BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting

Friday March 16 2012

PROGRAMME

&

ABSTRACT BOOK

Venue

Palace of the Academies
Rue Ducale / Hertogsstraat 1
1000 Brussels

Organisation

Prof. Dr. Jean-Michel RIGO
Hasselt University
BIOMED Research Institute
Research Group Cell Physiology
Agoralaan (gebouw C)
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BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

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Main Lecture

09.45-10.45 Prof. Dr. Helmut KETTENMANN (Max Delbrück Center for Molecular Medicine, Berlin, Germany).

Microglia – active sensor and versatile effector cells in the normal and pathologic brain.

Oral Communications

10.45-11.00 B. BRÔNE, E. DRIES, D. JANSSEN, Q. SWENNEN, E. BOCKSTEINS, P. LEGENDRE, D. SNYDERS, J.-M. RIGO (UHasselt, UAntwerpen, Université Pierre et Marie Curie, Paris, France & UMPC Université Paris, France).
Minocycline shifts microglia to an anti-inflammatory phenotype, according to their potassium channel expression profile.

11.00-11.15 N. SWINNEN, B. BRÔNE, P. LEGENDRE, J.-M. RIGO (UHasselt & INSERM U952, CNRS UMR 7224, Université Paris, France).
Microglia in the embryonic cortex.

11.15-11.30 A.O. DUMONT, S. GOURSAUD, E. HERMANS (UCLouvain).
Influence of inflammation on the regulation of glutamate transporters in primary cultured astrocytes and microglia during amyotrophic lateral sclerosis.

11.30-11.45 I. MATHAR, T. VOETS, M. FREICHEL, R. VENNEKENS (KULeuven & Univ. Heidelberg, Germany).
Transient receptor potential channel M4 is a regulator of catecholamine release.

11.45-12.00 M. DE BOCK, M. CULOT, R. CECCHELLI, L. LEYBAERT (UGent, Univ. d'Artois, Lens, France)
Control of blood-brain barrier by calcium and connexin channels.

12.00-12.15 R. DEUMENS, S. VAN GORP, A. BOZKURT, T. FUHRMANN, E. HERMANS, G.A. BROOK (UCLouvain, RWTH Aachen, Germany, UCSD San Diego, USA & Maastricht UMC, The Netherlands).
Pain and motor outcome following a novel repair strategy of the injured adult rat spinal cord.

12.15-12.30 J. PORTELLI, L. THIELEMANS, L. VER DONCK, E. LOYENS, J. COPPENS, N. AOURZ, J. AERSSENS, K. VERMOESEN, A. SCHALLIER, Y. MICHOTTE, D. MOECHARS, G.L. COLLINGRIDGE, Z.A. BORTOLOTTO, I. SMOLDERS (VUBrussel).
Inactivation of the ghrelin receptor suppresses limbic seizure activity in rodens.

12.30-12.45 P. PREZIOSI, C. MANCUSO (Catholic University School of Medicine, Roma, Italy).
Biliverdin reductase-A post-translational modifications in Alzheimer's disease and mild cognitive impairment.

12.45-14.15 **Lunch and Guided Poster Session**

Posters (height 120 cm – width 100 cm)

1. S. DILLY, C. LAMY, S. PONCIN, T. BRUYNS, V. SEUTIN, D. SNYDERS, J.-F. LIEGEOIS (ULiège & UAntwerpen).
Interactions of apamin with pore mutated SK3 channels.

2. D. GULDNER, C. DRESSEN, G. VEGH, P. LEBRUN, P. LYBAERT (ULBruxelles).
Kir6.2/SUR2 expression in male accessory reproductive glands in rats and mice.

3. G. OWSIANIK, J. PRENEN, D. HERMANS, J. EGGERMONT, T. VOETS, B. NILIUS (KULeuven).
Anoctamin 6 is a positive modulator of volume-regulated anion channels.

4. D. GOSH, G. OWSIANIK, P. VANDEN BERGHE, A. SEGAL, J. VRIENS, T. VOETS (KULeuven).
Characterization of the trafficking of human transient receptor potential melastatin 8 (hTRPM8) channel by total internal reflection fluorescence (TIRF) microscopy.

5. B.I. TOTH, J. VRIENS, T. VOETS (KULeuven).
Adenosine-5`-triphosphate (ATP) and phosphatidylinositol 4,5-bisphosphate (PIP2) are positive regulators of transient receptor potential melastatin 3 (TRPM3) channel activity.

6. X. FLORENCE, P. DE TULLIO, B. PIROTTE, P. LEBRUN (ULBruxelles, ULiège).
New benzopyran derivatives acting as calcium antagonists in vascular smooth muscle.

7. A-S. HERVENT, C.E. VAN HOVE, H. BULT, G. DE KEULENAER, P. FRANSEN (UAntwerpen).
Influence of arterial hypertension on L-type Ca^{2+} channel-mediated contractions of conduit arteries.

8. K. PHILIPPAERT, B. COLSOUL, T. VOETS, R. VENNEKENS (KULeuven).
Screening for pharmacological tools to target Ca^{2+} activated non-selective cation channels.

9. R. VENNEKENS, I. MATHAR, P. HERIJGERS, G. VAN DER MIEREN, B. NILIUS (KULeuven).
TRPM4 is a novel regulator of contraction strength in the heart muscle.

10. A. AVILA, P. VIDAL, L. NGUYEN, J.-M. RIGO (UHasselt, ULiège).
Glycine receptor influences interneuron cell migration in the developing brain.

11. S. SMOLDERS, N. SWINNEN, A. AVILA, J-M. RIGO, B. BRÔNE (UHasselt).
Migration of microglia in the embryonic neocortex.

12. A. SWIJSEN, A. AVILA, B. BRÔNE, D. JANSSEN, G. HOOGLAND, J.-M. RIGO (UHasselt & Univ. Maastricht, The Netherlands).
Experimental early-life febrile seizures induce changes in $GABA_AR$-mediated neurotransmission in the dentate gyrus.

