1].

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Viterbo, Italy; [2]Laboratory of Protistology and Biology Education, Department of Education, Cultural Heritage and Tourism, University of Macerata, Italy; [3]Unit of Clinical Pharmacology, University Hospital "Luigi Sacco"-ASST Fatebenefratelli Sacco, Milano, Italy; [4]Department of Biomedical and Clinical Sciences "Luigi Sacco" (DIBIC), University of Milano, Italy; [5]School of Sciences and Technologies, Università degli Studi di Camerino, Italy.

Due to its pathophysiologic significance, the apoptosis/autophagic machinery is a promising target for new chemotherapeutic agents. Using both in vitro and in vivo techniques we showed that climacostol, a small organic product of the ciliate Climacostomum virens, has potent cytotoxic/pro-apoptotic effects, positively targeting the tumour suppressor p53. It exerts a sustained accumulation of autophagosomes in cells that are committed to die by apoptosis. Climacostol affects autophagosome turnover via p53-AMPK axis, although the mTOR pathway also plays a role. The up-regulation of p53 in the nuclei couples to p53 stability

(phosphorylation at Ser15 site). Of interest, climacostol effects on autophagy and apoptosis are two separate events acting on life/death decisions of the cell. Since the p53 system is at the molecular crossroad regulating the autophagy impairment of climacostol and its role in apoptosis, it is important to explore the dual targeting of autophagy and apoptosis (via p53) for the selective killing of tumours. Thus, we synthesised three novel analogues of climacostol finding that the introduction of a methyl or a hydroxyl moiety to the aromatic ring effectively modulates its potency and mechanism of action. Also, we noticed that the methoxymethyl ether protecting group allowed us, thanks its easy removal in a weakly acidic environment, to optimise the synthesis of climacostol. The protected molecule (MOMO) was then analysed for its biological behaviour, since the pH control of drug responses is of great interest in the biomedical field. Here we provide a proof-of-concept study on the acidic pH-activation of MOMO to identify a new prodrug strategy for the generation of pH-sensitive small molecules as pharmacologically active cytotoxic compounds

PP.201

The Effect of Thymoquinone on Autophagy-related Proteins in 6-hydroxydopamine-Induced Neurodegeneration

Tuzcu Gürkan F[1] , **Bilgiç T**[1], **Şahbaz U**[1], **Birim D**[2] , **Ertürk M**[3] , **Armagan G**[2] , **Dağcı T**[1],

[1]Department of Physiology, Ege University, Bornova, Izmir [2]Department of Biochemistry, Ege University, Bornova, [3]Department of Anatomy, Ege University, Bornova.

Objective:Parkinson's disease(PD) is the second most common neurodegenerative disease in the world. Pathogenesis of neurodegeneration includes abnormally folded protein aggregation, inflammation, dysregulation in cell death mechanisms. Increased autophagy activity is observed in substantia nigra in PD patients. Thymoquinone(TQ), an active ingredient obtained from Nigella Sativa, has been shown to have antiinflammatory, antitumor and antioxidant activities. The neuroprotective effects of TQ were shown in animal models of PD. In this study, we aimed to examine the effects of TQ on autophagy related proteins in in-vitro model of dopaminergic damage. Method:In our study, 6-hydroxydopamine(6-OHDA) was used to induce dopaminergic damage in SH-SY5Y cells. Following dose-response and timeresponse studies, cells were pretreated with thymoquinone at 2.5 µM for 1 h and then with 100µM 6-OHDA for 6 h. The effects of treatments on autophagy proteins (beclin1, LC3, ATG-5, ATG-7, ATG-16) were examined by Western Blot. The data were analyzed by one way ANOVA and Tukey tests. Results:After 6 hours of 6-OHDA exposure, there was a significant increase in ATG7, ATG-16 and LC3A/B levels compared to untreated cells($p<0.05$). Moreover, a significant decrease in ATG-5, ATG-7 and ATG-16($p<0.05$) protein levels were observed in TQ group compared to 6-OHDA group. In addition, a tendency of decreasing in beclin-1 and LC3A/B levels were found following TQ treatment against 6-OHDA. Conclusion:Autophagy is important for the degradation of dysfunctional cellular components. The regulation of autophagy by natural compounds may be used as a potential therapeutic strategy for neurodegenerative diseases.This study was conducted within the scope of Ege University Scientific Research Project No. 18-TIP006.

PP.202

Alternations of the alpha2 Na,K-ATPase Observed in Distinct Mice Models of Myodystrophy

Kravtsova V[1], **Bouzinova E**[2], **Matchkov V**[2], **Krivoi I**[1].

[1]Department of General Physiology, St. Petersburg State University, Russia; [2]Department of Biomedicine, University of Aarhus, Denmark

The Na,K-ATPase (NKA) is a membrane transporter critically important for excitability, electrogenesis and contractility of skeletal muscle, where the alpha1 and alpha2 isoforms of NKA are expressed. Mdx and Bla/J mice are the experimental models of myodystrophy, specifically of Duchenne muscular dystrophy and dysferlinopathy, respectively. The molecular mechanisms behind myodystrophy are of therapeutic importance; however the role of NKA in these dysfunctions has not previously been addressed in detail. This study examines the function of the alpha1 and alpha2 NKA isozymes in diaphragm muscle of mdx and Bla/J mice compared to control C57Bl/6 mice. We used conventional electrophysiology, quantitative PCR and Western blotting as well as confocal microscopy with cytochemistry. In both mdx and Bla/J mice muscle fiber membrane was depolarized due to specific loss of the alpha2 NKA electrogenic activity. These disturbances were most pronounced in the motor endplate membrane region, where the alpha2 NKA localization was also strongly altered. However, in contrast to Bla/J mice, the alpha2 NKA protein content as well as mRNA expression was specifically and significantly lowered only in mdx mice. Duchenne muscular dystrophy and dysferlinopathies differ fundamentally in their molecular mechanism. Nevertheless, both mdx and Bla/J mice are characterized by similar abnormalities in membrane localization and impaired function of the alpha2 NKA that can be resulted from adaptive skeletal muscle remodeling following chronic motor dysfunction. This

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PP.203

Involvement of Chloride Channels in Alveolar Cells Response to Oxidative Stress

Canella R[1], Benedusi M[2], Martini M[1], Cervellati F[1], Valacchi G[1-3]

[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy. [2]Department of Biomedical and Specialist Surgical Sciences, University of Ferrara, Italy. [3]Department of Animal Science, Plants for Human Health Institute, NC State University, Kannapolis, North Carolina, USA

Background: Our previous studies have analyzed the effects of O3, one of the most harmful pollutants for the respiratory system, on human lung epithelial cells Cl- currents. Ozone exposure significantly alters the flow of Cl- ions, inducing an outward rectifier effect. Among the different types of Cl- channels of the cell membrane, ClC-2 and ORCC (Outward Rectifier Chloride Channel) seemed the possible mediators of the O3-induced response although the channel involved in this response was not univocally identified. Aims: In the present work, by the use of siRNA technique we down regulated the expression of the gene coding for the functional part of ORCC and ClC-2 and Cl- current was analyzed upon O3 challenge by patch clamp technique. Results: We observed that the enhancement of the current disappears when ORCC was silenced. Surprisingly, even under control conditions, a consistent part of the Cl- current was due to the activity of this channel, usually inactive in the absence of stimulation. Silincing ClC-2 and patch clamp experiments indicated its important contribute to the overall membrane Cl- current in control. Discussion and Conclucion: This results allowed us to unequivocally affirm the involvement of ORCC channel in the response of pulmonary epithelial cells to oxidative stress. With the same techniques we will be able to define the role of the ClC-2 channel. These findings open the possibility of identifying the biomolecular changes undergoing the O3 effect and open the way to similar studies on other types of channels or tissues directly exposed to atmospheric pollutants, such as skin cells.

PP.204

Anti-oxidant and anti-steatotic effects of fucoidans isolated from marine algae and terrestrial plants

El Rashed Z[1], Khalife H[2], Kanaan H[2], Allaria G[1], Voci A[1], Grasselli E[1], Canesi L[1], Demori I[1]

[1]Department of Earth, Environmental and Life Sciences, University of Genova, Italy; [2]Department of Biology, Lebanese University, Lebanon; [3]Laboratory of Chemical Synthesis and Extraction of Polysaccharides from Seaweed, Faculty of Pharmacy, Lebanese University, Lebanon

Fucoidans (FUs) are fucose-rich sulfated polysaccharides very abundant in brown algae such as Cystoseira compressa. FUs have been studied for their various biological effects in mammalian cells. Recently, the presence of FUs has been identified also in terrestrial plants, i.e. Eucalyptus globulus and Ferula hermonis, growing in Lebanon. In this work, the anti-oxidant and anti-steatotic effects of FUs from marine and terrestrial sources were compared. FUs were purified from C. compressa, E. globulus and F. hermonis by extraction with HCl and subsequent neutralization with NaHCO3. Chemical characterization was done by FTIR spectroscopic analysis. Anti-oxidant activity was determined by DPPH test. Steatotic rat hepatoma FaO cells (obtained by 3 h exposure to an oleate/palmitate mixture) were used as an in vitro model of hepatic steatosis and exposed to 50 μg/ml FUs for 24 h. The anti-steatotic effect of FUs was investigated by measuring intracellular triglyceride (TG) content and by detecting lipid droplet formation through fluorescence microscopy. The expression of PPAR (Peroxisome Proliferator Activated Receptor) isoforms, that play an important role in lipid homeostasis, was evaluated by qPCR. Our results show that FUs isolated from all three species exhibited significant DPPH scavenging activity and reduced excess TG content in steatotic FaO cells. Moreover, FUs purified from terrestrial plants exerted stronger anti-oxidant and anti-steatotic effects than those obtained from marine brown algae, with E. globulus FUs showing the highest activities. The identification of FUs and the demonstration of their powerful anti-oxidant and antisteatotic abilities confirms Eucalyptus as one of the plant genus with most potential pharmacological properties worldwide.

PP.205

Vesicle-Cloaked Virus Clusters Are Optimal Units for Inter-organismal Viral Transmission: Focus on the lipid analyses.

Lopalco P[1], Santiana M[2] Ghosh S[2], Ho BA[2], Rajasekaran V[1], Du W-L[2], Mutsafi Y[2], De Je´ sus-Diaz DA[3], Sosnovtsev SV[3], Levenson EA[3], Parra GI[4], Takvorian PM[5,6], Cali A[5], Bleck C[7], Vlasova AN[8], Saif LJ[8], Patton JT[9], Corcelli A[1], Green KY[3] Altan-Bonnet N[2]

[1]Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari A Moro, Italy [2]Laboratory of Host-Pathogen Dynamics, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA [3]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA [4]U.S. Food and Drug Administration, Division of Viral Products, Silver Spring, MD, USA [5]Federated Department of Biological Sciences, Rutgers University, Newark, NJ, USA [6]Albert Einstein College of Medicine, Department of Pathology, Bronx, NY, USA [7]Electron Microscopy Core, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA [8]Food Animal Health Research Program, Ohio Agricultural Research and Development Center, CFAES, Veterinary Preventive Medicine Department, College of Veterinary Medicine, The Ohio State University, Wooster, OH, USA [9]Department of Biology, Indiana University Bloomington, Bloomington, IN, USA

In enteric viral infections, such as those with rotavirus and norovirus, individual viral particles shed in stool are considered the optimal units of fecal-oral transmission. We reveal that rotaviruses and noroviruses are also shed in stool as viral clusters enclosed within vesicles that deliver a high inoculum to the receiving host. Cultured cells non-lytically release rotaviruses and noroviruses inside extracellular vesicles. In the present report, we show results of an investigation in the lipidome of stool vesicles of rotavirus and norovirus by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF/MS), using 9-aa as matrix. Lipid analyses have been performed in negative and positive ion mode on very small amounts of intact vesicles by skipping lipid extraction and separation steps. MALDI-TOF/ MS lipid analysis of rotavirus-containing vesicles, isolated from mouse stools, revealed enrichment of plasma membrane lipids including sphingomyelin (SM), phosphatidyl ethanolamine (PE), and phosphatidylserine (PS). In addition, MALDI-TOF/MS analyses of murine Norovirus (MNV-1)-containing vesicles, isolated from RAW264.7 cell culture reveal the presence of bis(monoacylglycero) phosphate (BMP), a lipid enriched in multivesicular bodies (MVBs) and MVB-derived exosomes.

PP.206

Chk1 regulates 17β-estradiol-induced cell proliferation.

Bartoloni S, Leone S, Acconcia F

Department of Sciences, University Roma Tre, Rome, Italy.

The sex hormone 17β-estradiol (E2) regulates diverse physiological effects including cell proliferation by biding to the estrogen receptor (ER), which as a transcription factor drives E2-sensitive gene transcription and as an extra-nuclear localized signaling molecule triggers the membrane-dependent activation of diverse kinase cascades. While E2 triggers cell proliferation, it also induces ER degradation, which synchronizes gene expression with the E2-driven effects and limits the possibility of a cell hyper-response to the hormone. Here, we used the modulation of ER intracellular levels as a bait to pinpoint new kinasebased pathways involved in the regulation of E2-induced cell proliferation by screening a small-scale kinase inhibitor library in high-throughput assays. We identified a role for checkpoint kinase 1 (Chk1) in the regulation of ER intracellular levels and cell proliferation. Therefore, we next studied Chk1 in E2-dependent ER signaling and degradation. Results indicate that Chk1 inhibition accelerates ER breakdown, prevents E2-induced ER transcriptional activity as well as E2-induced signaling to cell proliferation. Analysis of the mechanism through which Chk1 controls ER stability further reveals an unexpected physiological role for transcriptional/replicative stress in the regulation of ER degradation. These discoveries indicate that Chk1 plays an intrinsic role in E2:ER signaling to physiological functions that drive cells to proliferation. Keywords: Estrogen-signaling; Chk1; Cell proliferation

PP.207

Chemical functionalization of HA-Mg/ColI scaffold for bone tissue engineering

Calabrese G[1], Petralia S[2], Dolcimascolo A[1], Zappalà A[1], Figallo E[3], Conoci S[4], Parenti R[1].

[1]Dept of Biomedical and Biotechnological Sciences, University of Catania, Italy [2]Applied Chemical Works, Paternò (CT), Italy [3]Fin-Ceramica Faenza, Italy [4]Distretto Tecnologico Micro e Nano Sistemi Sicilia, Catania, Italy

The most used method for treating large bone defects is still the bone grafting. Autologous bone grafting represents the gold standard way, although it can trigger some problems, including donor site morbidity, pain and risk of infection. For this reason, in recent years clinical needs have led to the development of alternative and innovative methods, such as the bone tissue engineering strategy by using biomaterials able to perfectly mimic the properties and structure of natural bone. Several natural and synthetic materials have been used to generate the scaffold for bone regeneration, but even today, a suitable material is not available to satisfy all needed properties for a good bone substitute in term of osteoconduction, osteoinduction and osteogenesis. In this study, we evaluated if chemical functionalization of HA-Mg/Col type I scaffold by coating with several metal ions (Ag and Ni) and metal oxides (γFe2O3 and GO) improves

its osteoconductive and osteoinductive properties. For this aim hADSCs have been cultured on functionalized scaffolds and induced to osteogenic differentiation in vitro for 24 days. The results indicate that only coating with γFe2O3 improves osteoconductive and osteoinductive properties of HA-Mg/Col type I scaffold, while Ag extremely reduces cell adhesion and proliferation. Our data suggest that the chemical functionalization of the biomaterials could represent a good tool to improve the osteoconductive and osteoinductive properties of scaffolds for bone tissue engineering.

PP.208

The Effect of Platelet-Rich Plasma in Inactive Form on the Burn Zone of Stasis in Rats

Orhan E[1], Gündüz Ö[2], Kaya O[3], Metin MS[4], Tarladaçalışır YT[4].

[1]Department of plastic and reconstructive surgery, University of Gaziantep, Gaziantep, Turkey. Departments of [2]Pharmacology, [3]Physiology, and [4]Histology and embryology, University of Trakya, Edirne, Turkey.

Aim: The protection of the stasis zone tissues reduces the width and depth of the burn injury. In this study, it is aimed to reduce tissue damage in the burn stasis zone using platelet-rich plasma (PRP). Methods: For this study, approval was obtained from T. University Experimental Animals Local Ethics Committee, and the study was carried out in the Experimental Animals. In total 72 Wistar rats were used in the study. PRP was obtained from the blood taken from 8 rats. The remaining 64 rats were divided into 4 groups. In Group 1, only the Comb burn procedure was performed. In Group 2, 0,3 cc of physiological saline solution, in Group 3, 0.3 cc of platelet-poor plasma and in Group 4, 0.3 cc of PRP were intradermally injected into stasis zone tissues after burn procedure. The zones of stasis of the rats in the subgroup 'b' were drawn on a millimetric paper, and the area of the living zone was measured, and the amount of living areas was calculated as a percentage by proportioning the zone of stasis to the total area. Immunoreactivity densities of Beclin-1, anti-Nrf2 (Nuclear factor erythroid 2– related factor 2) antibody and anti-HO-1 antibody were evaluated by the histological score (HSCORE) on the sections of each animal. For immunohistochemical evaluation of apoptosis, TUNEL assay kits were used as indicated by the kit manufacturer, and apoptotic cells were identified. Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). Whether the distribution of continuous variables was normal or not was determined by the Shapiro-Wilk test. The assumption of homogeneity of variances was examined by Levene's test. Descriptive statistics were expressed as median (interquartile range). Whether the differences in clinical measurements (i.e. the proportion of alive area, apoptosis, autophagy, HO-1 and NRF-2) among the groups were statistically significant was evaluated by the Kruskal-Wallis test. When the p-values from the KruskalWallis test statistics were statistically significant, Conover's multiple comparison test was used to learn which group differed from which others. A p-value less than 0.05 was considered statistically significant. Results: 21.5% of the tissues in Group 1, 20.8% in Group 2, 27.0% in Group 3, and 69.6% in Group 4 were found to be alive. The autophagic cell number average was calculated as 340 in Group 1, 340 in Group 2, 335 in Group 3 and 450 in Group 4, while the average number of cells stained with Nrf2 was calculated as 225 in Group 1, 245 in Group 2, 250 in Group 3 and 370 in Group 4. When the groups were compared in terms of the living tissue ratio, autophagy and number of cells stained with Nrf2, the values in Group 4 were found to be statistically significantly higher compared to Group 1, Group 2 and Group 3, while there was no difference between Groups 1,2 and 3. Conclusion: As a result of our study, it was shown that PRP, intradermally administered in an inactive form, increased Nrf2, HO-1 levels and autophagy in the burn stasis zone and reduced tissue damage in the burn stasis zone by decreasing apoptosis.This study has shown that PRP has a protective effect on the burn stasis zone tissues.

PP.209

Temperature-dependent Increase in the Calcium Sensitivity and Acceleration of Activation of ANO6 Chloride Channel Variants

Lin H[1], Kim SJ[2], Nam JH[3,4]

[1]Department of Otorhinolaryngology, Yonsei University Republic of Korea [2]Department of Physiology, Seoul National University, Korea [3]Department of Physiology, Dongguk University Korea [4]Channelopathy Research Center (CRC), Korea

Anoctamin-6 (ANO6) belongs to a family of calcium (Ca2+)-activated chloride channels (CaCCs), and has three splicing variants (V1, V2, and V5), which are expressed on the plasma membrane. Unlike other CaCCs, ANO6 requires a non-physiological intracellular free calcium concentration ([Ca2+]i > 1 μM) and several minutes for full activation under a whole-cell patch clamp. Therefore, its physiological role as an ion channel is uncertain and it is more commonly considered as a Ca2+-dependent phospholipid scramblase. Here, we demonstrated that physiological temperature (37 C) increased the Ca2+ sensitivity of ANO6 under a whole-cell patch clamp; V1 was

activated by 1 μM [Ca2+]i, whereas V2 and V5 were activated by 300 nM [Ca2+]i. All ANO6 variants were activated by 100 nM [Ca2+]I when the temperature was increased to 42 C. The delay in the activation of the three variants decreased significantly at 37 C. Notably, the temperature-dependent Ca2+-sensitisation of ANO6 was not significant under inside-out patch clamp, suggesting a critical role of unknown cytosolic factors. Conversely, unlike channel activity, room temperature (27 C), but not physiological temperature (37 C), induced the scramblase activity of ANO6 with submicromolar [Ca2+]i (300 nM), irrespective of the variant type. Our results highlight the physiologically meaningful ion conducting property of ANO6 at 37 C and indicate that the channel function and scramblase activity of ANO6 may be functionally separated.

PP.210

Human neuronal CDKL5 knockout cells: a novel tool for the characterization of cellular and molecular mechanisms underlying CDKL5 deficiency disorder phenotype

Loi M, **Trazzi S**, **Fuchs C**, **Medici G**, **Gennaccaro L**, **Elisa Ren E**, **Tassinari M**, **Galvani G**, **Ciani E**.

Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy

CDKL5 deficiency disorder (CDD) is a rare encephalopathy characterized by early-onset epilepsy and severe intellectual disability. CDD is caused by mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene. To date, the lack of a robust and relevant in vitro neuronal model of the disease has limited the possibility of discovering the molecular mechanisms underlying the CDD neuronal phenotype. Using CRISPR-Cas9 technology, we created a neuronal in vitro model of CDD, human neuroblastoma SH-SY5Y cells, CDKL5 KO. SH-SY5Y cells are a useful in vitro model of neuronal function and maturation as they can be induced to differentiate in cells with morphological and biochemical characteristics of mature neurons. We found that neuronal maturation of engineered CDKL5 KO cells is strongly impaired when they are induced to differentiate by retinoic acid or BDNF. Compared to their WT counterparts, differentiated CDKL5 KO cells are characterized by reduced neurite outgrowth and a decreased number of neurite varicosities. Moreover, CDKL5 deficiency increases the vulnerability of CDKL5 KO cells to apoptosis induced by different types of neurotoxic stress, e.g., oxidative stress. Interestingly, H2O2-terated CDKL5 KO cells showed an increased nuclear H2AX phosphorylation in comparison to their WT counterparts. Since phosphorylated H2AX is considered to be a biomarker for DNA double-strand breaks, compromised DNA repair may underlie the higher neuronal vulnerability associated with Cdkl5 loss of function. Defective neuronal maturation and increased vulnerability are neuronal phenotypes that characterize the brain of the mouse model of CDD. Therefore, our results indicate that this new CDKL5 KO cell model may be a very useful tool to dissect the molecular mechanisms underlying the CDD phenotype.

PP.211

The effect of zero-glucose on uterine contractility

Kaleta T, **Wray S**

Department of Molecular & Cellular Physiology, University of Liverpool, UK.

Uterine contractility issues, such as preterm or dysfunctional labours, remain one of the primary obstacles to female and neonatal health. The mechanisms of myometrial contraction and its impairment, need to be better elucidated. As contractions produce ischemia, glucose delivery to the myometrium becomes restricted. A direct cause-and-effect relationship between glucose depletion and muscle function impairment remains to be established in the myometrium. We investigate this, and compare effects under hypoxic and depolarized conditions. Myometrial strips from pregnant and non-pregnant C57BL6 mice were equilibrated in oxygenated physiological saline (pH7.4) at 37 C. Contractile activity was measured isometrically and the effects of zero-glucose, zero-glucose and hypoxia (N2) and zero-glucose on the response to high K^+ (40 mM) were tested. N is number of mice. The amplitude of spontaneous contractions decreased significantly (t-test) to 48±7% (n=14) of control in pregnant and 80±4% (n=5) in non-pregnant mice. This inhibition was significantly more pronounced with hypoxia and zero-glucose; 16±10% pregnant (n=9) and 11±6%, nonpregnant (n=10). The peak and plateau contraction to high K^+ were also significantly reduced by zero-glucose, (21±4% and 25±1%., in pregnant mice). Our results demonstrate that zeroglucose significantly inhibits contraction, irrespective of how it is produced. The results indicate that depletion of glucose in vivo will be a contributing factor to the pathway underlying contractility related disorders. The mechanism of its effect requires elucidating.

PP.212

Expression levels of selected calcium homeostasis- and mitochondrial dynamics- related proteins in Wolframin1 deficient rats

Kureková S[1], **Zahradníkova AJr.**[1,2], **Cagalinec M**[1,2,3]

[1]Centre of Biosciences, Slovak Academy of Sciences, Slovakia. [2]Biomedical Research Center, University Science Park of Biomedicine, Slovak Academy of Sciences, Slovakia. [3]Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia.

Wolframin (Wfs1) is a membrane protein of endoplasmic reticulum, mutations of which are responsible for the Wolfram syndrome, characterized by diabetic and neurological symptoms. Wfs1 was shown to be involved in calcium handling: depletion of Wfs1 led to decreased and delayed cytosolic Ca2+ elevations in response to glucose stimuli and to increased expression of SERCA. Moreover, deletion of Wfs1 led to perturbations in mitochondrial dynamics and morphology in neurons. Although Wfs1 is highly expressed in cardiac muscle, its role in this tissue is not clear. In this work we have characterized the effect of invalidation of Wfs1 on expression of calcium signalling- and mitochondrial dynamics-related proteins in ventricular myocytes of exon5-Wfs1 deficient rats (Wfs1-e5/-e5). Myocytes were lysed in Ripa buffer. Proteins from each cell lysate were separated by an 8% or gradient SDS-PAGE and transferred to PVDF membrane. We used primary antibodies to calcium transport and mitochondrial fusion proteins. The bands were visualized with an ECL system and images were analysed with the ImageLab software from Bio-Rad. Expression of the proteins of interest in isolated cardiomyocytes was analysed on the level of mRNA using real-time PCR. We have used commercially available intron-spanning TaqMan Expression Assays on cDNA obtained from total RNA extracted by TRI reagent precipitation method. We have determined that the expression of SERCA and calsequestrin were not affected in Wfs1-e5/-e5 animals. The protein and mRNA expression levels of other calcium handling and mitochondrial dynamics proteins (RyR2, IP3R2, NCX1, PLB, Mfn1, Mfn2) will be presented. This work was supported by grants SASPRO 0063/01/02; APVV 15-0302; VEGA 2/0169/16, 2/0143/17 and 2/0090/18 and ITMS 26230120006.

PP.213

Amphibian skin peptide macrotympanain A1 powerfully reduces lipid accumulation in a cellular model of hepatic steatosis

Demori I[1], **Lobasso S**[2], **Lopalco P**[2], **Corcelli A**[2], **Bellese G**[3], **Queirolo L**[1], **El Rashed Z**[1], **Millo E**[4], **Salvidio S**[1], **Canesi L**[1], **Cortese K**[3], **Grasselli E**[1]

[1]Department of Earth, Environmental and Life Sciences, University of Genova, Italy; [2]Dipartimento di Scienze Mediche di base, Neuroscienze e Organi di Senso - Università degli Studi di Bari Aldo Moro – BA, Italia [3] Dipartimento di Medicina Sperimentale - Università degli Studi di Genova, Italia [4] Dipartimento di Medicina Sperimentale – Sezione di Biochimica - Center of Excellence for Biomedical Research (CEBR) - Università degli Studi di Genova, Italia

Macrotympanain A1 (MA1) is an amphibian skin peptide displaying significant anti-oxidant and anti-inflammatory abilities, which are useful for amphibian skin protection and health. Evidence suggests that amphibian skin peptides exert various biological effects also in mammalian cells. In this work, we demonstrate a lipid-lowering activity of MA1 in a cellular model of hepatic steatosis and begin to investigate a possible mechanism of action of MA1 on liver cells. MA1 (FLPGLECVW) was synthetized using the standard method of solid phase peptide synthesis, which follows the Fmoc strategy. Rat hepatoma FaO cells were made steatotic by incubation with oleate/palmitate mixture for 3 h, and then exposed to 10 µg/mL MA1 for 24 h. The lipid extracts of cells were analysed by TLC and MALDI-TOF/MS. Expression of PPAR (Peroxisome Proliferator Activated Receptor) isoforms, that play an important role in lipid homeostasis, was evaluated by qPCR. MA1 effects on intracellular signal transduction pathways was evaluated by western blotting. The results show that MA1 was able to significantly reduce the content of triacylglycerols and cholesterol-esters in steatotic cells, whereas the polar lipid profile was not altered. The anti-steatotic effect of MA1 was associated with modulation of Pparg expression and of PI3K and ERK/MAPK signaling pathways. Experiments are in progress in order to better understand the lipid lowering mechanisms triggered by MA1 on mammalian liver cells

PP.214

Role of disulfide bonding in modulation of Cx36 gap junction channel conductance by n-alcohols and general anesthetics

Raškevičius V[1], **Jotautis V**[1], **Rimkutė L**[1], **Marandykina A**[1], **Kairys V**[2], **Skeberdis VA**[1].

[1]Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas LT-50162, Lithuania; [2]Institute of Biotechnology, Vilnius University, Vilnius LT-10257, Lithuania

We have demonstrated earlier that short carbon chain n-alcohols (up to octanol) stimulated while long carbon chain n-alcohols inhibited the conductance of connexin 36 (Cx36) gap junction (GJ) channels. In contrast, GJ channels composed of other types of Cxs all were inhibited by n-alcohols independently on their carbon

chain length. Structural modeling of Cx36 protein docking with hexanol and isoflurane that stimulated as well as nonanol and carbenoxolone that inhibited the conductance of Cx36 GJs revealed their multiple common docking sites and a single pocket accessible only to hexanol and isoflurane. The pocket is situated in the vicinity of three unique cysteine residues, namely C264 in the fourth, and C92 and C87 in the second transmembrane domain of the neighboring Cx36 subunits. To examine the hypothesis that disulfide bonding might be involved in the stimulatory effect of hexanol and isoflurane, we generated cysteine substitutions in Cx36 and demonstrated by a dual whole-cell patchclamp method that in HeLa and N2A cells these mutations reversed the stimulatory effect of hexanol and isoflurane to inhibitory one, typical of other tested Cxs (Cx26, Cx30.2, Cx31, Cx43, Cx45 and Cx47) that lack respective cysteines and/or a specific docking pocket for these compounds. Our findings suggest that the stimulatory effect of hexanol and isoflurane on Cx36 GJ conductance could be achieved by re-shuffling of the inter-subunit disulfide bond between C264 and C92 to the intra-subunit one between C264 and C87.

PP.215

Induction of the apoptotic volume decrease (AVD) under normotonic conditions in HeLa cells exposed to Trolox

Giordano ME, **Caricato R**, **Schettino T**, **Lionetto MG**

Department of Biological and Environmental Sciences and Technologies, Salento University, Lecce, Italy

Previous observations showed that Trolox, a synthetic analog of vitamin E, widely used as antioxidant standard in a number of bioassays, can exert a pro-oxidant behavior at higher concentrations (>40 μM) on HeLa cells exposed for 24h, producing an isotonic cell shrinkage. A number of cellular events are known to be triggered by oxidative stress, including impairment of ion transport mechanisms and alteration of cell volume homeostasis. This work aims to investigate the possible mechanisms through which Trolox at high concentrations acts on cell volume homeostasis alteration in HeLa cells. The study was carried out by 1) spectrofluorimetric determination of intracellular oxidative stress in cells charged with CM-H_2DCFDA, 2) morphometric analysis of cells observed under optical microscopy for cell volume determination, and 3) spectrofluorimetric and confocal analysis of cells charged with Annexin V/Propidium Iodide for apoptotic induction. HeLa cells exposed for 24h to high Trolox concentrations showed a significant dose-dependent isotonic reduction of cell volume associated to intracellular oxidative stress. The observed isotonic shrinkage was accompanied by apoptosis induction, as demonstrated by Annexin V/Propidium Iodide and was ascribed to Apoptotic Volume Decrease (AVD). The isotonic shrinkage appearance was demonstrated to occur early (after 2h) during the exposure to high Trolox concentrations. It was completely inhibited by pretreatment of the cells with a Cl- channel blocker SITS (0.5 mM). These results indicate that treatment of HeLa cells with high Trolox concentrations induces the activation of volume-regulatory Cl^- channels, most likely by an increase in endogenous ROS production, which in turn is able to generate AVD.

PP.216

Effect of O-GlcNAcylation of ICln in the regulation of cellular volume

Remigante A[1,2], **Costa R**[1], **Civello D**[1], **Morabito R**[2], **Nagy T**[3], **Marino A**[2], **Dossena S**[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medizinische Privatuniversität, Salzburg, Austria; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; [3]Department of Laboratory Medicine, University of Pécs, Hungary.

O-GlcNacylation (O-GlcNAc) is the post-translational conjugation of N-acetylglucosamine to serine or threonine residues of cellular protein. O-GlcNAc is known to be elevated in diabetes mellitus, but the pathophysiological role of this finding is not fully elucidated. Recently, the protein ICln, which is crucial in the activation of a chloride conductance ($I_{Clswell}$) after anisosmotic cell swelling, has been found to be O-GlcNacylated. Mass spectrometry and bioinformatics analysis of the amino acid sequence of ICln show several O-GlcNAc modification sites, of which the effect on ICln function is unknown. To elucidate this point, ICln wild type (WT) and different mutant forms have been expressed in human kidney cells and characterized by patch-clamp in conditions of normal or elevated O-GlcNAc levels. Moreover, the protein levels were assessed by western blot. The findings show that: O-GlcNAc elevation suppresses the ICln-induced current; IClnT223A is functional and sensitive to O-GlcNAc elevation; IClnS193X loses most of its activity, even though the residual current is sensitive to O-GlcNAc elevation; IClnS67A is functional but insensitive to O-GlcNAc elevation; IClnS67T is hypofunctional, insensitive to O-GlcNAc elevation, and, accordingly, its protein levels are reduced compared to the WT. Overall, these results indicate that O-GlcNAcylation of ICln at the level of Serine 67 leads to suppression of the ICln-induced current and may disclose the mechanism by which O-GlcNAc elevation affects the regulation of cellular volume. These findings

underscore that alterations of ion currents and homeostatic mechanisms may contribute to the onset and progression of diabetic complications and may therefore represent novel targets in the prevention or treatment of these conditions.

PP.217

A novel Nrf2 activator protects retinal explants from oxidative stress and neurodegeneration

Rossino MG[1], **Amadio M**[2], **Govoni S**[2], **Dal Monte M**[1,3], **Casini G**[1,3]

[1]Department of Biology, University of Pisa [2]Department of Drug Sciences, University of Pavia [3]Interdepartmental Research Center Nutrafood "Nutraceuticals and Food for Health", University of Pisa, Italy

Diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus, characterized by neurodegeneration and extensive vascular changes. Early diagnosis and intervention are important to prevent or ameliorate the development of DR. Recent studies suggest that pathophysiological mechanisms contributing to neurodegeneration include biochemical and metabolic alterations leading to oxidative stress. For this reason, some of the new therapeutic strategies are aimed at counteracting neurodegeneration by preventing the onset of oxidative stress using antioxidant compounds. In this respect, antioxidants of natural origin are receiving increasing interest. Recently, curcumin and diallyl sulfide were combined in a new chemical entity called nature-inspired hybrid (NIH), an activator of transcription nuclear factor erythroid-2-related factor-2 (Nrf2), the master regulator of the antioxidant response. We tested the antioxidant properties of NIH in retinal explants subjected to oxidative stress. In particular, we evaluated the activation of Nrf2 and the consequent expression of antioxidant enzymes like heme-oxygenase-1, superoxide dismutase, NADPH quinone oxidoreductase and glutamate-cysteine ligase catalytic subunit. Finally, we evaluated how treatment with NIH affects neurodegeneration quantifying the expression of apoptotic markers. The obtained results suggest that treatment with NIH not only generates an antioxidant response but also favors a neuroprotective action, confirming that a strategy aimed at counteracting oxidative stress could be a good choice for prevention or treatment of DR.

PP.218

Effects of alpha and beta glucans on immune modulating factors expression in enterocyte-like Caco-2 and goblet-like LS174 cells

Damiano S[1], **La Rosa G**[1], **D'Errico A**[1], **Del Buono A**[2], **Montanino C**[2], **De Felice B**[2], **Santillo M**[1]

[1]Dipartimento di Medicina Clinica e Chirurgia, Università di Napoli "Federico II," Naples, Italy, [2]Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche, Università della Campania Luigi Vanvitelli, Caserta, Italy

Glucans are complex polysaccharides consisting of repeated units of D-glucose linked by glycosidic bonds. The nutritional contribution in alpha-glucans is mainly given by starch and glycogen while in beta-glucans by mushrooms, yeasts and whole grains, such as barley and spelt well represented in the Mediterranean Diet. Numerous and extensive studies performed on glucans highlighted their marked anti-tumor, antioxidant and immunomodulatory activity. It has recently been shown that rather than merely being a passive barrier, the intestinal epithelium is an essential modulator of immunity. Indeed, epithelial absorptive enterocytes and mucin secreting goblet cells can produce specific immune modulating factors, driving innate immunity to pathogens as well as preventing autoimmunity. The aim of the study was to evaluate the effects of alpha and beta glucans, alone or in combination with other substances with antioxidant properties, on reactive oxygen species (ROS) levels, on the expression of ROS-generating enzyme DUOX-2 and of the immune modulating factors Tumor Necrosis Factor (TNF-), Interleukin 1 beta (IL-1β) and cyclooxygenase-2 (COX-2) in two intestinal epithelial cells, the enterocyte-like Caco-2 cells and goblet cell-like LS174T. The experiments were carried out incubating the cells with glucans for 18h in culture medium containing 0.2% FBS and measuring ROS levels fluorimetrically as dihydrodichlorofluorescein diacetate (DCF-DA) fluorescence, and mRNA levels of DUOX-2, TNF- , IL-1β and COX-2 by RT-PCR. Alpha and beta glucans decreased ROS levels in Caco-2 and LS 174 cells. The expression levels of COX-2, TNF- , and IL-1β were also reduced by alpha- and beta-glucans. Additive effects on the expression of these immune modulating factors were exerted by vitamin C. In Caco-2 cells, the dual oxidase DUOX-2 expression is positively modulated by ROS. Accordingly, in Caco-2 cells treated with alpha and beta-glucans alone or in combination with Vitamin C, the decrease of ROS levels was associated with a reduced expression of DUOX-2. The treatment of cells with the NADPH oxidase (NOX) inhibitor apocynin decrease COX-2, TNF- and IL-1β mRNA levels indicating that NOX dependent ROS regulate the expression of immune modulating factors of intestinal cells; however, the combination of vitamin C, alpha and beta glucans with apocynin did not exert additive effects. The present study showing a modulatory effect of alpha and beta glucans on ROS and on the

expression of immune modulating factors in intestinal epithelial cells suggest that the assumption of food containing high levels of these substances or dietary supplementation can contribute to normal immunomodulatory function of intestinal barrier.

PP.219

Rat motor cortex neurons lose resistance to oxidative stress during postnatal development

Gorton E[1], Castro C[2, 3], Pardillo-Díaz R[1], Nunez-Abades P[1, 3], Carrascal L[1, 3]

[1]Departamento Fisiología, Universidad Sevilla, Spain; [2]Departamento Fisiología, Universidad Cádiz, Spain; [3]Instituto de Biomedicina, Universidad Cádiz, Spain.

Oxidative stress and the production of reactive oxygen radicals play a key role in neuronal cell damage. To explore the effects of oxidative stress on the electrophysiological properties of the motor cortex neurons during postnatal development, brain slices from newborn (P2-P5), young (P11-P15) and adult rats were obtained. The whole cell patchclamp technique was used to record the membrane properties. Cumene hydroperoxide (CH, 10μM), was applied during 5-30 minutes to induce oxygen-derived free radical formation. No changes were observed in the membrane properties in newborn rats. In young rats an increase in resistance and a decrease in frequency gain and maximal frequency were observed 15 minutes after drug application. The effects of CH on adult rat neurons were previously described (Pardillo-Diaz et al., 2015); marked changes in the electrophysiological properties were observed. After 5 minutes of CH exposure, the input resistance increased and the frequency gain and maximal frequency decreased. At 15 and 30 minutes, the resistance decreased and frequency gain and maximal frequency of discharge continued to decrease. Additionally, half of these adult cells lost the ability to repetitively discharge action potentials. To understand the mechanisms involved in the resistance of the neurons to CH we measured free thiol content in brain slices treated with CH at different time points. Slices of younger rats showed a greater ability to respond to oxidation by increasing their free thiol concentration, thus showing a higher buffering capacity against oxidative stress. As a result, we conclude that newborn rats are more resistant to oxidative stress. However, during postnatal development the cells progressively lose the ability to fight against reactive oxygen radicals.

PP.220

The use of recombinant allergens for the study of the allergic immune response in ragweed allergy

Panaitescu C[1,2], Marusciac L[1,2], Buzan RM[1], Chen KW[1]

[1]Timisoara County Emergency Clinical Hospital Pius Brinzeu
[2]Victor Babes University of Medicine and Pharmacy Timisoara

Amb a 1 is the major allergen from ragweed (Ambrosia artemisiifolia) pollen, which sensitizes an important fraction of people in Europe. There are five known isoforms of Amb a 1, with different sequence homologies and distinct patterns of IgE binding and immunogenicity. 95% of ragweed allergic patients show IgE binding to Amb a 1 in immunoassays. This study aims to produce recombinant Amb a 1 isoforms and to investigate the allergic response induced by them. Amb a 1 isoforms 01 and 02 were expressed using Sf9 insect cells. The gene sequences were amplified using DH10 competent E. coli cells. Bacmid DNA was amplified, isolated and purified using a Midiprep kit. The DNA was then used for the transfection of Sf9 insect cells, after which the proteins were purified and concentrated. Protein antigenicity was analyzed by ELISA with sera from patients previously sensitized to ragweed pollen. Amb a 1 isoforms 01 and 02 were obtained as recombinant proteins. The protocol that yielded the highest amounts of protein used supernatant without dialysis. The obtained proteins were IgE reactive. Recombinant Amb a 1 isoforms are adequate tools for understanding the allergenic properties of Amb a 1 isoforms found in natural ragweed pollen, which is of great importance for the development of efficient products for diagnosis and therapy of ragweed pollen allergy.

PP.221

Kelch-like protein 33 may participate in regulating cytokinesis during epithelial mesenchymal transition

Vazquez-Carretero MD[1], Morales-Capita C[2], Torres B[2], Rodriguez-Mateos C[2], Polo S[2], Pintor-Toro JA[2]

[1]Department of Physiology. University of Seville. Spain. [2]Department of Cell Cycle and Oncogenesis. Andalusian Molecular Biology and Regenerative Medicine Centre. Spain.

The process of epithelial mesenchymal transition (EMT) is essential in several physiological and pathological processes such as tissue repair and cancer progression. So far, some long non-coding RNAs (lncRNAs) have been involved in EMT. Kelch-like proteins (klhl), belonging to Kelch superfamily, have been involved in skeletal muscle diseases or

cancer development, among others. We have previously identified the supposed lncRNA AK009210 as an EMT early induced gene in NMuMG cells. However, we found that AK009210 is part of the klhl33 gene itself, located at the 3´UTR end of the klhl33 gene. By RT-PCR we determined the relative abundance of klhl33 and AK009210 mRNAs and we found that both were highly expressed in skeletal muscle of mice. In NMuMG cells either klhl33 or AK009210 ablation induced changes in cell morphology which consists of bigger sized and bi-nucleated cells showing dislocation of E-cadherin and ß-actin in cytoplasmic- stress fibers. This abnormal phenotype was reverted when AK009210 DNA was supplemented. In addition, monitoring of NMuMG real-time cell proliferation showed that klhl33 depleted cells proliferated in a lower grade than control cells did. As far as we know this is the first study in demonstrating that klhl33 and AK009210 form of the same gene and may promote cell proliferation and the completion of cytokinesis during EMT.

PP.222

TRP Expression Signature in Tumor-Derived Endothelial Cells: Functional Roles in Prostate Cancer Angiogenesis

Bernardini M[1,2,3], Chinigò G[1], Brossa A[4], Grolez G[2,3], Genova T[1], Mattot V[6], Fromont-Hankard G[5], Munaron L[1], Bussolati B[4], Prevarskaya N[1,2], Fiorio Pla A[1,2,3], Gkika D[1,2]

[1]Department of Life Science and Systems Biology, University of Turin, Italy. [2]Inserm U1003, Université Lille 1, Villeneuve d'Ascq, France. [3]Laboratory of Excellence, Ion Channels Science and Therapeutics, Université de Lille 1, France. [4]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Centre, University of Turin Italy. [5] Inserm UMR 1069, Université de Tours, France. [6]Institute de Biologie de Lille, France.

TRP channels play a key role in cancer progression, modulating cell proliferation and survival, cancer invasion of surrounding tissues and angiogenesis. TRP expression could therefore characterize the prostate cancer (PCa) cell phenotype. Another well-established concept is that TRPs deeply modulate endothelial cell (EC) biology and tumor angiogenesis. However, a specific TRP expression signature of PCa angiogenesis is still lacking. Our aim was therefore to define a TRP expression signature during PCa angiogenesis providing novel therapeutic targets. By means of a qPCR screening and Western blotting, we fully profiled the expression of all TRPs in normal ECs and tumor endothelial cells (TECs) derived from PCa, as well as from breast and renal tumors. TRP channel function on TEC was analyzed by Ca^{2+} imaging and compared with healthy EC. Moreover, we characterized the role of the 'prostate specific' TRPs in the modulation of EC biological processes such as cell proliferation, motility and ability to form tubules in vitro, as well as in vivo angiogenesis. We identified five trp genes whose expression is deregulated in PCa-derived ECs compared to their healthy counterpart. We the specifically characterized the role of each TRP channel in both in vitro and in vivo angiogenesis, EC proliferation and migration as well as their role in PCa cell attraction by TECs. Taken together, our results propose novel molecular players to selectively target PCa progression and angiogenesis. Indeed, our expression profiling and functional data could explain the transition of prostate endothelial cells to their aggressive tumor phenotype.

PP.223

Neurogenesis in Brain Injuries Is Promoted by EOF2 A Diterpene that Activates Protein Kinase C Mediating Neuregulin Release

Gómez-Oliva R[1,6], Domínguez-García S[1,6], Geribaldi-Doldán N[1,6], Ruiz FA[2,6]. Nunez-Abades P[3,6], Carrascal L[3,6], Bolívar J[4,6], Macías-Sánchez AJ[5,6] Hernández-Galán R[5,6] Castro C[1,6]

[1]Área de Fisiología, Universidad Cádiz, Spain; [2]Área de Nutrición, Universidad Cádiz, Spain; [3]Departamento Fisiología, Universidad Sevilla, Spain; [4]Área de Bioquímica y Biología Molecular. Universiad de Cádiz, Spain; [5]Departamento de Química Orgánica, Universidad de Cádiz, Spain; [6]Instituto de Investigación Biomédica de Cádiz (INIBICA), Spain.

Acute or chronic injuries cause damage in the adult brain producing neuronal death. Neural stem cells (NSC) are activated within neurogenic niches in response to those injuries producing neuroblasts, which migrate towards the injured area. However, this response does not contribute to the generation of new neurons within the damaged tissue. Injuries constitute a gliogenic/non-neurogenic niche in which neuronal production is impaired because of the presence of signaling molecules, which act on intracellular pathways that prevent neuronal differentiation or neuroblast migration and survival. Kinases of the Protein kinase C (PKC) family mediate the release of growth factors that participate in different steps of the neurogenic process. We have isolated the diterpene with lathyrane structure 7,8,12-tri-O-acetyl-3-O-(4-methoxyphenyl) acetylingol (EOF2; CAS number 944799-48-8) from plants of the Euphorbia genus, which belongs to a family of non-tumorigenic PKC activators. The effect of intranasal administration of EOF2 on neurogenesis in brain injuries was analyzed in a mouse model of controled mechanical cortical injury, finding that treatment with EOF2 facilitates migration of neuroblasts from neurogenic regions

towards the perilesional area. PKC activity increased in the presence of EOF2 in a novel PKC-dependent manner. In addition, using fusion proteins in which membrane-anchored growth factors neuregulin 1 and TGFα were fused to a eGFP probe at the C-terminal and a to a mCherry probe at the N-terminal, we observed that EOF2 selectively mediated the release of the neurogenic growth factor neuregulin 1 without affecting the release of the gliogenic factor TGFα. Taken together our results dissected the molecular mechanism of a new neurogenic compound with potential clinical issues.

PP.224

Intraocular pressure lowering effect of new formulations of melatonin and agomelatine.

Locri F[1], Pezzino S[2], Pescosolido N[3], Pignatello R[4], Corsaro R[4], Cammalleri M[1], Bagnoli P[1], Dal Monte M[1], Rusciano D[2]

[1]Department of Biology, University of Pisa, Italy [2]Sooft Italia SpA, Montegiorgio, Italy [3]Department of Sense Organs, Sapienza University, Rome, Italy [4]Department of Drug Sciences, University of Catania, Italy

Glaucoma is a neurodegenerative disease in which an increased intraocular pressure (IOP) is a major risk factor. Lowering IOP is the only proven therapeutic intervention and medical IOP reduction remains the first-line treatment option for the majority of patients. Melatonin (MT) or its analogue agomelatine (AM) have shown significant neuroprotective features and their hypotonizing effect on the IOP have been demonstrated. Here, we evaluated the IOP lowering effect of innovative topical formulations of MT and AM in rats with normal IOP. Different formulations of MT were obtained in PBS, nanolipidic carriers (NLCs) and nanomicelles (NMCs). AM and mixtures of MT and AM were formulated in NMCs. Eye drops were instilled in Wistar rats and their IOP was measured at different time points. All MT eye drops showed a significant IOP lowering effect. The formulation in NMCs gave better and longer lasting effects than the NLC formulation, which in turn was better than the formulation in PBS. AM eye drops also showed an IOP lowering effect, which at the lower concentrations (0.01% and 0.1%) was comparable to that of MT while at 1% appeared more effective than the equivalent MT formulation. Finally, the association of MT and AM demonstrated a long-lasting effect than MT or AM given alone. Interestingly, an anecdotic observation on a single glaucoma patient in which eye drops with MT and AM were added to the standard therapy showed a further 25% IOP reduction after MT and AM thus suggesting that MT and AM could be introduced into the therapeutic armamentarium to treat glaucoma. Overall, the present data confirm the IOP lowering effect of MT and its analogues and evidence that NMC formulations may be the more appropriate for delivering MT to the eye. Funded by Sooft Italia SpA.

PP.225

Contribution of GABAA and GABAB receptors in the modulation of contractile activity in human colon.

Zizzo MG[1,2], Serio R[1]

[1]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università di Palermo, Italy; [2]ATeN (Advanced Technologies Network) Center, Università di Palermo, Italy

Gamma-Aminobutyric acid (GABA) is a transmitter released by enteric interneurons, regulating gut functions, mainly via GABAA and GABAB receptors. So far, its effective role in the gastrointestinal motility remains poorly understood, especially in human colon, where very few studies have been undertaken. We aimed to investigate the role of GABA in the contractility of the circular muscle of human colon by organ-bath technique. GABA (50 M- 5mM) induced dose-dependent excitatory effects, consisting in an increase in the amplitude of spontaneous contractile activity and, at higher concentrations, also of the basal tone. Such effects were antagonized by bicuculline, GABAA-receptor antagonist, by tetrodotoxin, a neural blocker, and by atropine, a muscarinic receptor antagonist. Muscimol, GABAA-receptor agonist, was able to mimic GABA-effects inducing as well contractile responses. Phaclofen, GABAB-receptor antagonist, per se induced an increase of the mechanical spontaneous activity and potentiated the GABA-induced excitatory effects. Moreover, Baclofen, GABABreceptor agonist, induced inhibitory effects sensitive to tetrodotoxin. In conclusion, these results demonstrated that neural GABAA and GABAB receptor activation is one of the multiple mechanism involved in the modulation of mechanical activity in the human colon circular muscle. Activation of GABAA receptors would lead to the release of acetylcholine from excitatory cholinergic neurons, in turn causing contractile responses. GABAB receptors seems to be tonically active increasing the release of inhibitory transmitters from enteric neurons, in turn counteracting the excitatory contractile activity.