13. W. STOOP, D. DE GEYTER, S. SARRE, R. KOOIJMAN (VUBrussel).
Neuroprotective effects of the insulin-like growth factor IGF-I and 17β-estradiol after transient focal cerebral ischemia in the endothelin-1 rat model.

14. J. VAN LIEFFERINGE, A. SCHALLIER, H. SATO, Y. MICHOTTE, A. MASSIE, I. SMOLDERS (VUBrussel).
System XC- is an important source of extracellular glutamate in mouse hippocampus.

15. G. BEHETS, G. RATH, C. BOUZIN, C. DESSY (UCLouvain).
Caveolin-1 participates to the regulation of gap-junctions in endothelial cells.

16. N. WANG, M. DE BOCK, G. ANTOONS, M. BOL, E. DECROCK, E. DE VUYST, K. LEURS, W. H. EVANS, K.R. SIPIDO, F. BUKAUSKAS, L. LEYBAERT (UGent, Univ. Graz Austria, Cardiff Univ. School Wales, United Kingdom, KULeuven, Albert Einstein College of Medicine, New York, USA).
Connexin mimetic peptides inhibit Cx43 hemichannel opening triggered by voltage and intracellular Ca^{2+} elevation.

17. E. DECROCK, D.V. KRYSKO, M. VINKEN, A. KACZMAREK, G. CRISPINO, M. BOL, N. WANG, M. DE BOCK, E. DE VUYST, C.C. NAUS, V. ROGIERS, P. VANDENABEELE, C. ERNEUX, F. MAMMANO, G. BULTYNCK, L. LEYBAERT (UGent, VIB, VUBrussel, Istitut. Veneto Med. Moleculare & Istituto CNR Neuroscience Padova, Italy, Univ. British Columbia, Canada, ULBruxelles, KULeuven).
Transfer of inositol trisphosphate through gap junctions is critical, but not sufficient, for the spread of apoptosis.

18. V. PENNEMANS, J-M. RIGO, J. PENDERS, Q. SWENNEN (UHasselt).
Novel urinary biomarkers for proximal tubular damage: Is age an issue?

19. C. DRESSEN, D. GULDNER, G. VEGH, P. LEBRUN, P. LYBAERT (ULBruxelles).
Calbindin and calretinin in mouse and rat sperm.

20. B. COLSOUL, G. JACOBS, K. PHILIPPAERT, G. OWSIANIK, F. SCHUIT, R. VENNEKENS (KULeuven).
Gene regulation of Trpm5 in animal models of type 2 diabetes.

21. A. MENIGOZ, K. PHILIPPAERT, T. AHMED, D. BALSCHUN, T. VOETS, R. VENNEKENS (KULEUVEN).
TRPM4, a new player in synaptic plasticity modulation in hippocampus

22. A. DEITEREN, W. VERMEULEN, J.G. DE MAN, T.G. MOREELS, P.A. PELCKMANS, B.Y. DE WINTER (UAntwerpen).
Inducing visceral hypersensivity in rats: study of the effects of inflammation, stress and their combination.

23. A. MAHESHWARI[1], H. SLAETS[1], C. VAN DEN HAUTE[2], V. BAEKELANDT[2], P. STINISSEN[1], J. HENDRIKS[1], N. HELLINGS[1] (U. Hasselt, KULeuven).
Local overexpression of interleukin -11 in the central nervous system prevents demyelination and enhance remyelination

24. J. VRIENS[1], T. VOETS[2] (KULeuven)
Novel gating mechanism of trpm3 channel opening

Oral Communications

14.15-14.30 E. BOCKSTEINS, A.J. LABRO, D.J. SNYDERS, D.P. MOHAPATRA, (UAntwerpen).
The electrically silent Kv6.4 subunit confers hyperpolarized gating charge movement in Kv2.1/ Kv6.4 heterotetrameric channels.

14.30-14.45 M. BOUDES, P. UVIN, S. PINTO, A. MENIGOZ, W. EVERAERTS, R. VENNEKENS, T. VOETS, D. DE RIDDER (KULeuven).
Expressed in urothelium and detrusor, TRPM4 is a key player of bladder physiology.

14.45-15.00 M. HEYLEN, J.G. DE MAN, S. STAELENS, S. DELEYE, P. PELCKMANS, T.G. MOREELS, B.Y. DE WINTER (UAntwerpen).
Are colonscopy and µPET/CT in mice valuable tools to assess colitis in the adoptive transfer model ?

15.00.-15.15 J. VAN LANGEN, P. FRANSEN, C.E. VAN HOVE, H. BULT (UAntwerpen).
Study of basal nitric oxide production in the mouse aorta.

15.15-15.30 G. RATH, J. SALIEZ, G. BEHETS, M. ROMERO-PEREZ, E. LEON-GOMEZ, C. BOUZIN, J. VRIENS, B. NILIUS, O. FERON, C. DESSY (UCLouvain, KULeuven).
Vascular hypoxic-preconditioning relies on TRPV4-dependent calcium influx and proper intercellular gap-junctions communication.

15.30-15.45 A. MARTINSEN, X. YERNA, N. MOREL (UCLouvain).
Voltage-operated calcium channel regulation by myosin light chain kinase in rat resistance mesenteric artery.

15.45-16.00 D. DE GEYTER, W. STOOP, S. SARRE, R. KOOIJMAN (VUBrussel).
The insulin-like growth factor-I as a treatment in a rat model for focal cerebral ischemia.

16.00-16.15 S. WENINGER, R.A. LEFEBVRE (UGent).
5-HT_4 receptor signaling in porcine left atrium: Influence of phosphodiesterases and particulate guanylyl cyclase activation.

16.15-16.30 M. KECSKES, G. OWSIANIK, P. VANGHELUWE, T. VOETS, R. VENNEKENS (KULeuven).
The role of the TRPM4 ion channel in cardiac hypertrophy.