PP.226

The potential role of O-GlcNAcylation in diabetes and depression comorbidity

Remigante A[1,2], Morabito R[2], Marino A[2], Dossena S[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medizinische Privatuniversität, Salzburg, Austria; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy

Diabetes leads to complications involving brain function, including cognitive decline and depression. It is widely accepted that depression is the consequence of an impaired neurogenesis in the gyrus dentatus of hippocampus, but the molecular mechanisms of this process are still poorly understood. Neurogenesis is the process by which neurons differentiate from neural stem cells, and VRAC channels, responsible for the activation of a chloride conductance (IClswell) after cell swelling during the regulation cellular volume, are essential in cell differentiation. It is well established that diabetes leads to increased O-GlcNAcylation (O-GlcNAc) levels in various tissues. OGlcNAc is a reversible post-translational modification of proteins that occurs via conjugation to the monosaccharide N-acetylglucosamine. In the present study, we investigated the behavior of the IClswell current in neuronal-like SHSY5Y cells by whole-cell patch-clamp in the presence of normal or elevated O-GlcNAc levels. The results show that: SH-SY5Y cells express the IClswell current in isotonic conditions; O-GlcNAc elevation did not lead to cell death but lead to a decline of cell metabolic activity and significantly suppressed the IClswell current. Overall, the evidence obtained indicates that O-GlcNAcylation affects the activity of VRAC channels, thus suggesting that O-GlcNAc elevation may impair hippocampal neurogenesis and contribute to the development of diabetes

PP.227

Development of a polarized 3D organoid pancreatic ductular epithelium that recapitulates the normal ductual architecture and function

Greco MR, **Cannone S**, **Cozzolino M**, **Casavola V**, **Guerra I**, **Caroppo R**, **Reshkin SJ**, **Cardone RA**.

Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari, Italy

Animal models have traditionally offered an important platform for understanding the physiological and cellular basis for tissue dynamics in a complete physiological environment. However, the differences between mice and humans, together with animal models being expensive, difficult and ethically not sustainable for large-scale studies have fostered the use of in vitro culture systems for dissecting the biochemical and physiological bases of tissue responses. This is particularly important for complex tissues such as pancreatic ducts where epithelial cells comprise the majority of ductal cells and tightly regulate transepithelial acid-base secretion of an apical hydroelectrolyte rich in HCO3- (pancreatic juice) into the duodeno and basolateral acid release into the ECM via a series of membrane transporters: the Na^+/H^+ exchanger, the $Cl^-/HCO3^-$ exchangers, the $Na^+/HCO3^-$ cotransporters, the H^+ATPase and the H^+/K^+-ATPase. However, these cells have been difficult to grow with the correct 3D ductual architecture and function. Using the normal pancreatic ductal epithelial cell line, HPDE, we have determined the necessary 3D growth conditions to have them grow as complex tubular structures lined with epithelial cells and ending in a structure similar to pancreatic acini. Here, we characterized these 3D structures for their expression of pancreatic duct markers and for the above transporters involved in their regulation of pHi/pHe homostasis. Future experiments in these pancreatic ductal epithelium organoids will characterize their bicarbonate secretion and the principal transporters involved in this secretion.

PP.228

Lipid accumulation in hepatocytes impairs endothelial cell function in a manner dependent on the grade of hepatic steatosis

Baldini F[1], **Khalil M**[1], **Fabbri R**[1], **Serale N**[1], **Voci A**[1], **Portincasa P**[2], **Vergani L**[1]

[1]Department of Earth, Environment and Life Sciences, University of Genova, Italy; [2]Dept. of Biomedical Sciences and Human Oncology, University of Bari, Italy.

Intercellular interactions play a central role in many pathophysiological processes including non-alcoholic liver disease (NAFLD), a chronic liver disease often associated with obesity and overnutrition. In response to lipotoxic conditions, the endothelium initiates inflammatory responses representing the first step in atherosclerosis. Cell-cell communication is mediated by a complex network and secreted factors play a major role. This study investigated the cellular mechanisms by which steatotic hepatocytes trigger endothelial cell dysfunction in vitro. To this aim conditioned medium from steatotic hepatocytes (HCM) was used to treat endothelial cells. FaO hepatoma cells exposed to different steatogenic agents alone or combined (3h oleate/palmitate-OP; 72h fructose-Fru; 24h TNF) mimic the progression towards more or less severe steatosis in vitro. After treatments, the HCM were incubated with HECV endothelial cells for 24h. Intracellular TG accumulation, cell viability, apoptosis, H2O2 production, oxidative stress markers, and nitric

oxide (NO) release were assessed by spectrophotometric/fluorimetric assays and/or realtime PCR. HCM from all steatotic hepatocytes caused lipid accumulation in HECV cells, with endothelial steatogenesis depending on the steatosis grade of hepatocytes. Lipid accumulation in HECV cells was modest with Fru-HCM, but greatly increased with OP–HCM (+182%), OP/TNF-HCM (+166%), and Fru/OP-HCM (+210%), compared to controls. A similar trend was observed for H2O2 production, lipid peroxidation, NO release and endothelial cell activation. The results indicate that the extent of endothelial dysfunction in vitro depends on the grade of hepatic steatosis, but also on the metabolic features of hepatic steatosis.

PP.229

Usnic Acid: Does Protect Neurons from Glutamate Excitotoxicity?

Gundogdu G[1], Tasci SY[2], Nalci KA[3], Demirkaya Miloglu F[4], Hacimuftuoglu A[3]

[1]Department of Physiology, Pamukkale University, Turkey [2]Department of Physiology, Ataturk University, Turkey [3]Department of Pharmacology, Ataturk University, Turkey, [4]Department of Analytical Chemistry, Ataturk University, Turkey.

Usnic acid is a kind of lichen metabolites that has many protective effects on traditional medicine. Glutamate, the major excitatory neurotransmitters in the central nervous system. Glutamate excitotoxicity is the term used for the neuronal death process caused by the huge release of the glutamate into the extracellular space. The aim of study was to invastigate the neuroprotective effects of usnic acid in primary cortical neuron cultures againist glutamate excitotoxicity was evaluated. The protocol of this study was confirmed by Local Ethics Committee of Laboratory Animals Experiments of Atatürk University Erzurum, Turkey. Primary cortical neuron cultures prepared from cerebral cortices of newborn rats. Cultures were exposed to 6x10-5 M glutamate to form excitotoxicity. Then different concentrations of (final concentrations in the well to be 10 µM-1mM) usnic acid were added into the medium and left to incubate for 24 and 72 hours. The proliferation-inducing effect on cell viability was determined by using MTT assay. In order to evaluate production of reactive oxygen species,Total Oxidant Status (TOS) and Total antioxidant status (TAS) analyses were used. According to MTT assay, it was determined that cell viability was increased following usnic acid administration and observed that at lower concentration (10-100 µM) of usnic acid had a statistically significant protective effect on cell viability compared to glutamate control ($p<0.05$). TAS assay results demostrated that lower concentration of usnic acid increased the antioxidant level in cells, which might help to protect neurons againist glutamate induced exitotoxicity. These results suggest that usnic acid can be used as a therapeutic agent against glutamate excitotoxicity, but further studies are need clarify the mechanism of action of usnic acid.

PP.230

Vesicles-mediated release of AQP4 from glioblastoma cells: a communication mechanism?

Simone L[1], Pisani F[2], Frigeri A[3], Svelto M[2], Vescovi AL[1], Binda E[1], Nicchia GP[2]

[1]Fondazione IRCCS Casa Sollievo della Sofferenza, Cancer Stem Cells Unit, San Giovanni Rotondo, Italy. [2]Department of Bioscience, Biotechnology and Biopharmaceutics and Centre of Excellence in Comparative Genomics, University of Bari Aldo Moro,Italy. [3]Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari Aldo Moro, Italy.

Extracellular vesicles (EVs) are involved in a variety physiological events occurring in the nervous system, allowing the cross talk among neurons and glial cells and the exchange of proteins and mRNA. The EV-mediated communication is modified under pathological conditions, such as brain cancer. EVs have been shown to sustain tumour growth and to drive tumour-surrounding cells toward a tumour-enhancing phenotype, in a variety of model systems, including glioblastoma (GBM) cells. Recently, we have demonstrated that Aquaporin-4 (AQP4) is able to influence GBM cell fate potentiating the invasiveness capability or activating the apoptotic path, depending on its aggregation dynamics into the plasma membrane. In order to verify whether AQP4 aggregation dynamics are involved in controlling glioma cell behaviour through EV-mediated communication, the aim of this study has been to assess if EV-mediated release of AQP4 occurs in glioma cell lines. Live widefield imaging and differential centrifugation followed by western blotting approach were employed to identify subtypes and to quantify protein content in EVs derived from AQP4-overexpressing GBM cells and from healthy cell lines used as a control. Results show that GBM cells secrete heterogeneous types of AQP4 containing vesicles, different in biogenesis, size, function and protein content. AQP4 positive EVs include microvesicles, apoptotic bodies and 'beads-on-a-string' structure, indicating a potential role for AQP4 in this context. The role for AQP4 aggregation dynamics in controlling EV release is currently under investigation. In this work we gain insight into the complex role of AQP4 in tumour biology and try to answer the question if AQP4 is able

to influence GBM microenvironment through an EV intercellular communication.

PP.231

The Cytotoxic Effects of Suberosin Isolated from Ferulago cassia on SH-SY5Y Human Neuroblostoma Cells

Gundogdu G[1], Yilmaz Tasci S[2], Nalci KA[3], Karakaya S[4], Hacimuftuoglu A[3]

[1]Department of Physiology, Pamukkale University, Turkey [2]Department of Physiology, Ataturk University, Turkey [3]Department of Pharmacology, Ataturk University, Turkey, [4]Department of Pharmacognosy, Ataturk University, Turkey.

Neuroblastoma is one of the common types of solid tumors in children accounting for around 15% of cancer-related deaths in children. Suberosin is a kind of coumarin derivatives that has antiproliferative effects on some cancer cell lines. The aim of this study is to determine the anticancer effect of suberosin isolated from Ferulago cassia on SH-SY5Y human neuroblostoma cells. Suberosin was isolated from roots of F. cassia CH_2Cl_2 extract. SHSY5Y cells were grown in Dulbecco's modified Eagle's medium (DMEM) F12 and suberosin was administered at dose range of 10 µM -1 mM. The cytotoxic effect of suberosin on SHSY5Y cell line was measured by using MTT method according to time and dose dependent manner In order to evaluate production of reactive oxygen species, total oxidant status (TOS) and total antioxidant status (TAS) analyses were used. In this study, the IC50 of suberosin was found as 10 µM in SH-SY5Y cells at the 72th hour of MTT assay. TAS and TOS analysis results showed that antioxidant level was significantly decreased at lower doses ($p < 0.05$). In this study, it has been shown that suberosin isolated from F. cassia CH_2Cl_2 extract has cytotoxic and oxidant effect in SH-SY5Y cells, and as a result, it has been concluded that suberosin alone or in combination with other drugs may be useful in the treatment of neuroblostoma.

PP.232

Intracellular functions of protein C inhibitor (PCI)

Resch U, Mihaly-Bison J, Sokolikova B, Furtmüller M, Uhrin P, Geiger M

Department of Vascular Biology and Thrombosis Research, Centre for Physiology and Pharmacology, Medical University Vienna, Austria

PCI is a secreted protease inhibitor with broad protease reactivity and wide tissue distribution. Glycosaminoglycans (GAGs) and phospholipids modulate its activity and specificity. Studies suggest that PCI plays a role in hemostasis, in male reproduction, in host defense, and as a tumor suppressor. PCI is internalized by cells and translocates to the nucleus. Internalization requires phosphatidylethanolamine and an intact N-terminus of PCI. Currently we are analyzing the intracellular role of PCI. We studied internalized nuclear PCI in Jurkat lymphoma cells and endogenous nuclear PCI in normal prostate (RWPE1) and prostate cancer cells (PC3, LnCaP, DU145) by Western blotting of subcellular fractions and by immunocytochemistry. PCI contains a functional nuclear localization signal which mediates nuclear translocation. In Jurkat cells internalized PCI is mainly found in the nuclear envelope fraction where it is cleaved by a cathepsin L-like protease yielding a ~35kDa fragment. In all prostate cell lines, endogenous PCI was present in the nucleus. However, while nuclear PCI in normal prostate cells had a molecular weight (~57kDa) comparable to that of intact PCI, the molecular weight of PCI in prostate cancer cells was equivalent to the size of PCI cleaved by cathepsin L in vitro (~35kDa) or in the nucleus of Jurkat cells. Nuclear localization of cathepsin L is mainly seen in cancer cells and in some transformed cell lines. In colorectal carcinoma cells, nuclear cathepsin L activity is required for cell cycle progression. Several substrates of nuclear cathepsin L have recently been identified, such as histone H3, 53BP1, and the transcription factor CUX1. Internalized, nuclear PCI may therefore regulate epigenetic modifications and/or cell cycle progression.

Poster Session IV (1/4)

Cardiovascular Physiology

PP.233

A platform for assessing pro- and anti-arrhythmic effects of drugs based on isogenic human iPSC-derived cardiomyocytes

Campostrini G[1], Sala L[1], Ward-van Oostwaard D[1], van Meer B[1], Tertoolen LGJ[1], Bartulos-Encinas O[2], Braam SR[2], IJzerman AP[3], Mummery CL[1], Bellin M[1]

[1]Dept of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands; [2]Ncardia BV, The

Netherlands; [3]Leiden Academic Centre for Drug Research, Leiden University, The Netherlands

Cardiotoxicity is an unexpected side effect of drugs and a major cause of drug failure in preclinical and clinical phases of drug discovery. Some drugs bind to the cardiac hERG ion channel and cause a prolongation of the heart QT interval, inducing arrhythmias that can eventually lead to sudden cardiac death. Notably, individuals with inherited long QT syndrome (LQTS) are more prone to develop drug-induced arrhythmia. We previously showed that the LUF7346 allosteric modulator can shorten the QT interval *in vitro* in LQTS human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Our aim is to establish a drug-screening platform based on healthy and diseased hiPSC-CM to i) identify molecules with hERG allosteric modulator activity and ii) assess drug pro- and anti- arrhythmic effects. We used two isogenic hiPSC lines: one representing a severe form of LQTS, called Jervell and Lange-Nielsen syndrome (JLNS), carrying a homozygous mutation in the cardiac ion channel *KCNQ1* gene, and its isogenic wild-type line, that we generated with CRISPR/Cas9 technology. Both lines were differentiated into cardiomyocytes and their electrophysiological properties were evaluated by multi-electrode array (MEA) recording of spontaneous beating activity and by patch clamp. JLNS hiPSC- CM action potential (AP) duration was prolonged compared to the isogenic wild type line. We then optimized seeding and recording conditions in 96-well MEA plates. Finally, we used a novel integrated system based on fluorescent dyes to simultaneously measure AP, calcium transient, and contraction upon application of the reference compound LUF7346. This platform can identify active molecules able to shorten AP. Our approach will provide evidence for the value of using hiPSC-CM in preclinical drug testing.

PP.234

Baroreflex sensitivity: a fast tilt test study on humans

Taboni A[1], Fontolliet T[1], Moia C[1], Ferretti G[1,2]

[1]Department of Anaesthetics, Pharmacology, Intensive Care and Emergencies, University of Geneva, Switzerland; [2]Department of Molecular and Translational Medicine, University of Brescia, Italy

Exercise moves arterial baroreflex curves upward and rightward (baroreflex resetting), reduces baroreflex sensitivity (BRS) and shifts the operating point toward the baroreflex threshold. Recently, Bringard et al. (2017 EJAP 117:619-630), with a closed-loop approach during exercise transients, demonstrated that the BRS decrease precedes the baroreflex resetting, suggesting that the former may be due to a central command mechanism mediated by vagal tone withdrawal. The aim of this study was to analyse baroreflexes during fast postural changes on a tilt table, at rest and at light exercise (50W), testing the hypothesis that the up-tilt and the down-tilt may follow different baroreflex patterns, with different arterial pressure (AP) vs RR-intervals (RRi) slopes. 8 subjects performed 6 up- and 6 down-tilts on a tilt table at rest and 50W. We measured AP by Portapres, RRi by ECG. We analysed baroreflexes during tilt transients by linear regression of consecutive beats during which AP and RRi varied consensually (Bringard et al., cited above). We used the sequence method to compute BRS at steady state, both at rest and at 50 W. Preliminary data show that, at rest, the RRi versus AP slope was 15.6 ± 5.4 ms $mmHg^{-1}$ during up-tilt and 17.7 ± 14.6 ms $mmHg^{-1}$ during down-tilt (NS). At 50W, the RRi versus AP slope was 5.3 ± 1.8 ms $mmHg^{-1}$ during up-tilt and 6.3 ± 2.2 ms mmHg-1 during down-tilt (NS). These slopes were similar to the corresponding BRS computed at steady-state, both at rest (19.0 ± 10.6 ms mmg^{-1}) and at 50W (8.0 ± 3.6 ms $mmHg^{-1}$). These results suggest shift of baroreflex operating point along either the resting or the exercise baroreflex curve. Up and down tilts showed similar responses. If confirmed, these results would suggest rejection of the tested hypothesis.

PP.235

Effects of phenylephrine on bioelectric activity of the rat atrial septum myocardium

Pustovit K[1,2], Malolina E[3]

[1]Department of Human and Animal Physiology, Lomonosov Moscow State University, Russian Federation; [2]Department of Physiology, Pirogov Russian National Research Medical University, Russian Federation; [3]Laboratory of evolutionary biology of development, Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Russian Federation

The bioelectric properties of forming parts of the heart undergo significant changes during pre- and postnatal ontogenesis. The early stages of embryogenesis determine not only the general scheme of the structure, but also the features of bioelectric activity in various parts of the mature heart. The presence of myocardium which is capable of spontaneous depolarization in atrial septum (AS), may affect its electrophysiological properties. The ability to automatic activity of the AS remains unknown. In the present study we show the adrenergic effects on the bioelectric activity of AS in rat. Action potentials (AP) were recorded with use of standard microelectrode technique in multicellular isolated left atrial (LA) and AS preparations from neonatal (n=15) and adult (n=12) male Wistar rat. AP duration and resting potential (RP) were estimated in LA and AS in control conditions (paced 4 Hz or quiescent preparations) in presence of phenylephrine

(PE) (10μM) alone, or with If-current blocker ZD 7288 (10μM) in cases of spontaneous AP generation in AS. PE has had an impact on both AS and LA; cause changes in APD and RP. PE caused spontaneous pacemaker-like activity in quiescent preparations of AS, but not in LP. Applying the ZD7288 decreased the rate of slow diastolic depolarization and frequency in AS, up to complete suppression of AP generation. In conclusion, PE affects the AS bioelectric activity via α1-adrenoreceptors that leads to IK1-current decreasing. ZD 7288 effect indicates the highly-likely presence of If-current in the AS myocytes in rats, which contributes to the automatic activity of the main pacemakers of mammals. This study was supported by Russian foundation for Basic Research [grant no. 18- 34-00696].

PP.236

The effect of chamber-rest on electrophysiology of the heart in young people

Skrda D, Svorcjr P, Jarkulis V, Michalcak T, Kmet S, Marko A

Dep. of Physiology and Pathophysiology, University of Ostrava, Czech Republic

The aim of the study was to determine the effect of Chamber-Rest therapy on electrocardiographic parameters in young people. It is a therapeutic method based on a stay in a complete darkness to improve health status of people living under constant stress. Aside from improving the psychological status, this method could also affect vital functions including electrophysiology of the heart. 29 students (19 to 26 years) were placed individually in a special room with maximal darkness for 96 hours. The room met the requirements for a stay (quiet, socially isolated place). The participant received food and drinks without using any device emitting light or showing the actual time. The first measurement was performed the day before starting the therapy. The next measurement was taken 30 minutes after completing the session, followed by two more measurements in the fourth and the seventh day after exiting the dark room. The measured variables included PQ, QT and QTc intervals as well as the heart rate based on the 2nd bipolar lead of the ECG. The heart rate was significantly lower in the day of completing the stay, as well as in the fourth and the seventh day. The QT interval was significantly prolonged in the day of completing the stay, and the rest of ECG intervals remained unchanged. 96 hours of darkness therapy lowered the heart rate of young people. This effect is beneficial because higher heart rate is associated with an increased risk of cardiovascular disease. The prolongation of the QT interval is a marker for development of ventricular arrhythmias. The QTc interval remained unchanged, therefore the predisposition to the emergence and progression of ventricular arrhythmias was not lowered.

PP.237

Beat-to-beat variability of pulse wave velocity

Svacinova J[1], Hidegová S[2], Sieglová H[1], Kasčáková Z[1], Fabšík M[1], Jakubík J[1,2], Novák J[1], Nováková Z[1], Hrušková H[1,2]

[1]Department of Physiology, Faculty of Medicine, Masaryk University, Czech Republic; [2]International Clinical Research Center, St. Anne's University Hospital Brno, Czech Republic

Pulse wave velocity (PWV) increase is a marker of decreased arterial compliance (C). C decreases with increased blood pressure (BP), due to endothelial dysfunction and structural changes of the arterial wall. Increased mean PWV is observed in diabetics as well as in hypertonics. Mean PWV was analysed in many studies, but beat-to-beat changes of the PWV were less described. Aim of this pilot study is analysis of PWV beat- to-beat variability in dependence of body position.

PWV was measured in II. type diabetics (DM: 7m/8f, age 68±10 years, BP 158/90±19/9 mmHg) and healthy controls (Con: 5m/6f, age 23±2 years, BP 117/76±9/5 mmHg). PWV was recorded by method based on multichannel full body bioimpedance. Protocol consisted of 6 min of supine (sup) and 6 min of head up tilt in 45°(hut). PWV of left forearm (PWVlf) and left calf (PWVlc) were measured. Variability was counted as mean power spectra of PWV in frequency bands LF (0.04 – 0.15 Hz) and HF (0.15 – 0.5 Hz). LF-PWVlf, HF-PWVlf and HF-PWVlc in sup were higher in DM than in Con. During hut LF-PWVlc and HF-PWVlc increased in both groups; LF-PWVlf and HF-PWVlf increased only in Con. During hut of DM, PWVlf was lower than PWVlc. Decreased ability of arterial wall to dampen blood pressure changes probably could lead to higher PWV variability. This could be caused by hypertension and changed arterial stiffness (differences between groups), or by blood redistribution during body position change (differences between sup/hut and lf/cl). This pilot study showed, that PWV variability was changed by health condition, body position and measured artery position. Clear understanding of mechanism influencing PWV variability requires further study. Supported by: MUNI/A/1255/2018, LQ1605 (MEYS CR, NPU II)

PP.238

Autonomic regulation in young healthy subjects during the short-term "dry" immersion

Gerasimova-Meigal L, **Sireneva N**, **Meigal A**

Department of Human and Animal Physiology, Pathophysiology, Petrozavodsk State University, Russian Federation

"Dry" immersion (DI) is used as a ground-based analogue of microgravity since it mimics some features of space flight. This study was focused on the evaluation of the autonomic control of the heart rate during the short-term DI using time- and frequency-domain parameters of heart rate variability (HRV). Ten healthy subjects (5 m, 5 f) aged 18-20 years were studied both during 45-minute DI (DI-group) and lying supine awake on a bed (no-DI group). The DI was induced with help of the "MedSim" device (IBMP, Moscow). The heart rate and blood pressure ranged within normal values in both groups during the whole study time. In the no-DI group, no changes of time- and frequency-domain HRV parameters were found, with the exception of slight increase of SDNN ($p<0.01$), caused by the increased parasympathetic input associated with the relaxed wakefulness. In the DI group, the notable autonomic response, both parasympathetic and sympathetic, was documented. This appeared as the increased HRV detected by the analysis of time- domain parameters, increased total power of the spectrum (TP, $p<0.01$), high- (HF, $p<0.01$) and low- (LF, $p<0.01$) frequency-domain HRV parameters. Right after the DI, time- and frequency-domain HRV parameters still differed from their initial values suggestingly due to compensation mechanisms after the DI. The significant decrease of the stress index ($p<0.05$) was found in both groups. In conclusion, the autonomic regulation during the short-term DI in young healthy subjects is characterized by the rise of neurogenic activity, both parasympathetic and sympathetic, in order to maintain the optimal hemodynamics. Further studies of the adaptation mechanisms to microgravity would have elucidated safety issues of this method for its translation to rehabilitation

PP.239

Glucose-stressed human brain microvascular endothelial cells: preventive role of nutritional compounds.

Bassino E[1*], **Bramardi B**[1], **Gasparri F**[2], **Munaron L**[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Italy; [2]Department of Pharmacy, University of Salerno, Italy

Hyperglycemia leads to brain microvascular complications by altering blood flow and blood brain barrier (BBB) permeability and promoting abnormal endothelial proliferation. This study aimed to elucidate the role of nutritional compounds on human brain microvascular endothelial cells (HBMEC) stressed with high glucose concentration. We evaluated the effects of natural compounds on HBMEC treated with 30 mM glucose (HG). Specifically, we analyzed cell viability and oxidative stress production; moreover, we tested in vitro angiogenic processes, endothelial permeability and tight junctions expression. The results suggest that HG reduces HBMEC viability, increases oxidative stress and promotes abnormal in vitro angiogenesis. Moreover, HG decreased, endothelial permeability evaluated by TEER, tight junction proteins content, while caspase activity was enhanced. Natural compounds differently affect the aforementioned parameters promoting a preventive activity in both acute or chronic glucose stimulation. Finally, the present study provides novel information about the role of nutritional compounds in the prevention of endothelial dysfunction associated to hyperglycemic condition. Key words: brain endothelial cells, glucose, endothelial dysfunction, natural compounds.

PP.240

Antagonism of prostaglandin $F_{2\alpha}$-FP receptor signaling inhibits spreading depolarization in cerebral ischemia

Varga DP, **Szabó I**, **Varga VE**, **Menyhárt A**, **Bálint AR**, **Farkas E**, **Bari F**

Department of Medical Physics and Informatics, University of Szeged, Hungary

The inhibition of the FP receptor of prostaglandin F2a has recently been shown to limit neurodegeneration in brain ischemia. Recurrent spreading depolarizations (SD) are increasingly more appreciated to contribute to ischemic brain injuries. We set out to test the hypothesis that FP receptor blockade may achieve neuroprotection by the inhibition of SD. Forebrain ischemia/reperfusion was induced in isoflurane-anesthetized, young, adult, male Sprague-Dawley rats (n=16) by the bilateral occlusion and later release of the common carotid arteries. Two open craniotomies on the right parietal bone served the continuous elicitation of SD for one hour with 1M KCl (caudal), and the acquisition of local field potential (rostral). The entire dorsal cranium was thinned to track regional cerebral blood flow (CBF) variations by laser speckle contrast imaging. The femoral vein was used for the infusion of an FP receptor antagonist (AL-8810; 1mg/bwkg) or its vehicle (0.1% dimethyl sulfoxide, DMSO). AL-8810

markedly reduced the duration of individual SDs (30±10 vs. 56±14 s; AL 8810 vs. vehicle), as well as the cumulative depolarization time (2711 vs. 4511 s, AL-8810 vs. vehicle). Both the incidence (9 vs.12, AL-8810 vs. vehicle) and the amplitude of SD-related hypoperfusion as a result of inverse neurovascular coupling were reduced in the AL-8810 group (-6.8±3 vs. -13.4±3.2 pp; AL-8810 vs. vehicle). Further, the amplitude of reactive hyperemia after reperfusion initiation was substantially greater (94.9±20 vs. 79.7±16%; AL8810 vs. vehicle). In summary, the antagonism of FP receptors (located in the vascular wall or neurons) emerges as a promising approach to inhibit the evolution of SDs in cerebral ischemia. Key words: cerebral ischemia, prostaglandin signaling, spreading depolarization

PP.241

H_2S induces pro-angiogenic effects and decreases ischemia/reperfusion injury in human microvascular endothelial cells

Zicola E, **Arrigo E**, **Mancardi D**

Department of Clinical and Biological Sciences, University of Turin, Italy

Endothelial cells (ECs) injury and vascular function strongly correlates with cardiac function following ischemia/reperfusion injury (IRI). Moreover, several studies indicate that ECs are more sensitive to I/R than cardiomyocytes and are critical mediators of cardiac IRI. Hydrogen sulfide (H2S) is involved, among other functions, in the regulation of the cardiovascular system and can act as a cytoprotectant during ischemia/reperfusion. Activation of ERK1/2 and AKT in endothelial cells after H2S stimulation exerts a stimulation of angiogenesis in normoxia. Moreover, blockage of ERK1/2 or Akt during preconditioning or I/R significantly decrease H2S cardioprotective effects. In this project, we investigated how H2S (1-100uM) preconditioning could prevent I/R injury and promote angiogenesis on microvascular endothelial cells (HMEC) following an ischemia/reperfusion protocol in vitro. We observed that H2S preconditioning positively affected cell viability both in normoxia and post-I/R conditions. Moreover, cells significantly increased their migration compared to the control condition especially in post-I/R conditions. There was also an enhancement on cells ability to form capillary-like structures in vitro. Furthermore, mitochondrial function was investigated through the use of mitochondrial targeted probes. Our experiments showed that mitochondrial function was preserved after I/R when cells were preconditioned. ERK1/2 phosphorylation was also evaluated to consider its relationship with the H2S pathway. Altogether, these data suggest that H2S can be used to protect endothelial cells from I/R injury to decrease myocardial injury after acute myocardial infarction.

PP.242

Angiotensin II Infusion Changes Hypoxia-Inducible Factor 1 (HIF-1) And Its Accompanying Genes Expression in Cerebral Microcirculation of High Salt Fed Sprague-Dawley Rats

Matić A[1], **Mihaljević Z**[1], **Šušnjara P**[1], **Kolobarić K**[1], **Stupin A**[1,2], **Drenjančević I**[1]

[1]Institute and Department of Physiology and Immunology, Faculty of Medicine Osijek, [2]Faculty of Dental Medicine and Health Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

Recently we showed that superoxide scavenging increases HIF-1α antioxidative enzymes' genes expression in cerebral circulation of rats fed high salt (HS) diet, suggesting that HIF-1α expression depends on the level of oxidative stress. Also, suppressor doses of angiotensin II (ANGII) restored NO production and increased antioxidant capacity in HS fed rats. This study aimed to assess the role of ANGII on HIF-1 alpha and its target genes' expression in rats fed HS diet. Healthy male Sprague-Dawley rats, 9-11 weeks old, were assigned in 3 groups (n=6-8 rats/group): low salt-diet group (LS group, 0.4%NaCl in rat chow); HS group (1 week of 4%NaCl in rat chow) and HS+ANGII group (1 week of HS diet, 4th-7th day infused by subpressor doses of ANGII via osmotic minipump (100ng/kg/min/3 days). Afterwards, rats were anesthetized with ketamine (75mg/kg) and midazolam (2.5mg/kg) and decapitated. mRNA expression of HIF-1 alpha, PHD1, 2 and 3, eNOS, iNOS, and HPRT were measured in cerebral microcirculation, by rtPCR. All experimental procedures were approved by the local Ethical Committee and conformed to the EU Directive 86/609. Data were analyzed using One-Way ANOVA, presented as mean±SD. $p<0.05$ was considered significant. Relative gene expression of HIF-1 alpha, PHD1, PHD2 and PHD3 was significantly increased in HS+ANGII group compared to other groups ($p<0.05$). iNOS expression was significantly increased in HS+ANGII group compared to HS group. eNOS gene expression was not changed ($p>0.05$). Results suggest that increase in HIF-1 alpha and its accompanying genes is related to decreased oxidative stress in the vasculature due to ANGII infusion. Results support pivotal role of ANGII in maintaining endothelial function by affecting signaling pathways. This work was supported by Croatian Science Foundation Project IP-2014-09-6380 (Faculty of Medicine, Osijek, Croatia).

PP.243

Role of endothelial cells in the process of osteo-differentiation and bone formation

Genova T[1,2], Petrillo S[3], Zicola E[4], Tolosano E[3], Altruda F[3], Carossa S[2], Mussano F[2], Munaron L[1]

[1]Department of Life Sciences and Systems Biology, UNITO, via Accademia Albertina 13, Turin, Italy; [2]CIR DS, Department of Surgical Sciences UNITO, via Nizza 230, Turin, Italy; [3]Department of Molecular Biotechnology and Health Sciences, UNITO, Via Nizza 52, Turin, Italy; [4]Department of Clinical and Biological Sciences, UNITO Orbassano

Bone development and remodeling are physiological processes involving the formation of a closely associated vascular network. Meanwhile, the osteo-differentiation of stem cells is a fundamental step in bone formation. The aim of the present study was to investigate the complex relationship between human microvascular endothelial cells and osteo-differentiating mesenchymal stem cells (MSCs). To this purpose we developed a co-culture system to evaluate the biological effects (proliferation, migration, in vitro tubulogenesis, vessels recruitment) of osteo-differentiating and non osteo-differentiated MSCs on co-cultured endothelial cells. Furthermore, the mRNA levels of main angiogenic and growth factors were detected through qRT-PCR in both cell types. To overcome the limitations of traditional 2D cell culture systems, we developed a 3D organotypic bone structure using a bioreactor that allowed us to study bone formation in a more reliable way. Our results demonstrated that osteo-differentiating mesenchymal stem cells stimulate vessels recruitment and that endothelial cells support the process of bone differentiation mainly through the activation of the BMP-2 pathway.

PP.244

Effects of Ketamine-Xylazine anesthesia on hemorheological parameters: A rat model

Akbudak I[1], Kilic-Erkek O[2], Tuzcu EB[2], Pakyurek H[2], Bor-Kucukatay M[2]

[1]Pamukkale University Hospital, Department of Anesthesia and Reanimation, Denizli, Turkey [2]Pamukkale University, Faculty of Medicine, Department of Physiology, Denizli, Turkey

Anesthetic drugs may affect microcirculation due to systemic cardiovascular and/or direct hemorheological influences. Although Ketamin-Xylazine anesthesia is commonly used for animal studies in many experimental animal laboratories, effects of this anesthesia on hemorheological parameters remain unknown. Adult, male Wistar-Albino rats were used to determine the effects of intraperitoneal administration of Ketamin-HCl/Xylazine-HCl (90 mg/kg-10 mg/kg) on red blood cell (RBC) deformability and whole blood viscosity (WBV). Rats were randomly divided into two groups as control (pre-anesthesia) and post-anesthesia (n=11). Intracardiac anticoagulated blood was drawn either without anesthesia administration (control group) or after the loss of righting, cornea and withdrawal reflexes following anesthesia (post-anesthesia group). RBC deformability was measured by an ektacytometry, whereas WBV in otolog hematocrit (Hct) was determined by a cone-plate rotational viscometer. Student t test was used for statistical analysis, mean±SD was determined. Mean time taken for the disappearance of righting reflex was 2.03±0.81, corneal reflex 4.05±1.45 and for withdrawal reflex 5.65±1.5 min. Mean Hct value of the rats was 39.4±5.5. Post-anesthesia RBC deformability measured at a shear stress of 0.3 (0.12±0.014 vs 0.141±0.174, p=0.009) and 30 (0.569±0.045 vs 0.598±0.022, p=0.044) Pa was decreased, 0.53 (0.174±0.026 vs 0.15±0.024, p=0.019) Pa was increased compared to control. Ketamin-Xylazine administration did not affect WBW measured at otolog Hct of the rats. These results should be kept in mind especially during cardiovascular and hemodynamic studies.

PP.245

Postocclusive reactive hyperemia of the cutaneous microcirculation: impact of different mechanisms

Lenasi H

Faculty of Medicine, University of Ljubljana

Postocclusive reactive hyperaemia (PORH) has been used as a clinical test to evaluate endothelial (dys)function. We elucidated specific mechanism contributing to PORH in skin microcirculation. Laser Doppler flux (LDF), arterial blood pressure (BP), and heart rate (HR) were assessed in healthy volunteers on the volar forearm and the finger pulp. Indices of PORH were obtained after a 3-min occlusion of the brachial artery in 5 separate experiments: in basal conditions, during handgrip exercise, during mental arithmetic test, after application of EMLA cream, and after inhibition of cyclooxygenase (COX) and endothelial nitric oxide synthase (eNOS). Handgrip exercise shortened tmax (p=0.006) and increased the peak LDF of PORH (p=0.03) in the forearm, with a trend of smaller area under the curve (AUC) and shorter tdur; in the pulp, shorter tmax and smaller AUC were shown. During mental stress, HR and BP significantly increased (p<0.05), and trends of shorter tmax and tdur in the pulp were found (p=0.06). EMLA application decreased AUC (p=0.02) without affecting other parameters. COX

and eNOS inhibition diminished AUC (p=0.02) in the forearm. The mechanisms contributing to PORH depend on the measuring site. Reduced PORH response after exercise implies the 'stealing phenomenon' of skeletal muscles. Mental stress reduces PORH suggesting impact of the sympathetic nervous system. Decreased PORH response after EMLA confirms the role of axon reflex. Partial inhibition of PORH after COX and eNOS blockade suggests either important contribution of other endothelial vasodilators or stronger impact of metabolic, myogenic and axon-reflex component. Accordingly, PORH in skin microcirculation should be interpreted cautiously when assessing endothelial (dys)function in clinical practice.

PP.246

Purinergic Calcium Signals in Tumor-Derived Endothelium

Scarpellino G[1], **Genova T**[1,2], **Avanzato D**[3], **Bernardini M**[1], **Bianco S**[4], **Petrillo S**[5], **Tolosano E**[5], **Bussolati B**[5], **Fiorio Pla A**[1], **Munaron L**[1]

[1]Department of Life Sciences & Systems Biology, University of Torino, Italy; [2]Department of Surgical Sciences, University of Torino, Italy; [3]Department of Oncology, University of Torino, Italy; [4]Department of Public Health and Pediatrics, University of Torino, Italy; [5]Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy

Tumor microenvironment is particularly enriched of ATP, but conflicting evidence has been provided on its functional effects on tumor growth and vascular remodelling. High ATP concentrations exert a strong anti-migratory, anti-angiogenic and normalizing activity on BTEC (Avanzato et al, 2016). Since both metabotropic and ionotropic purinergic receptors trigger Ca2+ signals, the present work investigates their expression profile on tumor-derived human endothelial cells from breast (BTEC) and renal (RTEC) carcinoma, the properties of the intracellular Ca2+ events resulting upon their activation and their role in anti-migratory activity. Purinergic stimulation of BTEC evokes different Ca2+ signals according to the agonist. High ATP doses strongly inhibit BTEC and RTEC migration and trigger biphasic Ca2+ signals, which require intracellular Ca2+ release and extracellular Ca2+ entry. The extracellular Ca2+ influx mechanisms include both SOCE and non-SOCE components, the latter being responsible for the ATP anti-migratory activity in BTEC. Conversely, the same high UTP doses, which are not effective on BTEC migration (Avanzato et al, 2016), trigger different Ca2+ signals suggesting a possible correlation between ATP-induced anti-migratory activity and Ca2+ signals, even if we cannot exclude a completely Ca2+ independent pathway.

PP.247

Skin hyperemic response to compression

Seddone S, **Messere A**, **Roatta S**

University of Turin, Italy

A brief compressive stimulus is known to induce a prominent rapid hyperemia in skeletal muscles, which is considered to contribute to the initial phase of the functional hyperemia. Whether the same mechano-sensitivity characterizes the cutaneous circulation is presently not known due to conflicting evidence from animal studies and to the lack of human studies. This study aims to investigate whether a rapid hyperemic response to compressive stimuli is also expressed by skin blood flow in humans. In 12 healthy subjects, (9 males and 3 females, age: 30±9 yr) two sequences of brief compressive stimuli were delivered to the forearm by a PC-controlled pneumatic cuff at varying pressure (200, 100, 50-mmHg; 2 s) and duration (1, 2 and 5 s; 200-mmHg); both series were repeated with the arm above and below heart level. By positioning the laser-Doppler probe underneath the cuff, cutaneous blood flow of the compressed skin could be monitored. The response was described in terms of peak blood flow normalized to baseline (nCBFpeak), excess blood volume received during the response and time-to-peak, from the release of compression. The results consistently evidenced the occurrence of a compression-induced hyperaemic response, with nCBFpeak = 2.9 ± 1.1, EBV = 17.0 ± 6.6, time-to-peak = 7.0 ± 0.7 (200 mmHg, 2 s, below heart level). Both nCBFpeak and EBV were significantly reduced (by about 50%) above compared to below heart level ($p<0.01$). To a lesser extent EBV also increased with increasing pressure ($p<0,05$) and duration ($p<0,01$) of the stimulus. For the first time, the rapid dilatatory response to compressive stimuli has been demonstrated in human cutaneous circulation. The functional meaning of this response remains to be elucidated.

PP.248

Effect of low-density lipoproteins (LDL) on lymphatic vessel intrinsic contractility

Solari E, **Marcozzi C**, **Negrini D**, **Moriondo A**

Department of Medicine and Surgery, University of Insubria, Varese, Italy

Lymph formation and propulsion along the lymphatic network are sustained by an extrinsic mechanism, due

to mechanical stresses arising in the interstitium surrounding the vessel itself, and an intrinsic one, due to the spontaneous contractions of the lymphatic muscle (LM) in the vessels wall. In the diaphragm, lymphatic vessels located at the muscular periphery display a spontaneous contractile activity, due to a well organised LM mesh. This intrinsic contractility can be modulated by interstitial molecules such as plasma low density lipoproteins (LDL) which, after crossing the vascular endothelium, enter the interstitium and are bound to LDL receptors on LM. In vivo experiments were performed on Wistar rats by microinjecting a 9.2 nl bolus of 0.25µg/µl of LDL in PBS into the interstitium next to the intrinsically contracting diaphragmatic lymphatic sites, which gave rise to an increase in contraction frequency (fc) of about +125% and a reduction in contraction time compared to PBS injected controls. LDL had no significant effect on the resting diameter, but significantly reduced the contraction amplitude of about -25%. Despite the consequent LDL-related stroke volume reduction, the increased fc played the pivotal role in determining lymph flow, which was found to be 64% larger than control. Data from this study indicate that LDL have a phasic, but not tonic, effect on LM intrinsic contractility and represent the first mechanical description of how the increase in lymph flow could be attained by an acute exposure of lymphatic vessels to LDL.

PP.249

Physiological levels of sympatho-chromaffin Chromogranin A exert potent cardioprotection against doxorubicin-induced cardiotoxicity

Rocca C[1], Scavello F[1], Colombo B[3], Gasparri AM[3], Dallatomasina A[3], Granieri MC[1], Amelio D[1], Pasqua T[1], Cerra MC[1,2], Tota B[1,2], Corti A[3] Angelone T[1,2]

[1]Department of Biology, E. and E.S., University of Calabria, Rende (CS), Italy; [2]National Institute of Cardiovascular Research (INRC), Bologna, Italy; [3]San Raffaele Vita-Salute University and IRCCS San Raffaele Scientific Institute, Milan, Italy

Doxorubicin (Doxo), an anti-cancer chemotherapeutic drug, is widely used for the treatment of various tumors, but its dose-dependent cardiotoxicity represents a severe limitation in its clinical application. The effects of physiological doses of CgA, a cardio-regulatory protein released in the blood by the neuroendocrine system and by the heart itself, on Doxo-induced cardiotoxicity and anti-tumor activity were investigated. The study was performed by using in vivo and ex vivo rat models, and murine models of melanoma, fibrosarcoma, lymphoma and lung cancer. CgA plasma levels after Doxo treatment were also measured. In vivo and ex vivo studies in the rat model showed that low-dose CgA can efficiently prevent Doxo-induced cardiotoxicity, as indicated by the marked reduction of Doxo-elicited heart inflammation, oxidative stress, apoptosis, fibrosis, and ischemic injury. In all murine models considered, CgA did not impair the anticancer activity of Doxo. On the other hand, Doxo could reduce the intra-cardiac expression and release of CgA in the blood, i.e. of an important cardioprotective agent. Overall, these results suggest that Doxo can reduce the levels of CgA, an endogenous cardioprotective agent, and that systemic administration of low-dose exogenous CgA can restore cardioprotection. Administration of low-dose CgA to patients with low levels of endogenous CgA might represent a novel pharmacological frontier to limit the cardiac damage typically associated with anthracycline therapy without impairing anti-tumor effects.

PP.250

The effect of the preimplantation factor on the cardiac expression of miR-21 in radiationinduced heart disease

Kovács MG[1], Kiscsatári L[2], Varga Z[2], Kővári B[3], Fábián G[2], Cserni G[3], Thum T[4], Csont T[1], Bátkai S[4], Barnea E[5], Kahán Z[2*], Sárközy M[1*]

[1]Department of Biochemistry, University of Szeged, Hungary; [2]Department of Oncotherapy, University of Szeged, Hungary; [3]Department of Pathology, University of Szeged, Hungary; [4]Hannover Medical School, IMTTS, Hannover, Germany; [5]BioIncept LLC, Cherry Hill, New Jersey, USA

A late-onset side effect of thoracic radiotherapy is the development of radiation-induced heart disease (RIHD). It often leads to heart failure with preserved ejection fraction and fibrosis. An elevated level of miR-21 is commonly associated with chronic inflammation and cardiac fibrosis in other heart failure models. Preimplantation factor (PIF) secreted by embryos under physiological conditions was shown to be protective in graft versus host disease due to its antioxidant and anti-inflammatory properties. Here, we aimed to investigate the effect of PIF on the development of RIHD and the cardiac expression of miR-21.In our present experiments, male Sprague-Dawley rats were divided into three groups: 1) control, 2) selective heart irradiation with a single dose of 50 Gy 3) selective heart irradiation and PIF-treatment (1 mg/kg/day for 2 weeks then 0.3 mg/kg twice a week). All groups were followed-up for 19 weeks. At the end of the experiment, cardiac morphology and function were monitored by transthoracic echocardiography. Cardiac hypertrophy and fibrosis were assessed by histology (HE and picrosirius red stainings, respectively). Cardiac expression of miR-21 was measured by qRT-PCR. Echocardiography and histology revealed diastolic dysfunction, left ventricular hypertrophy with preserved ejection fraction, and mild interstitial fibrosis

in the irradiated group. PIFtreatment could significantly ameliorate the development of cardiac hypertrophy and fibrosis. Cardiac miR-21 expression was significantly increased in the irradiated group (2.35±0.70 vs. 0.63±0.16, p<0.05) which was markedly reduced by the PIF-treatment (0.26±0.04 vs. 2.35±0.70, p<0.05). In conclusion, PIF can be a radioprotective agent of great promise in the prevention of the development of RIHD.

PP.251

Phoenixin14 induces cardioprotection as post-conditioning agent in rats with high-fat diet induced obesity

Pasqua T[1], Rocca C[1], Granieri MC[1], Palasz A[2], Cerra MC[3,4], Angelone T[1,4]

[1]Department of Biology, E. and E.S., University of Calabria, 87036 Rende (CS), Italy; [2]Department of Histology, Medical University of Silesia, ul. Medyków 18, 40-752, Katowice, Poland; [3]Department of Biology, E. and E.S., University of Calabria, 87036 Rende (CS), Italy; [4]National Institute of Cardiovascular Research (INRC), Bologna, Italy.

Phoenixin (PNX) is a novel peptide identified for the first time in 2013 via a bioinformatic approach. A major site of PNX expression is hypothalamus, i.e. the paraventricular and supraoptic nuclei, and the cells of the median eminence. This distribution partially overlaps that of Nesfatin-1, an anorexigenic peptide with a cardiomodulatory role. PNX is negatively associated with anxiety in obese men. Very recently, we demonstrated the expression of PNX in the heart and its peripheral function as a cardiomodulatory and cardioprotective agent after Ischemia/Reperfusion (I/R) injury in normoweight rats. To date, PNX role in cardioprotection under obese conditions is unexplored. In this study, by using an obese rat model, we aimed i) to detect PNX in the ischemic heart by ELISA, ii) to evaluate the ability of the peptide to protect the heart against I/R injury by using Langendorff method and iii) to study its intracellular signaling. In obese rats, the cardiac expression of PNX after I/R was reduced respect to the normoweight counterpart. PNX, when administered in postconditioning, reduced the infarct size and recovered the systolic pressure. By WB analysis, preliminary information indicated that PNX-dependent cardioprotection is mediated by the phosphorylation of Akt and AMPK, a cellular energy homeostasis sensor, and by the involvement of the mitophagy regulator PTEN-induced kinase 1 (PINK1). In addition, PNX modulated apoptotic indexes. Altogether, these data revealed the important cardioprotective effects of the exogenous PNX in the presence of a deteriorated cardiac performance typical of obesity, presumably by offsetting the reduced expression of the endogenous protein.

PP.252

The potential role of miR-125b and its target chemokine ligand 21 (CCL21) in the development of uremic cardiomyopathy

Kovács Z[1], Sárközy M[1], Gáspár R[1], Siska A[2], Kővári B[3], Szabó M[1], Bodai L[4], Zsindely N[4], Cserni G[3], Földesi I[2], Thum T[5], Bátkai S[5], Csont T[1]

[1]Department of Biochemistry, University of Szeged, Hungary; [2]Department of Laboratory Medicine, University of Szeged, Hungary [3]Department of Pathology, University of Szeged, Hungary [4]Department of Biochemistry and Molecular Biology, University of Szeged, Hungary [5]IMTTS, Hannover Medical School, Germany

Uremic cardiomyopathy is a common cardiovascular complication of chronic kidney disease (CKD). It is commonly presented as heart failure with preserved ejection fraction (HFpEF). Previous studies described the role of microRNA-125b (miR-125b) in the development of cardiac hypertrophy induced by pressure-overload and cardiac fibrosis induced by angiotensin-II in rodents. Therefore, here we investigated the effect of CKD on the left ventricular (LV) expression of miR-125b and its target gene expression changes. CKD was induced by 5/6 nephrectomy in male Wistar rats (250-300 g). Nine weeks later serum urea and creatinine levels were measured to verify the development of CKD and transthoracic echocardiography was performed to monitor cardiac morphology and function. Hypertrophy and fibrosis were also investigated by histology. LV miR-125b expression was measured by qRT-PCR. Next-generation sequencing (NGS) was performed to investigate the LV gene expression changes in response to CKD. Then hypertrophy- and fibrosis-associated target genes of miR-125b were selected among the genes showing significant LV expressional changes in response to CKD. In CKD, serum urea and creatinine levels were significantly higher, LV anterior and septal walls were significantly thicker, E/e' was significantly increased referring to diastolic dysfunction, histology showed LV hypertrophy and interstitial fibrosis, LV miR-125b was significantly repressed (2.02±0.76 vs. 4.50±0.65, p<0.05) and NGS revealed overexpression of the CCL21 gene among others. LV repression of miR-125b and overexpression of its inflammatory target gene CCL21 might play a role in the development of uremic cardiomyopathy. However, further molecular measurements are needed to prove this hypothesis.

PP.253

Comparison of Sertraline Usage on Human and Rat Atrium

Solak Gormus ZI[1], Solak H[1], Ozen Koca R[1], Gormus N[2]

[1]Department of Physiology, [2]Department of Cardiovascular Surgery, University of Necmettin Erbakan, Meram Medical School, Turkey

Patients who underwent cardiac surgery may have depression in the early postoperative period so they may need antidepressant drugs for prevention and treatment of depression. We aimed to investigate and compare in vitro effects of sertraline which is a selective serotonin reuptake inhibitor agent on human and rat atrium muscle contractility. Human- rat atrium tissue strips of 3-4 millimeters (n=28) (the patients between 47 to 72 and wistar albino rats 10 months old) were placed in isolated organ bath. Adrenaline 10-1 was administered in tissue cabs for producing isometric contractions. Contraction width measurements were used as contraction parameters. In both groups cumulative sertraline (10-9 to 10-4) doses were added in organ baths. Inhibition of contractions was statistically significant for 10-7-10-4M doses of sertraline following the initial administration of adrenaline both in human and rat atriums. Also, statistically significant inhibition of contraction occured at 10-6, 10-5, 10-4 M doses when compared with 10-9, 10-8, 10-7 M doses of sertraline. Sertraline may cause negative inotropic effects on human and rat atrium muscle. This negative effect is markedly seen when sertraline doses are augmented. Therefore, sertraline may be carefully used in cardiac surgery performed patients especially in early postoperative period. In order to reduce the risk of postoperative depression preoperatively, more studies are needed in preoperative period and also, we made crosscheck human atrium results with rat atrium.