ABSTRACTS

Legend
O = Oral communication numbered
P = Posters numbered

O-01

MINOCYCLINE SHIFTS MICROGLIA TO AN ANTI-INFLAMMATORY PHENOTYPE, ACCORDING TO THEIR POTASSIUM CHANNEL EXPRESSION PROFILE

E. Dries[1], D. Janssen[1],Q. Swennen[1], E. Bocksteins[2], P. Legendre[3,4,5], D. Snyders[2], J.-M. Rigo[1] and B. Brône[1]

[1] BIOMED, University of Hasselt, Diepenbeek, Belgium, [2] Laboratory for Molecular Biophysics, Physiology and Pharmacology, University of Antwerp, Antwerp, Belgium, [3] Institut National de la Santé et de la Recherche Médicale, U952, Université Pierre et Marie Curie, Paris, France, [4] Centre National de la Recherche Scientifique, UMR 7224, Université Pierre et Marie Curie, Paris, France, [5] UMPC Université Paris 06, Paris, France.

Minocycline has been generally thought to exert anti-inflammatory effects in the central nervous system by acting as a potent inhibitor of microglial activation. However, the mechanism of action of minocycline on microglial cells remains elusive. Since microglial activation phenotypes are characterized by the expression of different types of ion channels, we investigated whether minocycline can modulate the functional expression of K^+ and purinergic channels in primary cultured rat microglial cells using the whole-cell patch-clamp technique. Minocycline caused a significant decrease of delayed rectifier K^+ currents (K_{dr}) in LPS-activated microglial cells and a marked increase of inward rectifier K^+ currents (K_{ir}) in control and LPS-activated cells. This resulted in a new microglial I/V profile characterized by a prominent functional expression of Kir2.1 channels and the disappearance of the Kv1.3 and Kv1.5 channels. Moreover, this new I/V profile corresponded to the I/V profile of microglial cells stimulated with anti-inflammatory cytokines (IL-4 and IL-10). Our results indicate that, according to their ion channel expression profile, minocycline was not able to revert microglial cells to their surveying state, but in contrast shifted them into an anti-inflammatory phenotype.

O-02

MICROGLIA IN THE EMBRYONIC CORTEX

N. Swinnen[1,2], B. Brône[1], P. Legendre[2], JM. Rigo[1]

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Microglia are known as the macrophages of the central nervous system. These cells are derived from primitive myeloid progenitor cells that arise in the yolk sac before embryonic day (E) 8. In the mouse, the start of microglial colonization (E9.5) just precedes the beginning of a moment with increased activity in the embryonic cortex, the period of neurogenesis. By their production of growth factors, it has been suggested that microglia can influence axonal growth and synaptogenesis. The aim of this study is to investigate the colonization and distribution of the microglial cells in the embryonic murine neocortex and to determine their activation stages. Transgenic C57BL/6 CX3CR1$^{+/eGFP}$ mice embryos (E10.5 - E17.5) were isolated at the desired age. Coronal sections (10µm) were stained for Iba-1 and CD68. As activation markers we used Galectin-3/Mac-2, MHC II and Ki-67. Images were analyzed with ImageJ and statistics was performed with Prism, p-values < 0.05 were considered significant. The electrophysiological phenotyping of K^+ currents was performed on ex vivo E15.5 brain slices (300 µm). The cell density and number of microglia increase as the embryo ages. This is mainly due to invasion of microglia into the cortex, since only a small percentage of the microglia present in the cortical parenchyma is actively proliferating. Microglial cells are also present in the lateral ventricle and at the pial surface, it has been shown that microglia can enter the nervous parenchyma from these sites. The absence of the expression of Mac-2 and MHC II suggest these cells have a naïve/quiscent phenotype during embryonic development of the cortex. At the age of E15.5, microglial cells display a small inward rectifying K^+ current and this independent of their location in the embryonic cerebral cortex and their cell morphology.

O-03

INFLUENCE OF INFLAMMATION ON THE REGULATION OF GLUTAMATE TRANSPORTERS IN PRIMARY CULTURED ASTROCYTES AND MICROGLIA DURING AMYOTROPHIC LATERAL SCLEROSIS

A.O. Dumont, S. Goursaud and E. Hermans

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Functional alterations associated with glutamate excitotoxicity and inflammation have been documented in several neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). This study aims at investigating the influence of an inflammatory environment on the expression and activity of glutamate transporters in newborn derived astrocyte or microglia cultures, particularly in the context of ALS. In this aim, we have maintained astrocytes or microglia in culture and exposed these cells to tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine. The expression of the glutamate transporters GLT-1a, GLT-1b and GLAST was characterised by quantitative RT-PCR and Western-blotting and the activity was determined in D-[^{3}H]-aspartate uptake assays. Similar experiments were conducted using cells derived from a transgenic rat strain expressing an ALS-associated mutated form of human superoxide dismutase 1 (hSOD1^{G93A}). These experiments indicated that the expression of the glutamate transporters was modified after an exposure to an inflammatory environment in the culture from the wild-type (WT) rats. These regulations concerned mainly GLT-1a and GLT-1b, but GLAST was also implicated although regulations were opposed. Furthermore, we evidenced a different profile of regulation of GLT-1a and GLT-1b in hSOD1^{G93A} astrocytes, revealing a higher susceptibility to inflammation and distinct mechanisms of regulation in hSOD1^{G93A} cultures. A better understanding of the regulation of the glutamatergic transmission and the mechanism implicated could give rise to opportunities for intervention in neurodegenerative disorders.

O-04

TRANSIENT RECEPTOR POTENTIAL CHANNEL M4 IS A REGULATOR OF CATECHOLAMINE RELEASE

I. Mathar[1,2], T. Voets[1], M. Freichel[2], R. Vennekens[1]

[1]Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven [2]Pharmacological Institute, University Heidelberg, Germany.