PP.254

Inotropic Effects of Combined $MgSO_4$ and Sertraline Administration on Rat Atrium

Fidan E, Solak Gormus ZI

NEU Meram Faculty of Medicine, Department of Physiology, Konya TURKEY

The Aim of study was to determine the synergistic effects of Magnesium, an antioxidant ion combined with selective serotonine reuptake inhibitor sertraline in both anxiety/ depression treatment and prevention of early depression and regulation of inotropic potential of heart. The study was carried out in Necmettin Erbakan University Meram Medical Faculty Physiology Department Laboratory using experimental animals obtained from Experimental Medicine Research and Application Center. Adult Wistar Albino rats (10 months old, weighing 200-300 grams) were used. There are 6 groups including 40 animals and their distribution was determined randomly. In first 4 groups, under light ether anesthesia plasma from the hearts of animals was separated and stored at -20 ° C. At the same time, clotting times were investigated by help of lancet. 3-4 millimeters long strips were cut from atrium, placed in the hooks of isolated organ bath in Krebs solution in the transverse plane and the tension was adjusted to 2 grams. Isometric contractions were induced with 0.001 mM adrenaline and group 1 was observed only. Cumulative sertraline (10-9 and 10-4 M) to group 2, cumulative MgSO4 to group 3 (0.1, 1, 2, 4, 8,10 M), cumulative sertraline and cumulative magnesium sulfate to group 4 were added to baths. Group 5 received 10 mg/ kg / day sertraline injection for twenty-nine days before decapitation and to group 6 MgSO4 injection of 20 mg / kg / day for sixteen days. The 5th and 6th groups were subjected to the same procedures as control group after decapitation. Changes in weight, clotting time, biochemical findings and stresses were evaluated statistically. The relationship between control group and cumulative magnesium sulphate was significant when 4M magnesium sulfate was given. There was no significant difference between control group and cumulative sertraline group. The tension relationship between cumulative sertraline and cumulative magnesium groups was significant on 10-5 sertraline and 8 M magnesium sulfate. A rapid inhibition of strain was observed in isolated organ bath, where cumulative sertraline and cumulative magnesium sulfate were given in succession. The effects of sertraline on spontaneous contractions of 29 days and magnesium after 14 days of injection were found to be statistically significant. When clotting times were compared with each other, it was found that clotting times were limited to seconds and statistically significant decrease was observed in MgSO4 treated group. Combined usage of high dose Mg with antidepressants for pre/post operative depression may cause fatal risks. Magnesium may help sertraline to inhibit vasospasm in arteriolar grafts after coronary bypass surgery. However, shortening clotting time may increase the risk of embolism. In order to reduce the risk of post-operative depression preoperatively, more studies are needed to be considered.

PP.255

Trimethylamine N-oxide fails to impact viability, ROS production and mitochondrial membrane potential of adult rat cardiomyocytes

Querio G[1], Antoniotti S[1], Levi R[1], Gallo MP[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Italy

Trimethylamine N-oxide (TMAO) is an organic compound derived from dietary choline and L-carnitine, or by direct intake through fish consumption. Several studies underline that TMAO has different biological functions: when present in physiological concentrations it behaves as an osmolyte, a protein stabilizer and an electron acceptor. Recent works pointed out that high circulating levels of TMAO are involved in the development of cardiovascular diseases underlining its role in the progression of atherosclerotic lesions and cardiac outcomes. Nevertheless, studies on a direct role of TMAO on cardiomyocytes parameters are still limited. This work focuses on the effects of TMAO, alone or in combination with known insults, on isolated adult rat cardiomyocytes. TMAO 100µM and 10mM, for 1h and 24h, does not affect physiological cardiomyocytes parameters such as cell viability, sarcomere length, intracellular ROS and mitochondrial membrane potential. Furthermore, in a simultaneous treatment for 24h with Doxorubicin or H2O2, TMAO 100 µM does not exacerbate or counteract the damaging effect of both insults. In conclusion, no cardiotoxic effects of TMAO were detectable on isolated adult rat cardiomyocytes, underlining that in our experimental model the molecule cannot be considered a direct cause or an exacerbating risk factor of cardiac damage.

PP.256

Comparison of Saphena with Sertraline and Alarin On Human

Solak Gormus ZI[1], **Ozen Koca R**[1], **Solak H**[1]

[1]Department of Physiology, University of Necmettin Erbakan, Meram Medical School, Turkey

Alarin is a novel endogenous vasoactive newfound neuropeptide.Oriented information of vascular effects of alarin has been obtained from microvascular dermis vessels.Selective serotonin reuptake inhibitor group of antidepressant agents are commonly used in patients with cardiovascular disorders in order to diminish anxiete or depression.However both of their effects on saphena are not investigated particularly in vitro.We aimed to compare the effects of sertraline and alarin on human saphena muscle contractility with claimed indication.Human saphena tissues(n=28)were taken from the cardiac surgery performed cases for coronary bypass surgery.The patients ages were between 47-72.Saphena tissues were sectioned into 3-4 mm long rings.Rings were placed in organ baths containing Krebs solution, thermoregulated 37oC and aerated(95%O2,5%CO2).Changes in isometric tension were recorded.Phenylephrine(PE)10-6M was administered in tissue cabs for producing isometric contractions.Contraction with measurements were used as contraction parameters.Cumulative sertraline(10-9-10-3)and cumulative alarin(10-8-105)doses were added in organ baths.The contractions were recorded accordingly.Friedman-Kruskal Wallis tests were used for statistical evaluation.Amplitudes of contractions were determined and used for statistical analyses.Alarin inhibited contraction 10-5M and sertraline 10-4-10-3M concentration dose following PE administration($p<0.05$).Both significant effects of sertraline and alarin occured in a dose dependent manner.This means we can use sertraline and alarin in these doses confidently.These findings have a potential to contribute to studies owing to effect of alarin/sertraline on cardiovascular system.Further studies are needed to clarify the mechanism(s) of alarin/sertraline.

PP.257

Interrupted administration of sevoflurane improves circulating levels and functional properties of endothelial progenitor cells in patients undergoing coronary angioplasty

Vlad A[1,2], **Niculescu L**[3], **Stancu C**[4], **Popescu M**[1,2], **Stanca I**[2], **Corneci D**[1,2], **Ceafalan L**[1,4], **Gilca M**[1], **Surcel M**[4], **Popescu AC**[1,2], **Dimulescu D**[1,2]

[1]'Carol Davila' University of Medicine and Pharmacy, Romania;[2]'Elias' University Emergency Hospital, Romania; [3]'Nicolae Simionescu' Insititute of Cellular Biology and Pathology, Romania; [4]'Victor Babes' National Institute, Romania

Endotehlial progenitor cells (EPCs) are important players in the physiologic reendothelialization following percutaneous coronary intervention (PCI) and volatile anaesthetics stimulate survival and functional properties of stem cells. We investigated the effect of sevoflurane (Sevo) on EPCs circuating levels in patients undergoing PCI and the viability and adhesion of these cells. EPCs were quantified by flow-cytometry and culture assay before exposure to sevoflurane (baseline) and 24 h afterwards, in blood samples from patients scheduled for PCI. 7-days old cultured EPCs were exposed in vitro to sevoflurane 2% or 4% in air/5% CO2, or only to air/5% CO2 (control) for 2 h in a modular chamber. Proliferation, apoptosis and adhesion on monolayers of HUVEC were evaluated 24h later. Plasma CD45dim/CD34+/KDR+, CD45dim/CD34+/KDR+/CD133+ and CD45dim/CD34+/CXCR4+ mononuclear cells and the number of cultured DilAcLDL+/FITC-UEAI+ cells were higher in Sevo treated patients vs. control group at 24 h post-exposure vs. baseline ($p<0.01$ and $p<0.05$ respectively). In vitro exposure to Sevo raised EPCs proliferation (MTS assay: $n = 12$, $p < 0.01$ for 2% Sevo, $p < 0.001$ for 4% Sevo; DilAcLDL+/ FITC-UEAI+ cells counting: $n = 4$, $p < 0.01$ for 2% Sevo, $p < 0.001$ for 4% Sevo), diminished apoptosis ($n = 5$, $p < 0.05$ for 2% and 4% Sevo), and improved EPCs adherence to HUVEC

monolayers (n = 4, p < 0.05) vs. control. Thus, Sevo augments the levels of circulating EPCs in the late window of protection and their function, revealing a potential improvement of endothelial regeneration and prevention of in-stent restenosis following PCI by anesthetic preconditioning.

PP.258

Protective effect of delayed phase of remote preconditioning in the rat heart is not blunted by acute STZ diabetes

Ravingerova T[1], Murarikova M[1], Kindernay L[1], Farkasova V[1], Ferko M[1], Duris Adameova A[1,2]

[1]Institute for Heart Research, Centre of Experimental Medicine, Slovak Academy of Sciences; [2]Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Second phase of remote preconditioning (2RPC) has a potential of being used under clinical conditions of heart ischemia/reperfusion (I/R) injury. Protective signaling to distant targets is mediated via activation of cell "survival" RISK cascades. Diabetes mellitus (DM) as a comorbidity blunts the efficiency of conditioning interventions in humans. However, the effect of 2RPC has not been sufficiently studied in a setting of DM. We aimed to clarify whether acute (1w) DM (STZ, 65 mg/kg, i.p.) modifies the effect of 2RPC on I/R injury and affects "survival" signaling in the preconditioned myocardium. RPC was induced by 3 cycles of 5-min inflation/deflation of pressure cuff placed on hind limb of adult male Wistar rats. After 24 h, Langendorff-perfused hearts of all groups were exposed to 30-min global I/120-min R. Size of infarction (IS, TTC staining, in % of area at risk) and recovery of function (LVDP) served as indicators of myocardial injury. RISK protein levels were measured by WB. In non-DM hearts, 2RPC significantly decreased IS (by 65%) and improved LVDP recovery (by 52%). In nonpreconditioned diabetics, similarly lower extent of lethal injury was observed (IS: 16,6 ± 1,6%, vs. 33 ± 3,0% in the non-DM controls). That was associated with a better LVDP recovery and higher protein levels of phospho-Akt, eNOS and PKCε. In the diabetics, 2RPC failed to further reduce IS (16,4 ± 1,2%) and improve LVDP recovery, as well as to further up-regulate RISK proteins, different from non-diabetics. The results suggest that in the acutely diabetic heart, protection by 2RPC is not attenuated but may be masked by activation of similar "survival"mechanisms. Grants VEGA 2/0141/18, 2/0151/17, APVV-15-0607, APVV-15-0119, ITMS 26230120009 and EU Cardioprotection COST Action CA16225.

PP.259

Venous Pulse Wave Velocity

Ermini L[1], Pastore L[2], De Benedictis C[2], Ferraresi C[2], Roatta S[1]

[1]Dept. Neuroscience Sect. Physiology, University of Turin, Italy; [2]Dept. Mechanical Engineering, Polytechnic of Turin, Italy;

Central venous pressure and volume status are relevant parameters for the characterization of the patient's hemodynamic condition and for the management of fluid therapy however, their invasive assessment is affected by various risks and complications while non-invasive approaches provide only imprecise and subjective indications. Aim of the present study is to explore the possibility to assess changes in venous pressure from changes in the venous pulse wave velocity (vPWV). In 9 healthy subjects, pressure pulses were generated artificially in the veins by a PC-driven rapid inflation of a pneumatic cuff (300mmHg in <1sec) placed around a foot. Passage of the pulse wave in the superficial femoral vein distally to the inguinal ligament was detected by Doppler flowmeter and the latency from the pressure stimulus was measured. The vPWV was then calculated as the ratio between traveling distance and latency. Changes in leg venous pressure were obtained by raising the trunk of the subject from the initial supine position by 30 and 60 deg. In each position 15 pressure pulses were delivered every 30 s, at the end-expiratory phase for vPWV assessment. Venous pressure in the leg was non-invasively estimated by assessing the point of collapse of the jugular or axillary vein. The vPWV increased from 1.64±0.06(supine) to 2.13±0.26 (60 deg) (Student's ttest, p<.01) and exhibited a very strong correlation with leg venous pressure (overall r=0.76). Differences in vPWV among the three positions were statistically significant also on an individual basis in 8/9 subjects (ANOVA + Tukey's HSD post-hoc, p<.01). These preliminary results show that vPWV may be easily assessed in healthy subjects and may constitute a good non-invasive indicator of venous pressure changes.

PP.260

Platelet function and autonomic nervous system dysregulation in newly diagnosed hypertensive condition

Grossini E[1], De Zanet D[1], Migliavacca S[2], Pirisi M[2], Rolla R[3].

[1]Department of Translational Medicine, Laboratory of Physiology, University East Piedmont, Italy; [2]Department Translational Medicine, Internal Medicine Unit, University East Piedmont, Italy; [3]Department Health of Sciences, Clinical Chemistry, University East Piedmont, Italy

Platelets can contribute to the onset of hypertension not only through the induction of a prothrombotic condition but also by interfering with endothelial function. The autonomic system, which is widely known to be involved in the onset of hypertension too, can modulate platelets function. In spite of this, the relationship between platelet activity and sympathetic/parasympathetic drive in subjects at firstly diagnosed hypertension has not been clearly described, yet. In a total of 99 normal subjects, arterial blood pressure was monitored by 24h holter monitoring. Lipidic and glicidic profile and body mass index (BMI) were quantified. In addition, platelet activity was measured by means of various stimulation tests. A 6-min electrocardiogram registration was taken for further analysis of heart rate variability (HRV), which was performed in the time domain (HRV, the heart rate beat-to beat variance) and in the frequency domain (Very Low Frequency, VLF, Low Frequency, LF, and High Frequency, HF, components). Sympathetic, parasympathetic and stress index were calculated, as well. Among the 99 subjects (M:31; F: 68, mean age 54), 59 were hypertensives. Hypertensives and non-hypertensives did not differ as regarding age, sex, smoke. BMI and HRV variables related to sympathetic activation were higher in hypertensives. In addition, the ristocetininduced platelet aggregation and the thrombin receptor activating peptide-6 platelet aggregation tests shown higher responses in hypertensives versus non-hypertensives. In conclusion, the dysregulation of autonomic nervous system and an increased trend of platelet aggregation could play a physio-pathological role in the onset of newly diagnosed hypertensive condition. Relation to endothelial dysfunction could be hypothesized.

PP.261

Effect of Synchronized Muscle Contraction and Heartbeat on Blood Flow.

Giuriato G[1], Ruzzante F[1], Tarperi C[1], Bortolan L[1], Cevese A[1], Schena F[1], Venturelli M[1,2].

[1]Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Italy; [2]Department of Internal Medicine, University of Utah, USA

Blood flow (BF) to exercising muscles is susceptible to variations of intensity and duration of skeletal muscle contractions, cardiac cycle and arterial pulse wave (PW). During cyclic muscle contractions, these elements increase proportionally without an optimal matching, thus affecting BF. To maximize BF to the contracting muscle, we synchronized PW with electricallyevoked contractions of the quadriceps. In 10 young healthy participants (26±3 years), short rhythmic (200 ms) unilateral muscle contractions were evoked matching the peak of PW measured with an ultrasound doppler at the femoral artery (in-phase; IP). BF to the contracting muscle was compared with muscle contractions evoked matching the retrograde or minimal level of the PW (out-phase; OP). During steady-state exercise, in the presence of OP contractions, BF was significantly higher ($p<0.001$) 444 ± 84 ml/min compared to IP contractions 514 ± 96 ml/min. The results of the current study suggest that muscle contractions that doesn't interfere with the peak of PW facilitate the maximization of muscle perfusion, and a consequent increase in O2 delivery to contracting muscle. Moreover, the synchronization of skeletal muscle contractions and the PW may be a winning strategy to maximize skeletal muscle perfusion in populations with severe exercise limitations such as individuals with spinal cord injury.

PP.262

Effect of long-term passive stretching of the knee extensor and plantar flexor muscles on vascular function

Esposito F, Bisconti AV, Longo S, Shokohyar S, Rampichini S Borrelli M, Doria C, Limonta E, Coratella G, Cè E.

Department of Biomedical Sciences for Health, Università degli Studi di Milano, Italy

During each single bout of passive stretching (PS), blood flow (BF) in the feeding artery of the muscle involved in the maneuver decreases, then increasing during relaxation. These PS-induced BF changes may improve vascular function after long-term PS administration due to repetitive stimuli applied to the artery's wall. To this purpose, 36 participants (age: 23±2 yrs; stature: 1.68±0.12 m; body mass: 62±15 kg) were randomly assigned to PS (n=18; 6 wks, 5 times/w) or control group (CTRL, n=18). Before and after PS of the knee extensor and plantar flexor muscles or after a similar resting period in CTRL, vascular functionality of the arteries directly involved in (femoral and popliteal arteries) and distal to (brachial artery) PS administration was assessed by duplex eco-doppler. Single passive limb movement (SPLM) assessed the femoral artery BF changes. Flow mediated dilation (FMD) tested the popliteal and brachial arteries. SPLM peak BF (BFp) and maximum arterial vasodilation (FMD%) were calculated. Pulse-wave velocity (PWV) was determined by tonometry to quantify central (carotid-femoral artery PWV, PWVCF) and peripheral (carotid-radial artery PWV, PWVCR) arterial stiffness. Systolic (SBP) and diastolic (DBP) blood pressure were also measured. In PS, BFp and popliteal FMD% increased after PS by 30% and 25%, respectively ($p<0.05$). Brachial FMD% increased by 8% ($p<0.05$). PWFCF and PWVCR decreased by 10% and 7%, respectively ($p<0.05$). No changes in SBP and DBP

occurred. CTRL did not show any significant alteration. Six weeks of PS intervention improved vascular function and reduced arterial stiffness, particularly in the feeding arteries of the muscles involved in PS. Therefore, PS could be used as an effective means to passively improve vascular functionality.

PP.263

Non-genomic effects of calcitriol (1,25-dihydroxyvitamin D3) on transient receptor potential canonical 3 channels (TRPC3) in cardiac ventricular fibroblasts

Nakhl S[1,2], Saliba Y[1], Chahine R[2], Fares, N[1]

[1]Laboratory of Physiology and Pathophysiology, Faculty of Medicine, Saint Joseph University of Beirut, Lebanon; [2]Faculty of Medical Sciences, Lebanese University, Beirut, Lebanon.
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Recent data support rapid non-genomic responses of calcitriol in cardiac cells, by modulating membrane-based signaling pathways. Fibroblasts are main actors in cardiac remodeling where calcium (Ca2+) ions, through TRPC3 channels, play a pivotal role. Herein, we assessed the effect of calcitriol on TRPC3 channels in murine freshly isolated cardiac ventricular fibroblasts (CVF) using intracellular Ca2+ imaging and biochemical tools. Acute treatment of CVF with calcitriol elicited intracellular Ca2+ oscillations blocked by the pan-TRPCs inhibitor SKF96365. Specific TRPC3 pharmacological inhibition (pyr10) and knockdown (siRNA) blunted calcitriol inducedCa2+ entry whereas channel activation by GSK1702934A enhanced these oscillations. In addition, these oscillations persisted after inhibiting other potential extracellular sources of calcium entry. To rule out the contribution of the endoplasmic reticulum in these Ca2+ oscillations, the latter was depleted by CPA and the inositol triphosphate receptor was inhibited by 2-APB. Furthermore, plasma membrane insulation with Gd3+ completely abolished Ca2+ oscillations. Finally, VDR was essential in TRPC3-mediated calcitriol-induced Ca2+ oscillations. These findings give evidence for non-genomic effects of calcitriol in CVF in the normal heart.

PP.264

Arterial stiffness in obese adolescents: contribution of peripheral circulation

Czippelova B[1], Turianikova Z[1], Krohova J[1], Mazgutova N[1], Wiszt R[1], Lazarova Z[1], Pozorciakova K[2], Ciljakova, M[2,3], Javorka M[1]

[1]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Department of Physiology and Biomedical Centre Martin, Martin, Slovakia; [2]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin and University Hospital Martin, Clinic of Children and Adolescents, Martin, Slovakia; [3]National Endocrinological and Diabetes Institute in Lubochna, Slovakia

Obesity is a risk factor contributing to the development of atherosclerosis. Index CAVI (Cardio-ankle vascular index), estimating arterial stiffness, is increasingly used for the early atherosclerotic changes assessment. Several recent studies surprisingly found a negative correlation between CAVI and Body Mass Index (BMI). In our previous study, we found that lower CAVI values found in obese adolescents could be associated with decreased peripheral vascular resistance modulated by reduced sympathetic activity. The VaSera device enables the measurement of CAVI and kCAVI (knee CAVI). The CAVI is measured between heart and a. tibialis, whereas kCAVI is measured between the heart and the a. poplitea. Thus, kCAVI includes smaller part of the peripheral circulation than CAVI. The aim of our study was to assess the contribution of peripheral circulation on arterial stiffness differences between obese patients and controls by comparison of CAVI and kCAVI behaviour. We examined 29 obese (14f, age 16.44 ± 2.7 y., BMI: 33.31 ± 4.4 kg.m-2) and 29 nonobese gender and age matched adolescents (BMI: 21.01 ± 2.3 kg.m-2). Arterial stiffness indices CAVI and kCAVI were measured using VaSera VS-1500 (Fukuda Denshi, Japan). We found significantly lower values of both indices CAVI (CAVIob = 4.59 ± 0.88 vs. CAVIcont = 5.18 ± 0.63, p = 0.005) and kCAVI (kCAVIob = 4.77 ± 0.77 vs. kCAVIcont = 5.18 ± 0.56, p = 0.024) in obese group. In non-obese volunteers we found no significant difference between CAVIcont and kCAVIcont (p = 0.914), while in obese group kCAVIob was significantly higher than CAVIob (p < 0.001). Significant difference between CAVI and kCAVI in obese adolescents points to a substantial effect of vasodilation in the peripheral part of arterial tree in the lower limbs on CAVI values.
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PP.265

Effect of cerium oxide on ischemia reperfusion injury in skeletal muscle rats

Kucuk A[1], Polat Y[2], Sungu N[3], Kilicarslan A[3], Kartal H[4], Tatar T[5], Arslan M[6]

[1]Department of Physiology, Kutahya Health Sciences University Turkey; [2]Department of Cardiovascular Surgery, Mehmet Akif Ersoy Thoracic and Cardiovascular Surgery Training and Research Hospital, Istanbul-Turkey; [3]Department of Pathology, Yildirim Beyazit University Ankara Turkey; [4]Department of Cardiovascular Surgery, Gulhane

Gulhane Education and Research Hospital, Ankara-Turkey; [5]Department of Cardiovascular Surgery, Gazi University AnkaraTurkey; [6]Department of Anaesthesiology and Reanimation, Gazi University, Ankara-Turkey

Cerium oxide is the oxide form of cerium, which has protective effects in ischemia reperfusion (IR) injury. In this study we want to investigate the effects of this rareearth metal on skeletal muscle tissue in rat lower extremity IR injury model. Total of 24 Wistar albino rats were divided into 4 groups (n: 6); Control group (C), Cerium oxide group (CO), IR group (IR) and IR group with cerium oxide (IR-CO). Cerium oxide administered intraperitoneally (0.5 mg.kg-1). The rat tissues were taken for histopathological and immune-histopathological evaluations after 2 hours of ischemia and 2 hours of reperfusion period. Data were analyzed with Kruskal-Wallis and Mann-Whitney U test. Endothelial caspase 8 enzyme activity was significantly higher in IR group than C, CO and IR-CO groups ($p<0.0001$, $p<0.0001$, $p=0.034$, respectively). In addition, IR-CO group was significantly higher than C and CO groups ($p=0.001$, $p=0.034$, respectively). Muscle caspase 8 enzyme activity was significantly higher in IR and IR-CO groups than in C group ($p=0.001$, $p=0.018$, respectively). Inflammation ($p<0.0001$, $p<0.0001$, $p=0.020$, respectively) and Myosit injury ($p<0.0001$, all) were significantly higher in IR group than C, CO and IR-CO groups (Table 2). Vascular dilatation was significantly increased in IR group compared to C and CO groups ($p=0.011$, $p=0.011$, respectively). Vascular congestion was higher in IR group than only C group ($p=0.007$). Our results confirm that, cerium oxide has protective effects against the skelatal tissue damage resulting from IR injury in rats.

PP.266

Sex-Related Differences of Blood Pressure in Older Ren-2 Transgenic Rats

Rauchová H, **Hojná S**, **Kadlecová M**, **Vaněčková I**, **Zicha J**

Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Men have higher incidence of hypertension compared with age-matched women until the onset to menopause. Distinct sex-related differences in hypertension were observed repeatedly not only in humans but also in experimental animals. The aim of our study was to compare older (8-10 months old) male and female heterozygous transgenic rats (TGR) harboring Ren-2 mouse gene, with their normotensive Hannover Sprague-Dawley (HanSD) controls. Mean (MAP), systolic (SBP), diastolic blood pressure (DBP) and heart rate were measured by a direct puncture of carotid artery under deep isofluran anesthesia (2.5 % isofluran) and in awaking animals (0.5 % isofluran). Thiobarbituric acid-reactive species (TBARS) formation was monitored as indicator of lipid peroxidation damage in heart, kidney and liver and intracellular content of reduced glutathione was determined as the main intracellular antioxidant. Furthermore, plasma cholesterol (including its high density lipid (HDL) and low density lipid (LDL) fractions), were estimated. We found significantly higher blood pressure (BP) only in male TGR. BP elevation of TGR was more evident in awaking animals (MAP: 133±7 vs. 171±5, SBP: 145±8 vs. 208±7 and DBP: 121±6 vs. 140±4 mm Hg). Surprisingly, relative heart and kidney weights were not different between TGR and HanSD rats. We did not find any significant differences in TBARS concentrations and levels of reduced glutathione in heart, kidney or liver as well as in plasma cholesterol and its HDL and LDL fractions. Our results confirmed that the older TGR exhibit a marked sexual BP dimorphism. The study was supported by an international research grant GACR 19-08260J and by an institutional support (RV0:67985823).

PP.267

Effect of smoking on endothelial function in healthy adolescents

Mazgutova N, **Czippelova B**, **Krohova J**, **Wiszt R**[1], **Turianikova Z**, **Kosutova P**, **Javorka M**

Department of Physiology and Biomedical Center Martin, Comenius University in Bratislava, Jessenius Faculty of Medicine, Martin, Slovak Republic

Tobacco smoking is a leading risk factor for the development and the progression of cardiovascular diseases. The mechanisms by which smoking results in cardiovascular events include the development of atherosclerotic changes with a hypercoagulable state and an increased risk of thrombosis. Endothelial dysfunction has been recognized as a hallmark of preclinical systemic atherosclerosis and represents a preclinical reversible stage of the atherosclerotic process. It begins during the first two decades of life, while smoking is one of the risk factors of its development. The smoking influences endothelial function (EF) by inducing oxidative stress, inflammation, platelet coagulation and impairing serum lipid profile. These mechanisms contribute to atherogenic vessel wall changes. The information on the EF in young smokers is still lacking. The aim of our study was to non-invasively examine EF through Reactive Hyperemia Index – RHI (EndoPAT, Itamar Medical Ltd, Caesarea, Izrael) in young healthy subjects in relation to smoking status. Total of 68 adolescents (42 females, 26 males; mean age 18.9 ± 2.6 years) were examined with EndoPAT device during

standardized conditions. Smoking status was determined by a questionnaire and it was subsequently validated by plasma cotinine with a cutoff value of 15 ng/ml. We found no statistically significant differences in the endothelial function represented by RHI between young healthy smokers and non-smokers ($p>0.05$), even after objectification of their self-reported smoking status by cotinine level. This study can be a basis for future research extended to a larger sample size and an inclusion of subjects with other comorbidities (e.g. obesity), where we expect a potentiation of risk factors in the development of endothelial dysfunction. Research supported by grants VEGA 1/0117/17, VEGA 1/0200/19 and ITMS project "BioMed Martin" no. 26220220187.

PP.268

The role of the mitochondrial permeability transition pore - regulating proteins in relation to metabolic preconditioning

Andelová N[1], Waczulíková I.[2], Szeiffová Bačová B[1], Farkašová V[1], Ravingerová T[1], Ferko M[1]

[1]Center of Experimental Medicine, Slovak Academy of Sciences, Institute for Heart Research, Bratislava, Slovak Republic; [2]Department of Nuclear Physics & Biophysics, Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovak Republic

Metabolic preconditioning (MP) induced by acute streptozotocin (STZ) diabetes mellitus (DM) belongs to experimental models which in several studies has been proven to show positive compensatory mechanisms contributing to cardioprotection. The mitochondrial permeability transition pores (mPTPs) are associated with the signaling pathway of the adaptation mechanisms of cardioprotective models. Due to the multienzyme character of mPTPs, proteomic analysis is a suitable tool for their characterization. The aim of the present study is to contribute to the explanation of the cardioprotective function of cardiac mitochondria at the level of mPTP regulation in relation to MP. The experiments were performed on isolated cardiac mitochondria of Wistar rats. Samples from healthy controls were compared to the MP model represented by acute DM induced 8 days prior to the planned experiment with a single dose of STZ (65 mg/kg, i.p.). Proteomic analysis was performed using 1D gel electrophoresis and nano-liquid chromatography followed by mass spectrometry. Although the abundance of the whole mPTP protein complex was suppressed in the MP group ($p = 0.048$), expressions of individual proteins expressed by fold change parameter were maintained (analysed using TREAT (t-tests relative to a threshold) procedure). Further, we explored the interconnection of the identified proteins involved in the regulation and structure of the mPTP complex by correlation analysis presented by heatmaps. We have demonstrated that the MP-treated protein group shows different mutual interactions than those found in the control group. In conclusion, positive modulation of proteins of mPTP is an important part of endogenous protective processes leading to myocardial adaptation to the energy load represented by MP. The work was supported by grants: APVV-15-0119, VEGA 2/0121/18, ITMS 26230120009.

PP.269

Inhibition of RAS components attenuates progression of heart failure and its adverse consequences on myocardial extracellular remodeling and PKC signaling in normotensive rats with aorto-caval fistula

Sykora M[1], Kratky V[2], Kopkan L[2], Tribulova N[1], Barancik M[1], Cervenka L[2], Szeiffova Bacova B[1].

[1]Institute for Heart Research, Centre of Experimental Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic; [2]Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Extracellular matrix (ECM) exerts high impact on heart function. Thus, we aimed to explore whether HF due to volume overload (VO) induces alterations in MMP2, SMAD2/3 and PKC signaling involved in ECM remodelation as well as changes that may be revealed by enzyme histochemistry. Moreover, we tested the impact of treatment suppressing RAS on examined targeted proteins. VO-HF was induced in male normotensive Hannover Sprague-Dawley (SDR) rats by creating an aorto-caval fistula (ACF) and heart response was examined 20weeks later. Sham-rats were compared with non-treated rats with ACF and those treated for 15-weeks with ACEi (trandolapril 6mg/l, p.o.) or ARB (losartan 200mg/l, p.o.). Left (LV) and right (RV) ventricular heart tissue was analyzed using western blot and enzyme histochemistry. BW was not affected by VO and treatment. HW, LVW and RVW were higher in ACF and treated rats vs SDR. Expression MMP2 was decreased in ACF a normalized in ARB in LV and RV. Myocardial pro-hypertrophic PKCd expression was higher in ACF in LV and RV while ACEi decreased in LV and both drugs in RV. Expression of cardioprotective PKCe was decreased in ACF, RV and LV, and normalized ARB in RV and ACEi in LV. Profibrotic SMAD2/3 pathway was suppressed in ACF. Glycogen phosphorylase and capillary associated 5-nucleotidase, alkaline phospatase and dipeptidyl peptidase IV activities were reduced due to ACF and it was attenuated by treatment. ACF did not affect collagen deposition in either strain vs sham rats. Hypertrophic and hypofibrotic phenotypes are induced

by volume overload in both rat strains. Inhibition of RAS components attenuates progression of VO and its adverse consequences. Supported by grants VEGA 2/0158/19, 2/0076/16; APVV 15-0119, 15-0376 and EU ITMS 26230120006.

PP.270

Heart Rate Variability Mechanisms Analysed by Multiscale Information Decomposition

Krohova J[1], Czippelova B[1], Turianikova Z[1], Mazgutova N[1], Wiszt R[1], Faes L[2,3], Javorka M[1]

[1]Department of Physiology and Biomedical Center Martin, Comenius University, Jessenius Faculty of Medicine, Slovakia; [2]Department of Energy, Information engineering and Mathematical models (DEIM), University of Palermo, Italy; [3]BIOtech Center of the Department of Industrial Engineering, University of Trento, Italy

Heart rate variability (HRV) – a marker of the cardiac autonomic nervous system control – results from the activity of several mechanisms operating across multiple temporal scales. To better characterize the mechanisms beyond HRV, the aim of our study was to evaluate the strength of information transfer among systolic blood pressure (SBP), heart rate (its reciprocal value – RR interval from ECG) and respiration volume (RESP) oscillations across multiple temporal scales. Seventy-eight healthy young volunteers (32 male, age range: 16.0 – 25.8 yrs.) participated in this study. We applied the multiscale partial information decomposition to quantify the amount of information transferred towards RR from SBP and RESP signals during supine rest, orthostasis (head-up tilt, HUT) and cognitive load (mental arithmetics, MA). The analysis was performed separately for raw data and slower oscillations. The unique transfer entropy from SBP to RR (a baroreflex influence) was significantly higher for the slower oscillations compared to raw data at rest and increased for raw data during both challenges. The unique transfer entropy from RESP to RR (a component of respiratory sinus arrhythmia independent of baroreflex) decreased during stress. The redundancy and synergy between RESP and SBP interacting with RR further elucidated mutual interactions among analysed signals. We conclude that the contribution of baroreflex and respiration in the HRV origin varies with the time scale. To better understand HRV, the measures quantifying the influence of major source signals (SBP or RESP) on RR signal (unique transfer entropies) together with interactions between sources – redundancy and synergy – should be assessed. Grants: VEGA 1/0117/17 and VEGA 1/0200/19 and “BioMed Martin” no. 26220220187.

PP.271

The Effect of Hypnosis on Systolic Blood Pressure

Sunar F[1], Solak Gormus ZI[2]

[1]Karatay University Faculty of Medicine, Department of Medical Education, Konya, Turkey [2]NEU Meram Medical Faculty, Department of Physiology, Konya, Turkey

Hypnosis is frequently used in numerous fields of complementary medical treatments. Blood pressure is an important vital sign. The changes manifest itself with hypo/ hypertension. Hypertension is a common and important health problem in society. Our aim is to evaluate the effect of hypnosis on blood pressure in healthy volunteers and to make a preliminary study for treatment of hypertensive patients. Healthy twelve volunteers, six women and six men, aged between 18 - 65 years were included after getting ethical permits and consent. The room selected for hypnosis was quiet and room temperature was standart to minimize the effects on blood presure. We used rapid hypnosis technique. Measurements were made under hypnonic trans of 10-15 minutes. Volunteers were awakened by countdown method. The non-parametric Wilcoxon Signed Tanks test was used as statistics to chary comparison test. $P<0.05$ was accepted as significant. The statistical results of all changes made by measurement of blood presure was found to be $p>0.05$. Although $p> 0.05$ was not significant in pre-hypnosis, we observed average 4 mmHg decrease of systolic blood pressure during hypnosis. As a result of our data, it can be a preliminary study to show that longer hypnosis can be effective in treatment of people with systolic hypertension. It will be appropriate to repeat this study with more people.

PP.272

The effect of lower body negative pressure on phase 1 cardiovascular responses at exercise onset in healthy humans

Fontolliet T[1], Fagoni N[2], Bruseghini P[2], Capelli C[3,4], Lador F[5], Moia C[1], Tam E[4], Bringard A[5], Ferretti G[1,2]

[1]Département d’Anesthésiologie, Pharmacologie et Soins Intensifs, Université de Genève, Switzerland, [2]Department of Molecular and Translational Medicine, University of Brescia, Italy, [3]Department of Physical Performances, Norwegian School of Sport Sciences, Norway, [4]Dipartimento di Scienze Neurologiche e della Visione, Università di Verona, Italy, [5]Division de Pneumologie, Département des Spécialités de Médecine, Hôpitaux Universitaires de Genève, Switzerland

We tested the hypothesis that vagal withdrawal and increased venous return interact in determining the rapid cardiac output response (Phase I) at exercise onset. We used lower body negative pressure (LBNP)

to increase blood dislocation to the heart by muscle pump action and simultaneously reduce resting vagal activity. At exercise start, we expected larger response amplitude for stroke volume and smaller for heart rate at progressively stronger LBNP levels, so that the cardiac output response would remain unchanged. Ten subjects performed 50 W exercise supine in Control condition and during -45 mmHg LBNP exposure. On single beat basis, we measured heart rate (HR), stroke volume (SV), and we calculated cardiac output (CO). We computed Phase I response amplitudes (A1) using an exponential model. SV A1 was higher under LBNP than in Control ($p < 0.05$). Conversely, the A1 of HR, was 23 ± 56 % lower under LBNP than in Control (although NS). Since these changes tended to compensate each other, the A1 for CO was unaffected by LBNP. The rapid SV kinetics at exercise onset is compatible with an effect of increased venous return, whereas the vagal withdrawal conjecture cannot be dismissed for HR kinetics. The rapid CO response may indeed be the result of two independent yet parallel mechanisms, as hypothesized, one acting on SV, the other on HR.

PP.273

Effects of wild-type and mutant forms of atrial natriuretic peptide on cardiac fibrosis in type-2 diabetes

Bakhos JJ, Saliba Y, Hajal J, Fares, N

Laboratory of Physiology and Pathophysiology, Faculty of Medicine, Saint Joseph University of Beirut, Lebanon.

Diabetic cardiomyopathy is a slow progressive disease; it begins with a mild systolic dysfunction, followed by fibrosis and ends with hypertrophy and heart failure. Atrial natriuretic peptide (ANP) exerts a cardioprotective effect in pathophysiological processes such as fibrosis, diabetes and heart failure. A mutant form of ANP (mANP) was found to possess enhanced physiological properties as compared to the wild-type one. mANP appears to be more resistant to degradation and clearance than ANP, but its involvement in cardiac protection remains largely unclear. In this study, ANP and mANP similarly decreased in vitro proliferation and collagen secretion of freshly isolated murine ventricular fibroblasts treated with high glucose. This was mediated through a modulation of cGMP/PKG signaling and subsequently SMAD2/3 pathway inhibition. In vivo, type-2 diabetic mice showed systolic dysfunction and hypertrophy that were significantly ameliorated with mANP as compared to ANP. In addition, TGF-β1-related SMAD2/3 signaling showed less pro-fibrotic pattern in diabetic mice treated with mANP. This was associated to an increase in cardiac cGMP/PKG pathway culminating in an improvement of fibrosis. Our study shows for the first time that mANP activates cGMP/PKG more than wild-type ANP, in heart, and inhibits the fibrotic signaling pathways of TGF-β1 in type-2 diabetes. mANP could constitute an interesting therapeutic tool in cardiovascular diseases management.

PP.274

Cardiovascular responses and baroreflex sensitivity during apnoea phase 1 in spinal cord injury

Camelio S[1], Morelli D[2], Taboni A[3], Vinetti G[4], Fagoni N[4], Ferretti G[3,4]

[1]School of Specialization in Sports Medicine, University of Brescia, Italy; [2]U.O.R.R.F., Casati Hospital, Passirana di Rho (Mi), Italy; [3]Department of Anesthesiology, Pharmacology, Intensive Care and Emergencies, University of Geneva, Switzerland; [4]Department of Molecular and Translational Medicine, University of Brescia, Italy

The first phase (ϕ1) of the human cardiovascular responses to apnoea implies a rapid decrease in systolic blood pressure (SBP) until a SBP minimum (Pmin) is attained. This is accompanied by an increase in heart rate (HR), interpreted as baroreflex attempt at correcting SBP fall. After Pmin attainment, SBP, total peripheral resistance (TPR) and stroke volume (SV) increase, reflecting overall sympathetic stimulation. In patients with spinal cord injury (SCI), we hypothesized different cardiovascular responses to apnoea for impaired autonomic system regulation. In this preliminary report, we show data on 9 patients (age 58.1±21.7) with different severity (2 complete and 7 incomplete lesions) and neurological level of SCI (4 cervical, 1 thoracic and 4 lumbar lesion), who performed apnoeas in supine position. Beat-by-beat SBP, mean blood pressure (MBP), HR, SV and TPR were continuously determined before and during apnoeas by a Portapres device. Baroreflex sensitivity (BS) in ϕ1 was determined as the slope of the linear HR versus MBP relationship before Pmin. At apnoea start (control), SBP and HR were respectively 134.1±27.9 mmHg and 77.0±10.1 bpm; the SV and TPR were 86.0±17.5 ml and 12.4±2.7 mmHg*min/l (HRUs). In ϕ1 (30.3±12.7 s), at Pmin, SBP was 95.2±28.5 mmHg and SV was 58.9±16.0 ($p<0.05$ versus control), while HR and TPR did not change. At ϕ1 end, HR was 80.0±12.7 bpm and TPR was 14.4±3.4 HRUs (NS versus control); SBP returned to control value (133.3±39.5 mmHg). BS, detected in 5 out of 9 patients, was -0.37±0.16 beats/min*mmHg. We conclude that the tested hypothesis cannot be dismissed after these results.

PP.275

Search for the source of the retinal relaxing factor

Vanden Daele L[1], Boydens C[1], Devoldere J[2], Remaut K[2], Van de Voorde J[1]

[1]Department of Pharmacology, Ghent University, Ghent, Belgium, [2]Laboratory of General Biochemistry and Physical Pharmacy, Ghent University, Ghent, Belgium

The retinal relaxing factor (RRF) is an unidentified paracrine factor, which is continuously released from retinal tissue and causes smooth muscle cell relaxation. This study tried to identify the cellular source of the RRF. Furthermore, the possible RRF release by voltage dependent sodium channel activation and the calcium-dependency of the RRF release was investigated. Mice femoral arteries were mounted in myograph baths for in vitro isometric tension measurements. The vasorelaxing effect of chicken retinas, which contain no vascular cells, and of solutions incubated with MIO-M1 or primary Müller cell cultures were evaluated. The RRF release of other retinal cells was investigated by using cell type inhibitors. Concentration-response curves of veratridine, a voltage-dependent sodium channel activator, were constructed in presence or absence of mouse retinal tissue to evaluate the RRF release. The calcium-dependency of the RRF release was investigated by evaluating the vasorelaxing effect of RRF-containing solutions made out of chicken retinas in absence or presence of calcium. Chicken retinas induced vasorelaxation, whereas solutions incubated with Müller cell cultures did not. Moreover, the gliotoxin DL-α-aminoadipic acid, the microglia inhibitor minocycline and the tetrodotoxin-resistant voltage-dependent sodium channel 1.8 inhibitor A803467 could not reduce the RRF-induced relaxation. Concentration-response curves of veratridine were not enlarged in the presence of retinal tissue, and RRF-containing solutions made in absence of calcium induced a substantial, but reduced vasorelaxation. The RRF is not released from vascular cells and probably neither from glial cells. The retinal cell type which does release the RRF remains unclear. Veratridine does not stimulate the RRF release in mice and the RRF release in chickens is calcium-dependent as well as calcium-independent.

PP.276

Pannexin 1 is a participant of purinergic signaling in murine basilar artery

Kiryukhina OO[1,2], Tarasova OS[1,2,3]

[1]Institute for Biomedical Problems RAS, Moscow, Russian Federation; [2]Institute for Information Transmission Problems, RAS, Moscow, Russian Federation; [3]Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russian Federation

Pannexin 1 (Panx1) is a new player in vascular tone regulation, but the pattern of its expression|/functioning differs among the vascular beds. Surprisingly, vasomotor role of Panx1 has not been studied in cerebral arteries, although Panx1 in abundant in the central nervous system. Therefore, our study was aimed at the role of Panx1 in purinergic control of murine basilar artery (BA) tone. Experiments were performed on BA from 2-3-month-old male Panx1 knockout (KO) and C57BL/6 (WT) mice. The arterial segments were mounted in wire myograph (DMT). ATP- and acetylcholine (ACh)-induced relaxation was studied in U46619- precontracted arteries. The contribution of ATP/ADP was dissected using apyrase (ATPase/ADPase) and 8-SPT (adenosine receptor blocker). The content of CD39 mRNA was studied using qPCR (Corbett Research). Contractile response to ATP (10 microM) was higher in KO compared to WT (27% and 39% from the maximum contractile force, respectively). In precontracted BA, ATP (30 microM) induced biphasic effect: transient contraction was followed by relaxation, which also was increased in KO compared to WT (38% and 19% from the precontraction level, respectively). Apyrase in combination with 8-SPT decreased the response to ACh in WT but not in KO, while 8-SPT alone had not effect in either group. The level of CD39 mRNA in cerebral arteries was lower in KO compared to WT. Our data suggest, that Panx1 has important role in purinergic control of BA tone through ATP/ADP secretion. The ablation of Panx1 augments vasomotor responses to ATP, probably, as a result of the decline of CD39, a principal ectonucleotidase in murine arteries. Importantly, Panx1 participates in endothelium-dependent relaxiation of BA to vasoactive ligands. Supported by the RSF (grant N17-15-01433).

PP.277

Effects of mandibular extension on systemic arterial blood pressure in spontaneously hypertensive rats: probable involvement of calcitonin gene-related peptide

Lapi D[1], Saracino L[2], Federighi G[2], Del Seppia C[3], Sabatino L[3], Di Maro M[1], Colantuoni A[1], Scuri R[2]

[1]Department of Clinical Medicine and Surgery, "Federico II", Naples, Italy; [2]Department of Traslational Research on new Technologies in Medicine and Surgery, University of Pisa, Italy; [3]CNR Institute of Clinical Physiology, Pisa, Italy

Previous experiments, we studied the hypotensive effects of 10 min mandibular extension (ME), repeated twice, in spontaneously hypertensive rats (SHR), evaluating the mean arterial blood pressure measured in femoral artery. Furthermore, ME restored the pial arteriolar vasomotion in parietal and frontal cortical regions. These effects lasted at least 4 hours. The

present study was aimed to implement a non-invasive method to measure changes in systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MABP) from the tail of SHR. Rats were under light anesthesia for about 120 min and during the first 30 min were subjected to two 10 min ME at 10 min interval. ME was induced by placing a home-made U-shaped dilator between the dental arches of the rat. The cardiac parameters were measured immediately after sedation, at the end of double MEs and afterwards every 30 min up to 120 min. In this case, double MEs caused a significant decrease at the same extent of MABP, SBP and DBP. To study the mechanisms underlying ME effects, we also tested the expression of the calcitonin gene-related peptide known to be a potent vasodilator produced by sensory nerve terminals and involved in the prevention of hypertension. This has been evaluated in samples of brainstem and parietal cortex collected from rats sacrificed 100-120 min after double MEs. Our data corroborate previously published results demonstrating that ME has important hypotensive and vasodilator effects. Interestingly, ME produced an equal reduction in both SBP and DBP.

Poster Session IV (2/4)

Exercise Physiology

PP.278

Physical and psycho-physiological responses to self-paced running exercise following partial sleep deprivation

Souissi W, Hammouda O, Ayachi M, Khcharem A, de Marco G, Souissi M, Driss T

Research Center on Sport and Movement (CeRSM, EA 2931), UFR STAPS, Paris Nanterre University, Nanterre, France

Sleep has restorative effects including immunity, endocrine function and cognitive performance. Previous studies have shown a decrease in anaerobic performance following short-term maximal exercise. Only few studies investigated the effects of partial sleep deprivation (PSD) on aerobic performance, and have shown controversial findings. We aimed to investigate the effects of PSD at the beginning and the end of night on pacing, physical and psycho-physiological responses following self-paced running test. Fourteen runners (22±1 years) performed in a counterbalanced design 12-minute self-paced running exercise following two conditions: a control sleep night (CON) and a one night of PSD separated by three days. Main significant interactions were observed for covered distance, speed, simple reaction time, choice reaction time, RPE ($p<0.05$), mood, VE ($p<0.001$) and core temperature ($p<0.01$). Cognitive performance decreased following PSD compared to the CON condition ($p<0.001$) and VE was higher in PSD ($p<0.001$). However, blood lactate concentration and most of cardiorespiratory parameters (HR, VO2, RER) were not affected by sleep deprivation ($p>0.05$). There was only a significant main effect of time in HR, VO2 and RER ($p<0.05$). In conclusion, one night of partial sleep deprivation at the beginning and the end of night negatively affects pacing strategy and decreases endurance performance without altering cardiorespiratory function. Similarly, perceived exertion and mood seem to be impaired in response to a short period of sleep loss. Effective strategy such as napping should be introduced to overcome the deteriorations of physical performance and mood during self-paced exercise due to partial sleep deprivation.

PP.279

The VO_2 Slow Component: is there such a thing?

Colosio AL[1], Caen K[2], Bourgois J[2], Boone J[2], Pogliaghi S[1]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy; [2]Department of Movement and Sports Sciences, Ghent University, Belgium

It has been assumed, but not proven, that in the heavy/severe domain of exercise, the muscle displays an increasing energy demand over time, in association with the slow component of VO2 (VO2sc). While the physiological underpinnings of VO2sc are still debated, a recent study refuted that the energy demand of a constant heavy exercise changes over time. We tested the hypothesis that the overall cost of cycling is affected by time during metabolic transitions in different intensity domains. Eight active men (age 25 ± 2 years) performed 3 constant load trials of 3, 6 and 9 minutes in the moderate (m), heavy (h) and severe (s) exercise intensity domains. We characterised the VO2 and ventilation (VE) responses and blood lactate accumulation and calculated the adjusted oxygen cost of exercise (AdjO2Eq) for the 0-3, 3-6 and 6-9 time segments at the three intensities by: i) accounting for aerobic and anaerobic energy sources used over time ii) removing the VO2 cost of VE. Data were compared by two-way (time segment and intensity) RM-ANOVA. There was a significant main effect of intensity ($p<0.001$) on AdjO2Eq with s>h>m at all time windows. AdjO2Eq was unaffected by time for m (2626±939, 2687±1036, 2731±1035 ml 3 min-1 at 0-3, 0-6, 0-9 min). A significant effect of time was there for s (6544±1413 < 7061±1516 < 7372±1443 ml 3 min-1 at 0-3, 0-6, 0-9 min) but not for h (4959±1074, 5121±1268, 5225±1123 ml 3 min-1 at 0-3, 0-6, 0-9

min). The adjusted oxygen cost of exercise is unchanging from 3 to 9 min in the h as well as in the m domain as the rising contribution of oxygen uptake over time is countered by an energetically equivalent decreasing anaerobic contribution. Only in the s intensity domain a true loss of efficiency appears over time, in coincidence with the VO2sc.

PP.280

Cardiovascular drift and left ventricular performance during prolonged exercise at moderate intensity

Laginestra, FG[1,2], Wang E[2], Berg OK[2], Nyberg SK[2], Venturelli M[1], Helgerud, J[2]

[1]Department of Neuroscience, Biomedicine, and Movement Sciences, University of Verona, Verona. [2]Department of Circulation and Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

Prolonged exercise leads to a progressive upward drift in heart rate (HR) that may compromise stroke volume (SV) response. Some studies reported a diminished left ventricle (LV) systolic function in the post-exercise recovery phase. However, it is unclear whether a decrease in LV systolic performance exists also during exercise. The aim of this study was to examine the dynamics of LV regulation during prolonged exercise. We hypothesized that LV systolic function would be maintained while ventricular filling would be diminished. Eight healthy non-endurance trained young males (25±2yrs) completed a 60 min cycling bout on a semi-recumbent cycle ergometer at 57% VO2max. Measurements of HR, enddiastolic volume (EDV) and end-systolic volume (ESV) were obtained and used to calculate SV, cardiac output (Q) and ejection fraction (EF). Ear temperature (Te), skin temperature (Tsk) and oxygen consumption (VO2) were also collected. Fluid intake was tightly matched to the individual sweat rate. Percentage changes in blood volume (%BV) and body mass (pre to post bout) were recorded to assess eventual changes in loading conditions. From min 10 to min 60, both EF and EDV were maintained ($p=0.85$ and $p=0.17$). In the same time span, HR increased by 12% ($p=0.01$) while SV was kept stable ($p=0.82$). VO2 and Tsk increased respectively by 8% ($p=0.01$) and 6% ($p<0.01$) from min 10 to min 60. All the other control variables did not change throughout the trial. Our findings suggest that LV systolic performance is not blunted during prolonged exercise. In addition, we did not find a diminished ventricular filling but a maintained EDV in spite of the increased HR. This finding may indicate an improvement in the relaxation of the ventricle with the augmented sympathetic drive.

PP.281

Acute static stretching does not alter balance control ability: the role of neuromuscular activation.