TRP proteins form cation channels that are regulated through strikingly diverse mechanisms. Recently, genetic association studies identified many *Trp* genes including *Trpm4* as risk factors for disease states such as arrhythmias, hypertension and cardiomyopathy. The melastatin TRP channels TRPM4 and TRPM5 have distinct properties within the TRP channel family; they form non-selective cation channels activated by intracellular calcium ions and are expressed in heart, aortic endothelial cells, kidney and adrenal gland. Disruption of the TRPM4 gene in mice leads to increased basal blood pressure without evidence for impairment of endothelium- or smooth muscle-dependent regulation of contractility of peripheral resistance vessels, the renin angiotensin aldosterone system, basal cardiac output or body fluid homeostasis. Instead, TRPM4-deficient chromaffin cells exhibit increased acetylcholine-induced exocytosis of catecholamines which is associated with elevated level of epinephrine in the plasma and its metabolites in the urine. This indicates that TRPM4 serves as an inhibitory regulator of exocytotic catecholamine release, at least in chromaffin cells. Whether catecholamine release is also regulated by TRPM4 in other cells of the sympathetic nervous system such as perivascular neurons still needs to be clarified as well as the molecular mechanism underlying how TRPM4 regulates catecholamine release.

O-05

CONTROL OF BLOOD BRAIN BARRIER BY CALCIUM AND CONNEXIN CHANNELS

M. De Bock[1], M. Culot[2], R. Cecchelli[2], L. Leybaert[1]

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[2]Université d'Artois, Laboratoire de physiopathologie de la barrière hémato-encéphalique, 62307 Lens, France.

The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important factor determining the functional state of blood-brain barrier (BBB) endothelial cells (ECs) but little is known on the effect of Ca^{2+} oscillations on BBB function. Ca^{2+} oscillations are based on positive and negative $[Ca^{2+}]_i$ feedback on $InsP_3$ receptors. Connexin (Cx) hemichannels are Ca^{2+}-permeable plasma membrane channels that are also characterized by positive and negative $[Ca^{2+}]_i$ feedback on hemichannel opening; therefore, these channels may contribute to the generation of Ca^{2+} oscillations. We found that Cx hemichannels were actively involved in Ca^{2+} oscillations triggered in BBB ECs by the pro-inflammatory agent bradykinin (BK). These oscillations were inhibited by knock down of Cx37/43 with siRNA, by inhibiting hemichannels with Cx mimetic peptides (Gap26 or Gap27) or by preventing hemichannel closure at high $[Ca^{2+}]_i$ with a peptide identical to the last 9 amino acids of the C-terminus of Cx43. Work with antagonists of purinergic signaling demonstrated that ATP release via hemichannels and autocrine signaling was involved in the BK-triggered oscillations. By contrast, exposure of the cells to ATP triggered oscillations that were *not* associated with hemichannel opening. Interestingly, Ca^{2+} oscillations necessitated the presence of glial cells during EC culturing: ECs in mono-cultures without glial cells had strongly reduced oscillatory responses to BK or ATP. At the level of BBB function, we found that BK triggered an increase of BBB permeability measured *in vitro,* while ATP had no effect. Moreover, Gap27 inhibited the BK-triggered BBB permeability increase both *in vitro* and *in vivo* (in rat and mice). We conclude that Cx hemichannels form a novel target to influence BBB function and peptides targeting EC Cxs may thus serve as a tool to prevent BBB permeability increases that are associated with several neurological diseases.

O-06

PAIN AND MOTOR OUTCOME FOLLOWING A NOVEL REPAIR STRATEGY OF THE INJURED ADULT RAT SPINAL CORD

R. Deumens[1,3,5], S. van Gorp[4], A. Bozkurt[2], T. Führmann[1], E. Hermans[3], G.A. Brook[1]

[1]Institute of Neuropathology, RWTH Aachen University, Germany [2]Department of Plastic Surgery, Reconstructive and Hand Surgery, RWTH Aachen University, Germany, [3]Institute of Neuroscience, Université Catholique de Louvain, Brussels, [4]Department of Anesthesiology, University of California San Diego, USA, [5]Department of Anesthesiology, Maastricht University Medical Center, Netherlands

Repair of the injured mammalian spinal cord remains one of the most exciting challenges in the field of regenerative medicine today. Spinal cord injury triggers various cellular and molecular processes having debilitating consequences such as impaired motor function and onset of intractable neuropathic pain. We, here, used a novel repair strategy using olfactory ensheathing glia and collagen-based scaffolds. Previous studies showed that olfactory ensheathing glia have a tremendous capacity to support regeneration of injured axons and integrate nicely within the injured nervous system. As these studies mostly used animal models with defined, small spinal cord lesions, the present investigation focused on spinal cord injury with large lesion cavities, which represent more closely the clinical situation. We found that the olfactory ensheathing glia/collagen implant did not support an enhanced motor recovery as assessed with the CatWalk gait analysis. Moreover, pain hypersensitivity to mechanical paw stimulation remained unaffected by the repair therapy. Finally, pain-related microglial activation induced by the spinal cord lesion was unaltered in animals with or without repair therapy. Our data suggest that repair of spinal cord lesions with large cavities do not necessarily benefit from the implantation of olfactory ensheathing glia/collagen implants. These observations are particularly important considering the current use of olfactory ensheathing glia in human spinal cord injury in some countries.

O-07

INACTIVATION OF THE CONSTITUTIVELY ACTIVE GHRELIN RECEPTOR ATTENUATES LIMBIC SEIZURE ACTIVITY IN RODENTS

J. Portelli[1], L. Thielemans[2], L. Ver Donck[2], E. Loyens[1], J. Coppens[1], N. Aourz[1], J. Aerssens[2], K. Vermoesen[1], R. Clinckers[1], A. Schallier[1], Y. Michotte[1], D. Moechars[2], G.L. Collingridge[3,4], Z.A. Bortolotto[3], I. Smolders[1]

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Ghrelin is a pleiotropic neuropeptide that has recently been implicated in epilepsy. Animal studies performed to date indicate that ghrelin has anticonvulsant properties; however its mechanism of anticonvulsant action remains unknown. The present study showed anticonvulsant effects of ghrelin and the ghrelin-mimetic capromorelin against pilocarpine-induced seizures in rats and mice. Experiments with transgenic mice ascertained that ghrelin requires the growth hormone secretagogue receptor (GHSR) for its anticonvulsant effect. To our surprise, however, we found that the GHSR knockout mice had a higher seizure threshold than their wildtype littermates when treated with pilocarpine. Using both *in vivo* and *in vitro* models, we further discovered that abolishing the constitutive activity of GHSR by inverse agonism results in the attenuation of seizures and epileptiform activity. We confirmed that ghrelin's potential to rapidly desensitize the GHSR is followed by internalization of the receptor and a slow resensitization process. This, together with our present novel findings that different ghrelin fragments possess similar agonistic potencies but different desensitization characteristics on the GHSR, led us to elucidate that ghrelin probably prohibited limbic seizures in rodents and epileptiform activity in hippocampal slices due to its desensitizing effect on the GHSR. To the best of our knowledge, this constitutes a novel mechanism of anticonvulsant action whereby an endogenous agonist reduces the activity of a constitutively active receptor.

O-08

BILIVERDIN REDUCTASE-A POST-TRANSLATIONAL MODIFICATIONS IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

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The heme oxygenase/biliverdin reductase-A (HO/BVR-A) system is the main pathway involved in heme degradation. Heme oxygenase, a microsomal enzyme, degrades heme into ferrous iron, carbon monoxide and biliverdin-IXalpha. This latter is further reduced, by the cytosolic BVR-A, into bilirubin-IXalpha, a powerful free radical scavenger. Moreover, Mahin Maines and her group demonstrated that BVR-A is a serine/threonine/tyrosine kinase thus unraveling new roles for this enzyme in the regulation of the adaptive response to stress as well as in cell growth and metabolism. Over the last ten years, several lines of preclinical evidence proposed a cytoprotective role for the HO/BVR-A system in neurodegenerative disorders, but only few studies were performed in humans. In this work, the post-translational modifications of BVR-A in the hippocampus and cerebellum of subjects with Alzheimer's disease (AD) and amnestic mild cognitive impairment (MCI) were evaluated. Biliverdin reductase-A expression was significantly increased in AD and MCI hippocampi whereas no changes were found in cerebella. Moreover, hippocampal BVR-A was target for nitrosative post-translational modifications in both AD and MCI. In addition, BVR phosphorylation on serine/threonine/tyrosine residues was significantly reduced only in AD/MCI hippocampi. In agreement with these modifications, BVR-A activity was significantly reduced only in AD/MCI hippocampi. A possible explanation of this last result lies in the evidence that both the nitrosative modifications occurred in hippocampal BVR-A and the phosphorylation status of this latter reduced the reductase activity. Finally, these post-translational modifications interfered also with the BVR-A's kinase activity as demonstrated by the reduced formation of BVR-A-ERK complexes in AD /MCI Hippocampi. Not just the total levels of antioxidant enzymes, such as BVR-A, but also post-translational modifications should be studied when considering their effects in AD and MCI brain.

O-09

THE ELECTRICALLY SILENT K_V6.4 SUBUNIT CONFERS HYPERPOLARIZED GATING CHARGE MOVEMENT IN K_V2.1/K_V6.4 HETEROTETRAMERIC CHANNELS.

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Voltage-gated K^+ (Kv) channels are tetramers of α-subunits that detect changes in membrane potential (V) by a positively charged (Q) voltage-sensing domain (VSD). Molecular movements of these VSDs lead to charge displacement that can be recorded as transient gating currents (I_Q), which subsequently results in channel gating. The silent Kv subunits Kv6.4 does not form functional homotetramers but co-assembles with Kv2.1 with a proposed 3:1 stoichiometry to form functional Kv2.1/Kv6.4 heterotetrameric channels. Compared to Kv2.1 homotetramers, Kv6.4 exerts a ~40 mV hyperpolarizing shift in the voltage-dependence of Kv2.1/Kv6.4 channel inactivation, without any significant effect on activation gating. Here we report distinct gating charge movement of Kv2.1/Kv6.4 heterotetrameric channels, compared to Kv2.1 homotetramers, as revealed by I_Q recordings from HEK293A cells expressing these channels. The half-maximal potential ($Q_{1/2}$) of gating charge displacement for Kv2.1 homotetramers was -26 mV as determined from the charge-voltage (QV) curve. Analysis of the decay time constant of $I_{Q\text{-}ON}$ as a function of voltage resulted in a bell shaped curve with a maximal time constant around -20 mV, similar to $Q_{1/2}$. The gating charge movement of Kv2.1/Kv6.4 heterotetrameric channels displayed an extra component, characterized by a second component in the QV curve with a $Q_{1/2}$ of -93 mV and a double bell shaped curve for the decay time constant, with maximal time constants around -20 mV and -70 mV. This distinct gating charge displacement reflects movement of the Kv6.4 VSD and has a voltage-dependency that matches the hyperpolarizing shift in Kv2.1/Kv6.4 channel inactivation. These results provide a mechanistic basis for the modulation of Kv2.1 channel inactivation gating kinetics by silent Kv6.4 subunits.