Cè E, Longo S, Shokohyar S, Coratella G, Bisconti AV, Rampichini S, Borrelli M, Doria C, Limonta E, Esposito F

Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy

Balance control (BC) is the resultant of an integrative network involving sight, hearing, vestibular function, and proprioceptive feedback. Perturbations of one of these contributors could turn into a worsening of BC regulation. Passive stretching (PS) has been reported to affect proprioceptive feedback, thus possibly decreasing BC ability. This study evaluated the acute effects of PS and active stretching (AS) of the lower limbs on static and dynamic BC parameters. Thirty-eight participants (age: 26±3 yrs; stature: 1.72±0.10 m; body mass: 69±17 kg) underwent PS, AS and control sessions randomly on different days. Stretching routines had similar durations and involved bilaterally the muscles acting around the knee and ankle. Before and after stretching, hip, knee and ankle range of motion (ROM), maximum voluntary isometric contraction (MVC), and maximum muscles activation [surface electromyography, sEMG, root mean square (RMS) from the investigated muscles] were measured. Static and dynamic BC parameters were determined by stabilometry in bipedal and monopedal conditions (with both open and closed eyes). sEMG was recorded during balance test and normalized to MVC. After stretching, ROM increased in all the joints ($p<0.001$) and MVC decreased (PS: $p<0.001$; AS: $p=0.03$) together with RMS (PS: $p=0.01$; AS: $p=0.02$) in all the investigated muscles. BC resulted unaffected. However, an overall significant increment in sEMG RMS was found in all the tested muscles during balance tests (p from 0.02 to <0.001).These findings suggest that, muscles directly involved in BC were more activated to maintain a similar performance, likely as a possible compensation to an altered proprioceptive feedback from the stretched muscles and joints.

PP.282

Estimating metabolic rates during daily living activities in people with multiple sclerosis

Manca A[1], Martinez G[1], Ventura L[1], Aiello E[2], Deriu F[1]

[1]Department of Biomedical Sciences, University of Sassari, Sassari, Italy; [2]Department of Experimental, Medical and Surgical Sciences, University of Sassari, Sassari, Italy

Compared with healthy controls, persons with multiple sclerosis (PwMS) exhibit marked reductions in peak aerobic capacity (VO_2peak), ventilatory anaerobic threshold (VAT), peak respiratory exchange ratio (RERpeak), peak heart rate (HRpeak) and peak work rate (WRpeak). Whether they use more energy than healthy controls during submaximal activities is less clear. Moreover, no data are yet available on the metabolic rate of PwMS during daily living activities (ADL), which are basic tasks that must be accomplished every day for an individual to maintain independence and are used as a measurement of a person's functional status. The present cross-sectional case-control study was aimed at measuring the energetic cost of a composite set of basic ADL, including dressing, toileting, transferring and mobility, as derived by nvalidated tools (Katz Index of independence in ADL; Physical Self-Maintenance Scale; Barthel ADL Index). A portable, open-circuit gas analyser system (MetaMax 3B, Cortex Medical, Germany) was employed to monitor the metabolic rate during 13 basic ADL assessed over 2 non-consecutive days in 10 PwMS (5 with moderate-to severe disability; 5 with very mild disability) and in 5 age- and gender-matched healthy control subjects. Activities were monitored for at least 5 minutes and followed by a 5-minute complete rest. Compared to controls and mildly disabled PwMS, more disabled individuals showed significantly higher VO_2, VCO_2 and HR, but not RER, values during dressing, car usage, doing laundry and climbing stairs. Interestingly, significant differences in metabolic rate were also found between mildly disabled PwMS and controls. Open-spirometry outcomes during every-day life may contribute to enhance our understanding of the pathophysiology of MS-linked fatigue.

PP.283

Oxygen Uptake efficiency slope can accurately track changes of cardiorespiratory fitness early after heart transplant.

Allam M[1], Teso M[1], Borrelli E[2], Grassi B[3], Pogliaghi S[1]

[1] Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy; [2]Department of Surgery and Bioengineering, University of Siena, Italy; [3]Department of Medicine, University of Udine, Italy

Cardiorespiratory fitness (CRF) is a "sentinel" of the integrated function of the respiratory, cardiovascular and muscle systems, a marker of current and prospective health and a determinant of the quality of life in heart transplant recipients (HTR). The reference detection method (i.e. direct VO2max determination) poses particular challenges in patients. Oxygen uptake efficiency slope (OUES) is a valid index of CRF, used in different healthy and clinical populations; objective and submaximal in nature, it is an excellent candidate for the monitoring of CRF in the frail. We tested the hypothesis that OUES is correlated with the gold standard method and that it can accurately track changes in cardiorespiratory fitness over time after heart transplant. Fifteen male HTR (age 52.0 ± 9.9 yr) performed an incremental test to exhaustion 2-4 times within the first two years from transplant. Based on breath by breath measures of ventilation (VE) and gas exchange, we measured VO2max and OUES (i.e. the slope of the linear relationship of VO2 as a function of log10VE). Measures within 6, 12, 18 and 24 months from transplant were compared by RM ANOVA and correlated by Pearson correlation coefficient. Both VO2max and OUES increased significantly over time (main effect $p<0.05$). Furthermore, OUES was significantly correlated with VO_{2max} ($r2=0.67$, $p<0.01$). Finally, changes in OUES were significantly correlated with changes in VO_{2max} ($r2=0.61$, $p<0.01$). Our study confirms the validity of OUES as an effort-independent index of cardiorespiratory fitness in heart transplant recipients that can also track changes of VO_{2max} over time from transplant. In this population, in which a maximal effort may be problematic, OUES offers a valid and objective alternative to the direct VO2max method.

PP.284

Equivalent Load calculation for exercise prescription: validation of a new model

Pogliaghi S[1], Colosio AL[1], Iannetta D[2], Caen K[3], Keir DA[4], Boone J[3], Murias JM

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Italy; [2]Faculty of Kinesiology, University of Calgary, Canada; [3]Department of Movement and Sports Sciences, Ghent University, Belgium; [4]Department of Cardiology, University Health Network, Toronto, Canada

Failing to account for the difference in the VO_2/load (W) relationship that exists between incremental and constant W exercises paradigms is a very common occurrence, that leads to inaccurate W prescription. We tested the hypothesis that an equivalent W in the moderate (*m*) and heavy (*h*) intensity domains of exercise can be accurately estimated based on a mathematical model and parameters derived from incremental testing. On a bike ergometer, 60 healthy males (39±19 yrs, range 19-75) performed: incremental test to exhaustion (INC); constant W tests (CL) in the *m* and *h* intensity domains. For INC we determined: VO_2 at warm-up, gas exchange threshold (GET) and respiratory compensation point (RCP), mean response time (MRT), first ($s1$) and second ($s2$) slope of the VO_2/W relationship. For CL we determined: VO_2 at 6^{th} min (mVO_2). We used the following equation to

estimate CL VO2 ($_eVO_2$) based on INC test data: if W CL < W@GET, y_1, y_2 y_1 = V_{O2}@warm-up + {[W CL - W warm-up) + (MRT x ramp slope)] / s_1} y_2 = V_{O2}@GET + {[W CL - W@GET) + (MRT x ramp slope)] / s2} We compared mVO_2 and eVO_2 in *m* and *h* domains (RM ANOVA and correlation). $_eVO_2$ (1914±372) was not different from and highly correlated with $_mVO_2$ (1901±344 ml·min- 1, p=0.58, r^2=0.90) in the *m* domain. In the *h* domain $_eVO_2$ (2778±435) was lower than and highly correlated with $_mVO_2$ (2835±466 ml·min-1, p=0.02, r^2=0.91); this difference, though statistically significant, is below measurement capabilities. Our mathematical model, based on variables derived from an incremental test, accurately predicts the metabolic intensity associated with a given workload in the moderate and heavy domains of exercise. This approach can assist the translation of metabolic intensity targets to accurate equivalent load prescriptions.

PP.285

Physical activity among medical students

Gotia SL[1], Borza C[2], Mederle A[2]

[1]Department of Physiology, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania; [2]Department of Pathophysiology, University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

Physical inactivity is one of the global health problems, which increases overall incidence of several diseases like obesity, type II diabetes mellitus, or hypertension. Medical students receive substantial knowledge of the benefits of regular physical activity (PA) in preventing these types of diseases. The objectives of this study were to evaluate physical activity level in students of the University of Medicine and Pharmacy "Victor Babes", Timisoara, in Romania and to focus on the role of medical students in promoting physical activity. A cross sectional study was conducted among 40 undergraduate medical students. Were applied Global physical activity questionnaire (GPAQ) in order to assess the degree of physical activity in medical students. The information on PA participation was collected in three domains: activities at work, travel to and from places, recreational activities, and sedentary behavior. Body mass index was calculated from self-reports of height and weight. Statistical Package for Social Sciences (SPSS) version 16 was used for data entry and analysis. The minimum 10 minutes of each activity was required to be included in the study. The level of physical activity is presented in the corresponding metabolic equivalent (1 MET=energy produced assuming oxygen consumption of 3.5 ml/min/kg weight), and we divided the activity in 3 levels (low, moderate, high) according on the intensity, minutes of physical activity and days per week. In the study were included only males, aged 21 to 25 years (23.20 ±1.83), with a normal BMI. The majority of students (88.7%) were classified as having a moderate level of physical activity. More than half of the students had insufficient physical activity, especially in the first years of faculty, because the prolonged study-related activities and insufficient organized sports. Recreational activity was a major type of PA among the medical students. Despite being aware of the benefits and risks, the students didn't meet the minimal recommended level of physical activity. There is a direct correlation between physical activity level in medical students and medical advice they give to patients.

PP.286

Peripheral capillary oxygen saturation in relation to aerobic capacity during Himalayas trek

Girgis Kalim A[1], Ruzic L[2]

[1]Antonine University, Baabda, Lebanon; [2]Dpt. Of Sport and Exercise Medicine, Faculty of Kinesiology, University of Zagreb, Croatia

High altitude tourism is becoming increasingly popular among non-athletic population but its potential impact on health is often neglected. This study investigated the relations of physical fitness and mean peripheral capillary oxygen saturation (SpO2) during the trek in altitudes.17 recreational lowland men (age 48±11) participated in 26 day Himalaya trek, the highest point reached being Mera Peak (6476m). The initial measurements included also a graded test till complete exhaustion on treadmill. From achieved time and given the treadmill speed and grade the maximal aerobic capacity was estimated and expressed in MET. During the tour, the SpO2 was recorded on every new altitude by pulse oximetry, altogether on 21 occasion. The results showed that the intensity that was achieved at maximal exertion (mean reached intensity was 14.91±2.14 METs) was significantly correlated with the mean peripheral capillary oxygen saturation (mean SpO2 88.48±2.63%) calculated from all measured SpO2 during the trek (Pearson r=0.713 at P<0.05). The higher mean SpO2mean values during the trek were seen in participants reaching the higher intensities on exertion test prior to the expedition. Interestingly, some previous papers reported the possibility to predict acute mountain sickness (AMS) by monitoring arterial oxygen saturation during ascent, but also, they found that the better aerobic capacity and younger age were related to more symptoms. That is contradictory to our findings as in this study the higher aerobic capacity meant better

peripheral oxygenation of the participants during the trek which would imply lower chance for AMS. The explanation might lie in the older age of the participants in this study, but the further research should look into it in more details.

PP.287

Effect of pulsed electromagnetic fields (PEMFs) on the VO_2 kinetics

Trofè A[1], Raffi M[2], Campa F[2], Toselli S[2], Muehsam D[2], Piras A[2].

[1]Department for Life Quality Studies, University of Bologna, Italy; [2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Italy.

The pulsed electromagnetic fields (PEMFs) are a non-invasive therapy used for medical treatments which increases blood flow, rate of tissue oxygenation and velocity of gas exchanges. The aim of this study was to investigate the influence of PEMFs stimulation on VO_2 pulmonary and muscular kinetics during a constant-load exercise performed during cycling. Nine semi-professional male cyclists [mean age 22.3±4.6; mean VO_{2max} 56.8±6.1ml/kg/min] participated in the study. Experiments were performed on a cycle ergometer (Lode-H-300-R) in different days: at day 1 we measured the VO_{2max}, at day 2 and 3 we performed the experiments with and without the PEMFs stimulation. When active, the PEMFs were applied to the quadriceps of the right leg for the entire duration of recording. We recorded the oxygen uptake, the hemodynamic activity of the vastus lateralis and the electromyographic activity of vastus medialis and bicep femoris. Athletes performed 1 minute without load, then they cycled at workload corresponding to ~50% of the difference between ventilatory threshold (VT) and peak VO_2 (>VT) for at least 6 minutes, time necessary to recording the "slow component" of VO_2 kinetics. During PEMFs stimulation, we observed a lower oxygen uptake kinetics at steady-state ($p=0.045$), highlighted by a lower amplitude of the primary component ($p=0.026$). In the stimulated muscles, PEMFs caused a greater amplitude of the primary component of deoxyhemoglobin ($p=0.046$). This result indicates that more oxygen was released to muscle contraction, leading to higher aerobic efficiency. The values of lactic acid, measured at the third minute of effort, were higher during PEMFs ($p=0.001$) as a possible consequence of the PEMFs effect on fibers IIa in association with a significant slow component of VO_2.

PP.288

Effects of additional loads during self-resistance exercises

Zbidi S, Zinoubi B, Vandewalle H, Driss T

Research Center on Sport and Movement (CeRSM, EA 2931), UFR STAPS, Paris Nanterre University, Nanterre, France

Recent investigations have demonstrated significant gain in the strength of elbow flexor and extensor muscles during maximal isometric voluntary co-contractions (MIVCC) training of the agonist and antagonist muscles. The effects of muscle strength training programs are generally more pronounced when the level of strength achieved during exercises is higher. Therefore, the addition of an external load during MIVCC could be interesting to strengthen muscles. We aimed to verify if MIVCC combined with an external load leads to higher muscle activation than during isolated MIVCC. Muscle activations were estimated by the integrated electromyographic activity (iEMG) of the triceps brachii (TB), biceps brachii (BB) and brachio-radialis (BR) in ten subjects. iEMG during MIVCC were compared with iEMG during MIVCC with additional loads equal to 30 and 50 % of the maximal voluntary force (MVF) of the elbow extensor (E30, E50) and flexor (F30, F50) muscles. During F30 and F50, BB and BR were considered as agonist muscles and TB as an antagonist muscle. During E30 and E50, TB was considered as an agonist muscle, and BB and BR as antagonist muscles. The increase in iEMG of the agonist muscles during E30 and F30 were small and not significantly higher than the iEMG of the same muscles during MIVCC. The small increases in Iemg of the agonist muscle during E50 or F50 were significant ($p<0.05$). During MIVCC with external loads, the decrease in iEMG of the antagonist muscles were large and highly significant ($p<0.001$). Therefore, the relevance of MIVCC with additional loads as strength training exercises must be verified because the increase of agonist muscle activations was low and the antagonist muscle activations largely decreased when loads were added.

PP.289

Effect of whole-body vibrations on muscular activation during walking

Bazzucchi I[3], Nicolò A[3], Chadefaux D.[4], Marchetti E.[1], Fattorini L[2], Tarabini M[4], Di Giovanni R[1], Tirabasso A[1], Felici F[3]

1Italian National Institute for Insurance against Accidents at Work, Department of medicine, epidemiology, workplace and environmental hygiene, Monte Porzio Catone (RM), Italy; 2Sapienza University of Roma, Department of Physiology and Pharmacology "V. Erspamer", Rome, Italy; 3University Foro Italico of Roma, Department of Movement, Human and Health

Sciences, Italy; 4 Politecnico di Milano, Department of Mechanics, Polo Territoriale di Lecco, Italy

The human body is exposed to vibrations during a variety of working, sporting and leisure time activities, but prolonged exposure to vibrations may lead to a series of diseases. While it is well established that vibrations affect the neuromuscular activity during static tasks, only a few attempts have been made so far to evaluate the effect of vibrations on muscle activation during everyday life tasks. The present study aimed to investigate the effect of vibrations on leg muscular activation during walking. Seven male subjects walked on a treadmill fixed on a 6 degrees of freedom shaker. Vibration was imposed at 6 frequencies (2, 4, 6, 8, 10, and 12 Hz) along vertical and transversal directions. Walking speed was set at 1.25 m/s. Surface electromyography (sEMG) was recorded from four muscles of the lower limbs. Stride phases and stride length were identified by analysing pressure sensor data. All measurements were normalized to a walking condition performed without the vibration (control). Preliminary results showed that vibration does not affect stride length and step phases. When comparing the control condition with the 12-Hz condition, a significant right shift in the peak of EMG activity was observed for vastus lateralis (12 ± 10 %), tibialis anterior (12 ± 11 %) and gastrocnemious medialis (12 ± 8 %), while no significant difference was found for the EMG amplitude. In conclusion, vibrations appear to have an effect on the temporal profile of muscle activation, with a moderator effect of vibration frequency. This effect may be due to the tonic vibration reflex induced by vibrations and/or to changes in motor control induced by the mechanical perturbation.

PP.290

Effect of pedalling cadence on respiratory frequency during different exercise intensity domains

Nicolò A, **Girardi M**, **Bazzucchi I**, **Felici F**, **Sacchetti M**

Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy

Pedalling cadence is considered an important factor influencing respiratory frequency (fR) during exercise, with muscle afferent feedback from groups III and IV possibly mediating this effect. However, findings are controversial. The present study aimed to systematically assess the effect of pedalling cadence on fR by proposing sinusoidal changes in pedalling cadence during passive and active exercise of different intensities. Ten well-trained male cyclists performed a preliminary ramp incremental test and three sinusoidal experimental tests on separate days. The experimental tests consisted of 16 min of sinusoidal variations in pedalling cadence performed during passive exercise (PE), moderate exercise (ME) and heavy exercise (HE), in separate visits. In the three sinusoidal tests, pedalling cadence varied between 55 and 115 rpm, with a sinusoidal period of 4 min. Frequency analysis was used to obtain the amplitude (A) and phase lag ( ) of cardiorespiratory and electromyographic variables. During sinusoidal exercise, the A of fR decreased from PE (3.9 ± 1.4 breaths min-1) to ME (2.6 ± 1.3 breaths min-1) and HE (1.8 ± 1.0 breaths min-1), while the increased from PE (3.5 ± 9 s) to ME (16.9 ± 17 s) and HE (46.7 ± 29 s). During sinusoidal exercise, no entrainment was found in any of the three conditions tested. In conclusion, the effect of pedalling cadence on fR is moderated by exercise intensity; it is remarkable during PE, but it reduces with the increase in exercise intensity. This effect appears to be mediated by muscle afferent feedback from groups III and IV.

PP.291

The physiological impact of a short-term cardiac rehabilitation program on activities of daily living in elderly patients with chronic heart failure.

Paneroni M[1,2], **Scalvini S**[1], **Simonelli C**[1], **Rivadossi F**[1], **Lovagnini M**[1], **La Rovere M**[1], **Venturelli M**[2], **Ambrosetti M**[1], **Pedretti R**[1]

[1]Department of Cardiology, ICS Maugeri Pavia; [2]Department of Neurosciences, Biomedicine and Movement Sciences

A high proportion of elderly patients with Chronic Heart Failure (CHF) experience dyspnea and fatigue during the activities of daily living (ADLs). We aimed to determine 1) the VO2 peak of some basic ADLs comparing it to VO2peak at Cardio-Pulmonary Exercise Test (CPET) and 2) the effects of 3-week inpatient cardiac rehabilitation program on ADLs' performance. At entry and at the end of a 20-day cardiac rehabilitation program patients performed an ADL-test consisting of five task-related ADL activities and two time-related ADL activities while wearing a metabolimeter mobile device (K5, Cosmed). Task-related activities were: 1) to put on and take off socks, shoes and jacket (ADL 1); 2) to fold eight towels (ADL 2); 3) to put 6 bottles on a shelve (ADL 3); 4) to make a bed (ADL 4); 5) to go up and down 1-floor stairs (ADL 5). Time-related ADL activities were: 1) to sweep the floor for 4 minutes (ADL 6) and 2) to walk for six minute (6MWT). Metabolic load, oxygen uptake, ventilation, heart rate and symptom of dyspnea were computed for each ADL. During the program, patients performed an incremental CPET (10 watts/minute protocol). Fifty-six CHF patients (89% men; age 72±6 years; Ejection Fraction (EF) 38%±12; 66 % with EF<40%) were enrolled. At CPET, VO2 peak was 13,4±3,5 mL/kg/min

and W max was 81±20. At entry, the least demanding ADL [expressed as proportion of peak oxygen uptake (VO2 peak) reached at CPET] was ADL 3 with 53 ±19 % , while the most challenging was the 6MWT with 117± 34% . Forty-two (75%) patients reached the VO2peak of CPET during 6MWT. After rehabilitation, there was a significant decrease in the time required to perform the task-related activities (ADL 1-5) [from 382.25 ±114.90 to 354.48±116.92 seconds, p= 0.0175] and a significant increase in the distance covered during 6MWT [from 421.35 ±81.64 to 448.84 ±89.69 meters, p= 0.000]. Moreover, following rehabilitation a significant decrease of heart rate in ADL1, ADL 3 and ADL 5 and a significant decrease of dyspnea in ADL 5, ADL 6 and 6MWT were recorded. In conclusion, common ADLs lead to an higher or maximal oxygen uptake in heart failure patients and a comprehensive cardiac rehabilitation program can improve some physiological variables during ADLs, without a clear similar response trend.

PP.292

The role of mTOR dependent autophagy pathway on chronic resistance exercise induced muscular hypertrophy in rats

Altınoluk T[1], Kuntsal Dertsiz E[2], Arslan S[3], Şirvancı S[2], Karademir B[3], Yıldırım A[1]

1Marmara University, School of Medicine, Department of Physiology, Istanbul, Turkey; 2Marmara University, School of Medicine, Department of Histology & Embryology, Istanbul, Turkey; 3Marmara University, School of Medicine, Department of Biochemistry, Istanbul, Turkey

Although widely accepted that protein degradation events are regulated by rapamycin sensitive mammalian target of rapamycin (mTOR)-mediated pathway on resistance exercise induced hypertrophy in skeletal muscle; other rapamycin-sensitive mTORindependent mechanisms, such as TORC2, HSP70 (as chaperone mediated autophagy), ubiquitin-proteasome pathway, have been less studied. Adult Sprague-Dawley rats were randomly divided into control exercise (CE) (3 days/week, for 8 weeks and 4-9 climb per session), rapamycin treatment (RT) (1.5 mg/kg intraperitoneal injection, 3 days/week, for 8 weeks) and exercise+rapamycin treatment (RT+E) (same dose of rapamycin injected 1h before exercise) groups. Resistance exercise was applied using ladder climbing model with help of attached weights to tail. Gastrocnemius muscle cross-sectional area, wet muscle weight and total body weight increased in CE, compared to RT and RT+E; however, rapamycin did not affect maximum carrying capacity. Atg13 expression was not found different among groups; however, when assessed with confocal microscopy, increased Atg13 positive signal was detected in all three groups, besides, increased LC3 signal was at only in CE and RT groups. HSP70 and proteasomal activity were at same level in all groups. p-Akt Ser473, p-p70s6k Thr389, p-ULK1 Ser 757 and LC3 were analysed with western blot. Chronic rapamycin administration may have suppressed other previously unknown components of mTOR. Inhibition of mTOR by rapamycin did not prevent muscle functional capacity, and this indicates that among all degradation systems, rapamycin sensitive mTOR-dependent pathway is not the particular one that manages muscle hypertrophy process.

PP.293

The force of the myosin motor modulates the cooperativity in thin filament activation

Caremani M, Gallart C, Pertici I, Piazzesi G, Lombardi V, Linari M

PhysioLab, Department of Biology, University of Florence, Florence, Italy

Activation of striated muscle implies that Ca2+ binding to troponin in the thin filament leads to a structural change in tropomyosin that makes the actin filament available for the interaction with the myosin motors. Biochemical and structural evidence suggests that the complete removal of the steric block of tropomyosin on the binding sites on actin depends on myosin motor attachment (McKillop and Geeves Biophys J 65:693, 1993). Myosin binding is likely responsible also for the spread of thin filament activation (Desai et al. J Biol Chem 290:1915, 2015), which determines the steepness of the sigmoidal force-pCa relation in skinned fibres (measured by the Hill coefficient nH), but the mechanism remains unclear. The question is investigated here by determining the force- and stiffness-pCa relations in skinned fibres from soleus muscle of rabbit (sarcomere length 2.4 μm), in which the force per motor is modulated by both temperature and addition of 1 μM Omecamtiv Mecarbil (OM). OM is known to (i) suppress force while remaining strongly bound to actin and (ii) increase Ca2+ sensitivity of thin filament shifting the force-pCa relation leftward while reducing its steepness (Caremani et al. Biophys J 114:644a, 2018; Woody et al. Nat Commun 9:3838, 2018). By using the half-sarcomere compliance analysis, we estimate the isometric force per myosin motor (F0), which ranges from 0.8 ± 0.1 pN (1 μM OM,

12 C) to 2.6 ± 0.1 pN (control, 25 C). Most importantly we find a linear relation between F0 and nH estimated from the force-pCa relations, whatever is the protocol used to modulate the two parameters. These results indicate that cooperativity in thin filament activation depends on the local stress induced by the attaching motor. Supported by ECRF and University of Florence.

Poster Session IV (3/4)

Respiratory Physiology

PP.294

Carotid Body as a model for aging studies: the hypoxia-hyperoxia aging interaction

Di Giulio C

Department of Neurosciences, University of Chieti-Pescara, Italy

Carotid Body (CB), as a sensory neurepithelial organ, regulates the ventilation responding to the variation of blood gases O2-CO2 and pH, and influencing the gateway for the respiratory neurons in the brainstem. The aging process is characterized by a decline in several physiological functions resulting in a reduced capability to maintain homeostasis. This lowered homeostatic capacity seems to involve the (CB), whose role is to modulate ventilation and tissue oxygen supply thus playing a prime role in all aging processes. Aging causes marked changes in CB morphology. Indeed, it is enlarged and shows a concomitant decrease in the percentage of chemoreceptor tissue, as well as a proliferation of Type II cells. The carotid glomitis is present with aggregates of lymphocytes and fibrosis of the lobules. Type I cells are dehydrated, with a profound vacuolization, a shrinking nucleus, and lipofuscin accumulation. With increased age man CB shows a reduction in the number and volume of mitochondria, fewer synaptic junctions between glomi, along with a reduction in CB content of neurotransmitters, leading to a sort of 'physiological denervation'. Instead, in rats the hyperplasic response of CB cells during chronic hypoxia is less evident in aged CB samples as compared to young ones. The increase in HIF-1 - VEGF - ET and NOS-1 expression during chronic hypoxia is less evident in CBs of old rats as compared to the young ones. This favors changes in the set-point sensitivity for the chemosensory peripheral drive. Aging could be interpreted as a cumulative result of oxidative damage to cells, which derives from aerobic metabolism. Moreover, metabolism rate is tightly correlated with life span, thus a loss in mitochondrial function is one of the prime factors affecting CB aging processes. The age-related reduction in synaptic junctions might be a self-protective mechanism through which cells buffer themselves against accumulation of reactive oxygen species during aging. The correlation between hypoxia and life-span of CB cells remains open until the question of how and why cells sense oxygen is solved. In other words, in order to better understand aging, knowledge of which O2 species are being sensed by cells is needed.