O-10

EXPRESSED IN UROTHELIUM AND DETRUSOR, TRPM4 IS A KEY PLAYER OF BLADDER PHYSIOLOGY

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We investigated the role of TRPM4 in mice bladder function. Using *in situ hybridization* and immunohistochemistry, we found that both urothelium and detrusor express TRPM4. We proved that TRPM4$^{-/-}$ mice displayed altered micturition pattern and urodynamic parameters. The genetical and pharmacological inhibition of TRPM4 induced an increased bladder capacity. Calcium imaging performed on urothelial cells cultures demonstrated that calcium homeostasis in response to ATP stimulus was modified in knock-out mice. Finally, the contractile properties of TRPM4$^{-/-}$ mice were diminished following activation of muscarinic receptors in physiological range. Our data suggest that TRPM4 is a key mediator of the calcium signalling in bladder cells and supports bladder function.

O-11

ARE COLONOSCOPY AND µPET/CT IN MICE VALUABLE TOOLS TO ASSESS COLITIS IN THE ADOPTIVE TRANSFER MODEL?

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Reliable chronic animal models of inflammatory bowel disease (IBD) and techniques allowing monitoring of inflammation in the same animal are indispensable for in vivo research. The aim of this study is to examine if colonoscopy and micro-positron emission tomography/computed tomography (µPET/CT) in mice are valuable tools to assess colitis in an adoptive transfer model. Methods: Immunodeficient SCID mice are transferred with naive $CD4^+CD25^-CD62L^+$ T cells. These T cells were isolated from normal donor mice using magnetic separation procedures and transferred into SCID mice, to induce colitis (n = 5). A control group injected with saline was included (n = 5). Mice were monitored and after 4 weeks, colonic inflammation was examined by µPET/CT followed 24h later by colonoscopy. After the imaging procedures, mice were sacrificed and colonic inflammation was evaluated using a macroscopic and microscopic inflammation score and myeloperoxidase (MPO) activity assay. Results: Colitis mice but not control mice displayed a significant weight reduction at 3 weeks. Colitis mice showed a significant elevation of the clinical disease score at week 2, 3 and 4 compared to control mice. µPET/CT images of colitis mice showed increased distal colonic PET signals, which were not detected in control mice. A significant elevation of the colonoscopic score was seen in colitis mice compared to control mice. Macro- and microscopically, colitis mice showed significant alterations, which were absent in control mice. MPO activity was significantly increased in colitis mice compared to control mice. Correlation analysis showed significant correlations between the different assessment techniques. Conclusion: The adoptive transfer model may be useful for studying new treatment strategies for IBD, where colonoscopy and µPET/CT in mice can be used as reliable techniques allowing longitudinal monitoring of colitis and reducing the need to sacrifice animals.

O-12

STUDY OF BASAL NITRIC OXIDE PRODUCTION IN THE MOUSE AORTA

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Endothelial nitric oxide (NO) synthase (eNOS) is a key protector against atherosclerosis. This study addresses the basal release of NO in the isolated mouse aorta. Aortic segments were mounted in the organ bath and isometric forces were recorded. Basal NO was assessed by its ability to suppress phenylephrine (PE) induced contractions. Exactly 1h after dissection contractions were very small but gradually increased every hour. In the presence of the pan-NOS inhibitor N^{Ω}-nitro-L-arginine methyl ester contractions were much larger and did not increase. In contrast, PE contractions were unaffected by selective inhibitors of neuronal NOS (nNOS) or inducible NOS (iNOS). In the presence of superoxide dismutase PE contractions were lower, but they increased similarly to the untreated segments. Catalase had little effect on basal contractions. Acetylcholine (ACh) and the alpha-2 adrenoceptor agonist (UK 14,304) induced relaxations of pre-constricted segments, which remained unaltered throughout the experiment. Catalase did not affect ACh relaxations at 1h, but largely inhibited ACh relaxations at 3h. From these results we can conclude that basal NO production is initially very high, but quickly disappears in the organ bath. The selective NOS inhibitors showed that basal NO is eNOS-dependent and not formed by nNOS or iNOS. Basal NO selectively declined in the organ bath without apparent change in stimulated eNOS activity, implying different regulatory mechanisms. The observation that catalase had no effect on ACh relaxation at 1h, but strongly inhibited relaxations at 3h point to uncoupling of eNOS, leading to a shift from NO to O_2^- production and subsequent formation of H_2O_2, which acts as a potent vasodilator. These results underline the importance of choosing an early and consistent time point in organ bath experiments when studying bioavailability of NO. The time-dependent effect of catalase could explain discrepancies in publications on the contribution of H_2O_2 to endothelium-dependent vasodilatation.

O-13

VASCULAR HYPOXIC-PRECONDITIONING RELIES ON TRPV4-DEPENDENT CALCIUM INFLUX AND PROPER INTERCELLULAR GAP-JUNCTIONS COMMUNICATION

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We investigated the impact of hypoxia-reoxygenation on endothelial relaxation and aimed to clarify the role of transient receptor potential cation channels V4 (TRPV4) and gap-junctions in the protective effect associated to hypoxic-preconditioning on the vascular function. By mimicking ischemia-reperfusion in C57Bl/6 male mice in vivo, we documented an alteration of NO-mediated relaxation and an up-regulation of EDHF-mediated relaxation. Hypoxic-preconditioning however restored NO relaxation and further improved the EDHF response. We also examined specifically two major effectors of the EDHF pathway, TRPV4 and connexins. We found that in endothelial cells, expression and activity of TRPV4 channels were increased by hypoxic stimuli independently of preconditioning which was interestingly associated to an increase of structural caveolar component caveolin-1 at membrane locations. Gap-junctions however directly supported EDHF-driven preconditioning as in vivo gap-junction uncoupling by carbenoxolone completely inhibited the EDHF pathway and significantly reduced the protection afforded by preconditioning for the concomitant NO-mediated relaxation. Our work provides evidences on how TRPV4 and connexins might participate to preserve vasorelaxation under hypoxia and restore the NO-mediated pathway in hypoxic-preconditioning conditions pointing out caveolae as a common signaling location.

O-14

VOLTAGE-OPERATED CALCIUM CHANNEL REGULATION BY MYOSIN LIGHT CHAIN KINASE IN RAT RESISTANCE MESENTERIC ARTERY

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The Ca^{2+}-dependent kinase myosin light chain kinase (MLCK) is the main activator of smooth muscle contraction, and has been reported to be involved in Ca^{2+} channel regulation. We observed that the inhibition of MLCK by ML-7 (3 µM) depressed the contraction and the increase in cytosolic Ca^{2+} induced by noradrenaline (NA) in rat resistance mesenteric artery (RMA). We further characterized the role of MLCK on Ca^{2+} signal and contractile tension in RMA. To this aim, RMA were transfected by reversible permeabilization with either a siRNA directed against MLCK (MLCK-siRNA) or a scramble siRNA, and organ-cultured for 85h. Results showed that MLCK expression was reduced by 43% (n=23; p=0.009) in RMA transfected with MLCK-siRNA compared to scramble-siRNA. NA-induced contraction was significantly depressed in RMA transfected with MLCK-siRNA but NA-pD2 value was unchanged. In addition, 10 µM NA-induced Ca^{2+} signal measured in fura2-loaded RMA was decreased from 118 $\pm$ 19 nM (n=4) in scramble-siRNA-transfected RMA to 33 $\pm$ 12 nM (n=7; p<0.001) in MLCK-siRNA-transfected RMA. However, the Ca^{2+} signal induced by high KCl solution was also depressed from 78 $\pm$ 26 nM (n=4) in scramble-siRNA-transfected RMA to 27 $\pm$ 4 nM (n=6; p=0.04) in MLCK-siRNA-transfected RMA, suggesting that inhibition of MLCK expression modifies voltage-operated Ca^{2+} channels. In cells isolated from MLCK-siRNA-transfected RMA, the voltage-dependent Ca^{2+} current was reduced by 53% (n=5; p<0.01) compared to cells isolated from scramble-siRNA-transfected RMA. Altogether, these results indicate that, in RMA, MLCK participates in the regulation of voltage-operated Ca^{2+} channels.

O-15

THE INSULIN-LIKE GROWTH FACTOR-I AS A TREATMENT IN A RAT MODEL FOR FOCAL CEREBRAL ISCHEMIA

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The main goal of this study was to evaluate the use of the pleiotropic peptide insulin-like growth factor-I (IGF-I) for the treatment of ischemic stroke in an animal model. To facilitate translation to the clinic, we have developed a rat model for preclinical studies in conscious animals in the presence of a co-morbidity factor. We chose to work with hypertensive rats since hypertension is an important modifiable risk factor for stroke which influences the clinical outcome. We aimed to compare the susceptibility to ischemia of hypertensive rats with those of normotensive controls and measured the neuroprotective effects of IGF-I in both types of rats. The vasoconstrictor endothelin-1 was stereotactically applied in the vicinity of the middle cerebral artery of conscious control Wistar Kyoto rats (WKYRs) and Spontaneously Hypertensive rats (SHRs). Infarct size was assessed histologically by cresylviolet staining. Sensory-motor functions were measured using the Neurological Deficit Score (NDS). Activation of microglia in the striatum and cortex was investigated by immunohistochemistry using antibodies against CD68 and Iba-1. IGF-I was administered systemically (subcutaneously or intravenously) in a dose of 300 and 900 µg. We found that the SHRs displayed 50% less activated microglia. These results were confirmed in the lipopolyssacharide model for neuroinflammation indicating that microglia in SHRs were less susceptible to activation compared to their normotensive counterparts. Systemic administration of IGF-I resulted in lower infarct volumes and better NDSs in the WKYRs which means that IGF-I administration could become an effective therapy for ischemic stroke. In the SHRs however, the effects of IGF-I were smaller, maybe due differences in vasculature or different responses of microglial cells. Ongoing research to the differences between normotensive en hypertensive rats may provide indications for optimizing the effects of IGF-I for different types of patients.

O-16

5-HT_4 RECEPTOR SIGNALING IN PORCINE LEFT ATRIUM: INFLUENCE OF PHOSPHODIESTERASES AND PARTICULATE GUANYLYL CYCLASE STIMULATION

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Phosphodiesterase (PDE) 3 and PDE4, degrading cAMP, in concert regulate the fade of inotropic responses to 5-HT_4 receptor activation in pig left atrium. The particulate guanylyl cyclase (pGC) stimulator C-type natriuretic peptide (CNP) can influence responses to 5-HT via cross-talk of cGMP with cAMP, presumably through cGMP-inhibited PDE3 and cGMP-activated PDE2. The role of PDEs and pGC stimulation in controlling responses to 5-HT was further investigated. Porcine left atrial muscles were mounted in organ baths, received specific PDE inhibitors and/or CNP followed by 5-HT and were freeze-clamped for further processing. Phosphorylation of the protein kinase A targets troponin (TnI) and phospholamban (PLB) was determined by immunoblotting and cAMP content was measured by enzyme immunoassay. The PDE inhibitors per se had no consistent effect on PLB or TnI phosphorylation, nor on cAMP content. 5-HT increased cAMP content and phosphorylation of PLB, and this was significantly further enhanced under PDE3 and PDE4 inhibition. 5-HT did not significantly increase TnI phosphorylation while it did so under PDE3 and PDE4 inhibition. These results are in line with the functional data. Functional responses to 5-HT in the presence of CNP plus PDE2- and PDE4-inhibitors, ensuring maximal cross-talk with cAMP, were significantly enhanced. In this condition, PLB and TnI phosphorylation and cAMP content were significantly increased; however this was not significantly different from the rises in the presence of PDE4 inhibition alone. PDE3 and PDE4 subtypes are controlling the cAMP response to 5-HT_4 receptor activation, bringing about a concomitant dampening of downstream mediators of this pathway. The results with CNP are less straightforward and need further evaluation.