PP.295

The effects of PDE5 inhibitor sildenafil on inflammation and apoptosis in experimental ARDS model

Kosutova P[1], Mikolka P[1], Balentova S[2], Mokra D[1]

[1]Department of Physiology and Biomedical Center Martin, Comenius University, Slovakia; [2]Department of Histology and Embryology, Comenius University, Slovakia

Acute respiratory distress syndrome (ARDS) is characterized by neutrophil-mediated inflammation, and lung epithelial cell dysfunction and death. The study evaluated if administration of sildenafil enhances lung inflammation and apoptosis in an ARDS model. Respiratory failure was induced by repetitive saline lung lavage until PaO2 reached values <26.7 kPa in oxygen ventilation. Rabbits were divided into 3 groups: healthy ventilated animals (Control), animals with respiratory failure nontreated (ARDS) or treated with sildenafil (ARDS+SILD; 1 mg/kg i.v.), and ventilated for additional 4 h. Counts of cells in bronchoalveolar lavage fluid (BALF) and concentrations of TBARS, 3-nitrotyrosine (3NT), IL-6, -8, and TNF- in the lung tissue were measured. Apoptosis of lung cells was evaluated by TUNEL assay and immunohistochemically by caspase-3 antibody. Sildenafil decreased total cell count, percentage of neutrophils in BALF, and pro-inflammatory cytokines (IL-6, - 8, TNF-), oxidative stress (TBARS, 3NT), apoptosis (apoptotic index by TUNEL assay, caspase-3) in the lung tissue. Sildenafil positively affected the lung cell apoptosis, release of cytokines, and production of reactive oxygen species suggesting perspectives of PDE5 inhibitors in the treatment of ARDS. Granted by: CEPV II (ITMS 26220120016), CEKR II (ITMS 26220120034), BioMed (ITMS 26220220187), APVV-15-0075, VEGA 1/0356/18.

PP.296

Treatment of meconium aspiration syndrome by recombinant human superoxide dismutase and n-acetylcysteine added to exogenous surfactant

Kopincova J[1,2], Kolomaznik M[2], Mikolka P[2], Kosutova P[2], Topercerova J[1], Calkovska A[1,2], Mokra D[1,2]

[1]Department of Physiology, Comenius University, Slovakia; [2]Biomedical Center Martin, Comenius University, Slovakia

Our study aimed to compare the molecular background of effect of two antioxidants, N- acetylcysteine (NAC) and recombinant human superoxide dismutase (rhSOD), combined with exogenous surfactant Curosurf®, in the treatment of meconium aspiration syndrome (MAS). Considering redox signalling a principal part of cell response to meconium, antioxidants with different targets and routes of administration were supposed to affect MAS pathogenesis diversely. Young New Zealand rabbits were instilled with meconium suspension (Mec) and treated by Curosurf® alone (Surf) or Curosurf® in combination with i.v. NAC (Surf + NAC) or i.t. rhSOD (Surf + SOD), and oxygen-ventilated for 5 h. PaO2/FiO2 and ventilation efficiency index were evaluated every hour and post mortem, inflammatory and oxidative markers (advanced oxidation protein products, total antioxidant capacity, hydroxynonenal (HNE), p38 mitogen activated protein kinase, caspase 3, thromboxane, endothelin-1 and secretory phospholipase A2) were assessed in pulmonary tissue homogenates. Addition of rhSOD to surfactant led to significant, but transient, improvement in gas exchange; levels of inflammatory and oxidative molecules were reduced by Surf + SOD with higher impact; Surf + NAC had stronger effect on HNE formation, whereas duration of treatment efficacy in respiratory parameters was prolonged compared to Surf + SOD. In both antioxidants, it seems that targeting reactive oxygen species may be strong supporting factor in surfactant treatment of MAS. Supported by VEGA 1/0356/18; VEGA 1/0055/19, APVV-17-0250, APVV-15-0075 and Biomedical Center Martin, Slovak Republic, ITMS code: 26220220187.

PP.297

Effect of endotoxin on human lung carcinoma A549 cells producing pulmonary surfactant

Nova Z[1], Skovierova H[2], Strnadel J[2], Halasova E[2,3], Calkovska A[1]

[1]Department of Physiology, Comenius University, Slovakia; [2]Division of Molecular Medicine, Biomedical Center Martin, Comenius University, Slovakia; [3]Department of Medical Biology, Comenius University; Slovakia

Alveolar epithelial type II (ATII) cells can be damaged by bacterial lipopolysaccharide (LPS) which may impair their survival, induce oxidative stress and modulate surfactant production. The study evaluates the effect of LPS on human lung carcinoma A549 cells, in short and long term culture, which are extensively used as a model for surfactant producing ATII cells. A549 cells were cultivated under standard conditions with LPS 10-500 μg/ml for 24, 48 and 72 hrs. For long term culture cells were cultivated for 25 days and then exposed to LPS 10 and 100 μg/ml for 24 hrs. We determined cell viability by MTT assay, level of oxidative stress by flow cytometry and expression of surfactant protein (SP) A, B, C and D genes by Real-Time PCR. For all experiments cells cultivated in LPS-free medium were used as control. LPS did not significantly affect cell viability at low concentrations; a decrease about 25% was observed with LPS 500 μg/ml. There was no difference in oxidative stress level between control and LPS treated cells and also between short and long term cells. LPS decreased SPs gene expression after 24 and 72 hrs and increased after 48 hrs. SPs gene expression in long term cells was higher than in short term cells and was further enhanced by LPS. Generally, LPS 100 μg/ml had stronger effect than LPS 10 μg/ml. A549 cells are relatively resistant to LPS and are able to maintain integrity even at high LPS concentrations. LPS modulates their SPs gene expression in dose and time dependent manner. Long term cultured A549 cells probably produce larger amount of surfactant than short term cells. Moreover, LPS-induced SPs gene expression is modulated in different way. Supported by VEGA 1/0055/19, APVV-17-0250.

PP.298

Pulmonary surfactant with polymyxin B attenuates endotoxin-induced lung injury

Kolomaznik M, Kopincova J, Topercerova J, Zila I, Kosutova P, Mikolka P, Mokra D, Calkovska A

Biomedical Center Martin and Department of Physiology, Comenius University, Slovakia

Intratracheal (i.t.) administration of pulmonary surfactant (SF) mitigates inflammation caused by lipopolysaccharide (LPS). An antibiotic polymyxin B (PxB) binds to SF phospholipids (PL) and increases resistance of SF to inactivation. We hypothesized that therapy with SF+PxB can be more effective in reducing the LPS-induced inflammation than SF treatment alone. Adult rats (Wistar, n=26) were anaesthetized, tracheotomised, the endotracheal tube was inserted. Lung injury was induced by i.t. instillation of LPS (500 μg/kg; 2.2 ml/kg). Controls received saline. Animals with LPS were further treated with exogenous SF (Curosurf®, 50 mg PL/kg b.w.) or SF with PxB 1% w.w. (SF+PxB). After 5 hrs of artificial ventilation the animals were sacrificed, right lung was homogenized, left lung was lavaged by saline. The markers were determined

in homogenized lung (HL) tissue and bronchoalveolar lavage fluid (BALF). Lung oedema was expressed as wet/dry weight ratio. In comparison to control, LPS increased lung oedema formation, oxidative stress and the levels of IL-1β, IL-6, TNF- , MCP-1 in HL and BALF (all p<0.01). In LPS-treated animals, SF reduced lung oedema, oxidative stress in HL and IL-6 (all p<0.05) in BALF. With exception of oedema, the effect was potentiated by PxB added to SF. SF+PxB also reduced IL-1β, MCP-1 (p<0.05) in BALF and TNF- , MCP-1 (p<0.01) in HL. Enrichment of exogenous surfactant with PxB potentiates the effect of surfactant therapy in LPS-induced lung injury by mitigating inflammation and oxidative stress. The results indicate the potential of surfactant preparations to carry the drugs directly to the site of its action. Acknowledgements: APVV-17-0250, APVV-15-0075, VEGA 1/0055/19, BioMed 26220220187.

Poster Session IV (4/4)

Blood Physiology

PP.299

Platelet-stored antibodies potently diminish viral infection *in vitro* and *in vivo*

Schrottmaier WC[1,2], Salzmann M[1], Badrnya S[1], Morava S[1], Luik A-L[3], Kral-Pointner JB[1], Mussbacher M[1], Karlsson M[3], Forsell M[2], Assinger A[1]

[1]Institute for Vascular Biology and Thrombosis Research, Medical University of Vienna, Austria; [2]Department of Clinical Microbiology, Umeå University, Sweden; [3]Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Sweden

Besides their primary role in haemostasis, platelets are actively involved in immune responses as they respond to various inflammatory stimuli, including microbial infection. Further, platelets contain intracellular IgG, but their physiologic function remains unknown. Thus, we aimed to elucidate the function of platelet-derived IgGs and their effect on viral infections. Human and murine platelets contained IgG which were released upon shear stress. However, IgG loss did not correlate with P-Selectin exposure or CXCL4 release and -granule deficient (Nbeal2-/-) platelets failed to show reduced IgG content and release, indicating an extragranular IgG storage site within platelets. While platelet IgG could derive from megakaryocytes that have taken up IgG from the bone marrow microenvironment, naïve platelets also took up IgG directly from plasma in vitro and in vivo. Murine platelets from anti-IAV IgG seropositive mice reduced IAV infection in vitro and in vivo more efficiently than plasma containing comparable IgG levels. Further, human platelets from anti-CMV IgG seropositive but not seronegative donors also potently neutralized in vitro CMV-infection of HUVEC under microvascular shear stress. Our data indicate that IgG storage in platelets may not be restricted to -granules. Further, our results show that platelets have the potential to mediate potent IgG-mediated antiviral effects both in vitro and in vivo directly at foci of infection. This indicates that platelet-derived IgG may represent a yet unexplored mechanism for focused serological immunity.

PP.300

Effects of ginkgo biloba on some hematological, biochemical and histopathological alterations in rats with acute copper toxicity

Cinar M[1], Yildirim E[2], Duru O[1], Ekici H[2], Kabakci R[3], Sumer T[4], Senel Y[5]

[1]Department of Biochemistry, Kirikkale, Turkey; [2]Department of Pharmacology and Toxicology, Kirikkale, Turkey; 3Department of Physiology, Kirikkale, Turkey; 4Department of Pathology Kirikkale, Turkey; 5Department of Animal Nutrition and Nutritional Diseases, Kirikkale, Turkey

Although it is an essential element for the body, copper (Cu) has many toxic effects on living beings. Ginkgo biloba (GB) is a vital leaf extract used to care for many different diseases. We studied the effects of GB on some blood and histopathological alterations in rats with acute Cu toxicity. For this purpose, 32 male Wistar rats were divided into 4 groups (n=8); 1) Control, 2) Cu, 3) GB, 4) Cu+GB. Physiological saline (PS) was given by orally to control and Cu groups for 5 days while 150 mg/kg/day GB was given by orally to GB and Cu+GB groups. On the last day of oral treatments, control and GB groups were injected 10 mg/kg PS i.p. while Cu and Cu+GB groups were injected 10 mg/kg CuSO4 i.p. After 6 h later, blood and tissue (brain and liver) samples were collected for hematological, biochemical and histopathological analysis. It is observed that RBC significantly increased in the Cu+GB group as compared to other groups. However, a similar increase of HGB was not significant. PCV level was higher in the Cu+GB group than that of control and GB groups. As compared to control, an increment in MCV level was observed in rats given Cu and GB separately. Plasma ALT and ALP activities increased in the Cu group compared to control, GB and Cu+GB groups. Administration of GB with Cu reduced ALT activity but didn't decrease ALP activity. Total protein level was higher in the Cu+GB group than control and GB groups, but albumin level didn't change with any treatment. According to histopathological examinations, there were hepatocyte degeneration, necrosis, mononuclear

cell infiltration, and hemorrhage in only liver tissue of rats exposed Cu. GB ameliorated these findings in Cu+GB groups. Consequently, GB may have a protective effect against the adverse effect of Cu on liver tissue.

PP.301

Association of PAI-1 4G/5G polymorphism and IVF outcome in patients using low- molecular weight heparin

Milenkovic J[1], Milojkovic M[1], Mitic D[2], Jevtovic Stoimenov T[3], Smelcerovic Z[4], Djindjic B[1], Jovic M[4], Kundalic J[4]

[1]Department of Pathophysiology, University of Nis, Serbia; [2]Gynecology and Obstetrics Clinic, Clinical Center in Nis, Serbia; [3]Department of Biochemistry, University of Nis, Serbia; [4]Scientific Research Center for Biomedicine, University of Nis, Serbia

The 4G allele in plasminogen activator inhibitor (PAI)-1 gene changes its expression leading to the higher activity and subsequent hypofibrinolysis. PAI-1 4G/4G genotype is proposed to be a risk factor for infertility, implantation failure, and worse pregnancy outcome. Despite this, 4G/5G polymorphism is not in the current guidelines for anticoagulant prophylaxis. We aimed to determined influence of PAI-1 4G/5G polymorphism on in vitro fertilization (IVF) procedure outcome in females using low-molecular weight heparin (LMWH) for thrombophilia. Eighty patients undergoing fresh IVF cycle and using LMWH were enrolled. The 4G/5G polymorphism was determined by restriction fragment length polymorphism technique. There was significant difference in PAI-1 genotype frequencies between patients with positive vs. negative IVF outcome (p = 0.0029), with higher proportion of 4G/4G genotype (31.4%) in positive outcome group than in negative (6.67%) (Bonferoni adjustment, p = 0.0009, vs. heterozygotes). There was also significantly higher frequency of 4G allele in positive outcome group (p = 0.0085). The odds of having 4G/4G genotype and positive outcome with LMWH use were 6.2 (p = 0.01, 95%CI, 1.5468-24.9483), but only the presence of 4G allele was of borderline significance (p = 0.055). However, when adjusted for other thrombophilia factors (factor V Leiden, factor II G20210A, and MTHFR C677T), PAI-1 homozygous 4G was showing only a trend toward higher odds (p=0.068, OR 5.4). No interaction between thrombophilia factors was determined in the analysis. We can conclude that 4G/4G genotype tend to be associated with better IVF outcome in females taking anticoagulants. Work supported by the Project No. III 41018 of the Ministry of Education, Science and Technological Development of Republic of Serbia, and the Project No. 3 of the Faculty of Medicine University of Nis Serbia.

PP.302

Inherited thrombophilia in patients with unexplained infertility

Milojkovic M[1], Milenkovic J[1], Mitic D[2], Jevtovic Stoimenov T[3], Smelcerovic Z[4], Stevan Vujic S[4], Dunjic O[1]

[1]Department of Pathophysiology, University of Nis, Serbia; [2]Gynecology and Obstetrics Clinic, Clinical Center in Nis, Serbia; [3]Department of Biochemistry, University of Nis, Serbia; [4]Scientific Research Center for Biomedicine, University of Nis, Serbia.

Inherited thrombophilia is a well-known risk factor for thrombotic complications during pregnancy. However, its role in the processes of fertilization and embryo implantation are less well established. We aimed to determined genotype and allele frequencies of factor V Leiden, factor II G20210A mutation, methyl tetrahydrofolate reductase (MTHFR) C677T, and plasminogen activator inhibitor-1 4G/5G polymorphism. We also assessed their association and risk using logistic regression analysis. Fifty-five patients with unexplained infertility (UI) undergoing fresh IVF cycle and 130 healthy controls were enrolled. Mutations and polymorphisms were detected using allele specific PCR reactions. There was significant difference in MTHFR genotype distribution between patients and controls (p=0.0001). After Bonferoni adjustment, we determined significantly lower frequency of CC vs. CT genotype in patients compared to controls (p=0.0003), as well as higher percent of TT vs. CC genotype (p=0.00002). The risk allele 677T showed a strong association with an increased risk of UI (OR = 2.4394, 95%CI 1.5457 to 3.8498, p=0.0001). There was also a trend toward higher frequency of mutated FVL allele in patients (p=0.08). Not having 677CC genotype was associated with the highest risk for UI (OR=8.12; 95%CI 1.8450 to 35.7367, p=0.0056). Also, 677TT genotype increased the risk for 2.78 times (OR=2.78; 95%CI 1.0342 to 7.4869, p=0.043). MTHFR C677T is linked with elevated homocysteine blood levels which may lead to endothelial injury, increased thrombin generation, and impaired fibrinolytic activity. Hyperhomocysteinemia has been associated with early and recurrent pregnancy lost. We can conclude that 677TT polymorphism may be at least one of the underlying causes of UI. Work supported by the Project No. III 41018 of the Ministry of Education, Science and Technological Development of Republic of Serbia; and the Project No. 3 of the Faculty of Medicine University of Nis Serbia.

PP.303

Band 3 protein function in oxidative and inflammatory diseases

Morabito R[1], Remigante A[1,2], Spinelli S[1], Vitale G[1], Loddo S[3], Trichilo V[3], Dossena S[2], Marino A[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; [2]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Austria; [3]Department of Clinical and Experimental Medicine, AOU Policlinico Universitario G. Martino, Italy

$SO4^{=}$ uptake through Band 3 protein (B3p) represents a tool to monitor erythrocytes function under different conditions, such as diseases associated to oxidative stress, related to metabolic dysfunctions or inflammation. In the present investigation in vitro experiments have been conducted to monitor B3p function in several pathological conditions. Blood samples were withdrawn from patients affected by Systemic Sclerosis (SSc), or with high glycated hemoglobin (HbA1c) levels or elevated serum C-reactive protein (CRP) levels. The rate constant for $SO4^{=}$ uptake, determined by turbidimetric method and accounting for efficiency in anion exchange through B3p, was significantly lower in SSc patients than in healthy controls. Under high HbA1c levels, the rate constant and $SO4^{=}$ content were higher than in control patients. Elevated serum CRP levels induced a significant increase in both anion exchange capability through B3p and in $SO4^{=}$ trapped by the cells with respect to healthy volunteers. Once serum CRP levels were brought back to control values, anion exchange capability was restored. Overall, these results indicate that: measurement of the rate constant for $SO4^{=}$ uptake is a suitable tool to monitor the effect of acute inflammation and oxidative stress on erythrocytes homeostasis; high CRP and HbA1c levels seem to accelerate anion exchange capability through B3p; B3p from SSc patients is significantly altered; inflammation remission seems to correspond to B3p function restoration. Future studies will evaluate whether this modification may depend on an altered B3p conformation in crosslink with Hb, or on cellular signaling reflecting on B3p function, in an attempt of better understanding the impact of inflammation and oxidative processes on erythrocytes homeostasis.

PP.304

Role of antioxidants in preventing H_2O_2-induced damage on Band 3 protein

Spinelli S[1], Vitale G[1], Remigante A[1], Morabito R[1], Marino A[1]

[1]Deptartment of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy

Hydrogen peroxide has been already proven to elicit oxidative damage on Band 3 protein (B3p), anion exchanger essential to erythrocytes homeostasis. B3p capability in mediating anion exchange can be measured by determining the rate constant for $SO4^{=}$ uptake. In the present investigation the role of different antioxidants in preventing oxidative damage induced by H_2O_2 has been evaluated. To this end, blood samples, pre-incubated or not with different antioxidants (melatonin, Mg^{2+}) for 1 h, were treated with 300 μM H_2O_2 for 30 min. The rate constant for $SO4^{=}$ uptake, GSH levels and –SH membrane groups have been determined. Magnesium improves the rate constant for $SO4^{=}$ uptake with a significant GSH and -SH groups levels restoration. Melatonin restored rate constant for $SO4^{=}$ uptake, Band 3 protein expression levels and cell shape alterations provided that concentrations not producing malondialdehyde (MDA, index of lipid peroxidation) are used. Our results confirm that: i) anion exchange capability measurement through B3p is a suitable model to prove the beneficial effect of antioxidant against oxidative stress; ii) H_2O_2 at not hemolytic concentrations reduces anion exchange capability through B3p; ii) Mg^{2+} and melatonin prevent oxidative damage and can be useful in therapy against oxidative stress-related pathologies. Further studies are recommended to better focus on pathways associated to this beneficial effect.

PP.305

Complete blood count in neonates

Satti S

Department of physiology, Al-Neelain University, Khartum, Sudan

Complete blood count (CBC) with differential is one of the most common laboratory tests performed today. This test is helpful in diagnosing anemia, infections, acute hemorrhagic states, allergies, and immunodeficiencies as well as conditions secondarily affecting blood and bone marrow such as renal failure and certain cancers. It helps in monitoring for side effects of certain drug and toxic substances exposure that cause blood dyscrasias, at birth, full term newborns (FTN} have significantly different CBC compared to older children and adults. There is relative polycythemia with macrocytosis (high MCV). CBC in neonates also showed marked polychromasia with nucleated RBCs, and a high WBC count. Platelet count in neonates is similar to the adult platelet count. The study objective was to compare hematological parameters in newborns (FTN} outcome of normal vaginal delivery (NVD) and cesarean section(C/S). We recruited 46 neonates (52%) born by NVD and 44 neonates (48%) CBC were estimated in blood samples

collected from the umbilical vein, analyzed by automated cell counter. The result showed decreased count of blood platelets (239.17 ± 50.37 103/μL) in neonate delivered by C/S compared to NVD neonate (248.45 ± 49.7 103/μL), p < 0.001. Furthermore, platelet hematocrit (PCT) showed substantial differences in both groups (C/S N = 0.2% vs. NVD N = 0.23%; p < 0.001). Mean platelet volume (MPV) was found to be nearly the same (LPN = 7.95fl, FTN = 7.92fl). However increased levels of hemoglobin, RBCs count, haematocrit and MCV were observed in vaginally born infants compared to infants born by elective caesarean section. But the difference was statistically insignificant.

PP.306

Anti-inflammatory effectiveness of daidzein in experimental knee osteoarthritis- induced with monosodium iodoacetate in rats

Gundogdu G[1], Gundogdu K[2], Yilmaz Tasci S[3], Demirkaya Miloglu F[4], Cetin M[5]

[1]Department of Physiology, Pamukkale University, Turkey; [2]Department of Orthopedics and Traumatology, Erzurum Regional Training and Research Hospital, Turkey; [3]Department of Pharmacology, Ataturk University, Turkey; [4]Department of Analytical Chemistry, Ataturk University, Turkey; [5]Department of Pharmaceutical Technology, Ataturk University, Turkey

Osteoarthritis (OA) is a chronic and degenerative disease that characterized by imbalance between the events of making and destroying the periarticular bone, degeneration of articular cartilage. It mostly occurs in the knee joint. Daidzein (DZ) is a kind of phytoestrogens of isoflovane group commonly that found in many plants. The aim of this study was to investigate the effects of DZ on the inflammatory markers that has an important role in the pathogenesis of OA. The protocol of this study was confirmed by Local Ethics Committee of Laboratory Animals Experiments of Ataturk University, Erzurum, Turkey. In this study; Experimental knee osteoarthritis was formed by monoiodoacete by intraarticular (ia) injection. Twelve weeks age rats divided into 7 groups (Control (untreatment), OA+saline, OA+DZ oral, OA+DZ ia, OA+hyaluronic acid (HA) ia, OA+DZ oral+HA ia and OA+DZ+HA ia. In oral groups, DZ was administered with gavage twice daily for 21 days. In ia groups, DZ, HA and saline were injected ia at 1st,7th, 14th and 21th day of the experiment. At the end of the experiment blood samples were taken for evaluating serum TNF- and IL-1β inflammatory markers with ELISA kits. Results showed that serum TNF- and IL-1β levels decreased in the treatment groups compared to the OA+saline group. But, OA+DZ oral+HA ia group was only statistically significant decreased than OA+saline group ($p<0.05$). It has been demonstrated that DZ has anti inflammatory effect. At the same time, the results of the study show that DZ alone and/or in combination with HA may be useful in the treatment of knee OA.

PP.307

Frequency of ABO and Rhesus (RH D) blood group alleles among students of demonstration secondary school, Ahmadu Bello University, Zaria

Baraka Akor-Dewu M[1], Lami Ciroma F[2], Sunday Adeyemo Simon S[1]

[1]Department of Human Physiology, Ahmadu Bello University, Nigeria; [2]Department of Human Physiology, Kaduna State University, Nigeria.

The frequency of ABO and Rhesus blood groups vary geographically and from one population to another. Some variations may even occur within one population and within one small country. Therefore, this study was conducted to determine the frequency of ABO and Rhesus blood group phenotypes, genotypes and alleles among students of Demonstration secondary school, Samaru Zaria, Kaduna State, Nigeria. One hundred and three (103) students volunteered and were tested for ABO and Rhesus blood groups antigens. The students' blood were collected by vene-puncture of the cubital fossa and stored in EDTA bottles. Blood groupings were done using open slide methods, where a drop of blood sample was placed in three different places on clean glass slide followed by a drop of blood grouping reagents, anti-A, anti- B and anti- D. The reagents and the blood were mixed using clean stick, spread by moving gently the test slide back and forth, and checked for agglutination within one minute. The frequencies of ABO and Rhesus blood groups phenotypes were expressed in percentages and the modified Hardy-Weinberg Law was used to determine allele and genotype frequencies. In the overall sample, the O, A, B, and AB blood group percentages were 48.54%, 22.33%, 23.30% and 5.83%, respectively. The Rhesus positive incidence was 95.15%, while Rhesus negative was 4.85% in the overall sample. The order of ABO blood group allele frequencies was IO>IA>IB > in the overall samples. The allele frequencies of IO, IA, and IB in the total sample were found to be, 0.6967, 0.1582 and 0.1524 respectively. The Rhesus blood group allele frequencies of the total sample were 0.7798 D and 0.2202 d. In conclusion O+ was found to be the most common and B- the least represented.

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