O-17

THE ROLE OF THE TRPM4 ION CHANNEL IN CARDIAC HYPERTROPHY

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Cardiac hypertrophy is characterized by an increase in heart mass and associated changes in the shape of the left ventricle. Pathological hypertrophy is triggered by various stimuli such as hypertension and valvular dysfunction. It can result in heart failure and sudden death; therefore better understanding of the disease has significant importance. The Transient Receptor Potential (TRP) superfamily of ion channels form a large family of cation channels related to the product of the trp gene in Drosophila. TRPM4 a member of this family is a calcium-activated non-selective cation channel. It is activated by membrane depolarization and by an increase in $[Ca^{2+}]_i$ The channel is equally permeable to Na^+ and K^+ but, it is not permeable to Ca^{2+}. TRPM4 is expressed in the heart both in the atria and ventricle. Our goal was to study the level of hypertrophy in TRPM4 deficient mice. We have not found significant hypertrophy with aging in the KO mice and neither difference in TRPM4 level between Angiotensin (Ang) treated and sham operated wild type (WT) animals. However KO mice displayed increased heart weight, body weight ratio after two weeks of Ang treatment, compared to WT mice. We have found increased expression of several hypertrophy marker genes as well, like myosin heavy chain 7, atrial natriuretic peptide and alpha actin after the Ang treatment. To further support these findings we have generated a cardiac specific TRPM4 deficient mice, and we have seen similar, increased hypertrophy after Ang treatment. At basal condition, KO mice displayed a decrease in T-wave amplitude in electrocardiographic measurement. Histological analysis of the hearts showed an increased myocite size in TRPM4 deficient compared to WT mice. We propose that TRPM4 play a role in the calcium homeostasis of the cardiac cells and therefore one of the contributors in the development of cardiac hypertrophy. Our result suggests that the functional channel may prevent against the electrophysiological disturbances during cardiac hypertrophy.

P-01

INTERACTIONS OF APAMIN WITH PORE MUTATED SK3 CHANNELS

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In the present work, we have tested the impact of the replacement of valine residues in the pore region of SK3 (520) by either an alanine or a phenylalanine residue in terms of the interactions of apamin with these mutants in comparison with the corresponding native channels. Replacing valine residue at position 520 of the SK3 channel by a phenylalanine significantly increased the sensitivity of the channel to be blocked by tetraethylammonium (TEA) as previously reported. Indeed, an aromatic residue, such as a phenylalanine or a tyrosine, is frequently found in the pore region of several potassium channels more sensitive to TEA than SK channels. We measured the affinity (K_d) of apamin in saturation experiments and studied SK currents in transfected cells using patch clamp techniques. In parallel, molecular modelling techniques were used to examine the impact of these local modifications on the interaction of apamin with the corresponding channels. The presence of a phenylalanine in the pore region of potassium channels led to a higher sensitivity for TEA by creating more hydrophobic interactions as found by the docking procedure. In the *in vitro* binding experiments, the phenylalanine mutant (SK3VF) displayed a very low affinity for apamin. In patch clamp experiments, the SK current was only very partially blocked by apamin in the SK3VF mutant. Furthermore, apamin displayed an affinity and a blocking activity for the alanine mutant close to that for the corresponding native channels. In conclusion, the presence of a bulky and hydrophobic residue at a position near the pore mouth of SK3 channels has a negative impact on their interactions with apamin.

P-02

K_{IR}6.2/SUR2 EXPRESSION IN MALE ACCESSORY REPRODUCTIVE GLANDS IN RATS AND MICE

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ATP-sensitive K^+ channels (K_{ATP}) are ion channels which exhibit the property to couple cell metabolism to membrane electrical activity. K_{ATP} channels are hetero-octameric complexes composed of 4 pore-forming subunits (Kir6.x, member of the inwardly rectifying K^+ channels) and 4 regulatory subunits (SURx for sulfonylurea receptor, member of the ABC binding cassette superfamily). These channels have been identified in various tissues but they remain poorly studied in the male reproductive tract. The aim of the study was to evaluate the putative presence and localization of K_{ATP} channel subunits in the male accessory reproductive glands (prostate, coagulating gland and seminal vesicle) in rats and mice. Both Kir6.2 and SUR2 subunits have been detected on the various accessory glands by the ABC-DAB technique. Indirect immuno-fluorescence confirmed the colocalization of these subunits in the epithelium of the glands. Western-blot technique demonstrated the expression of Kir6.2 protein in total protein extracts from rodent prostate, coagulating gland and seminal vesicle. This study introduces the first evidence of the presence of K_{ATP} channel subunits in the major rodent male accessory glands. Their possible role in seminal fluid elaboration needs to be further characterized.

P-03

ANOCTAMIN 6 IS A POSITIVE MODULATOR OF VOLUME-REGULATED ANION CHANNELS

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Anoctamin 6 (Ano6) is a close homologue of Ano1 that functions as a calcium-activated chloride channel in many physiological processes such as cardiac and neuronal excitations, smooth muscle contraction, sensory transduction and transepithelial secretion. More recently, Ano6 has been identified as an essential component of outwardly rectifying chloride channels that play a role in a cell shrinkage and apoptosis. Both, Ano6 and Ano1, are activated by hypotonic cell swelling (HTS) and therefore might be a part of an epithelial volume-regulated anion channel (VRAC). Using quantitative real-time PCR experiments, we showed that Ano6 expression levels correlate with electrophysiological recordings of large VRAC currents in smooth muscle-like BC3H1 and colon carcinoma Caco-2 cells. Overexpression of Ano6 in HEK293 cells results in a significant increase of HTS-evoked anion currents when compared to not transfected HEK293 cells. In contrast to Ano1, Ano6 is not activated by an increase of intracellular Ca^{2+} levels. Moreover, co-expression of ANO6 with caveolin-1, a major component of caveolae plasma membranes and a positive regulator of VRAC function, significantly increases HTS-evoked currents in Ano6-overexpressing HEK293 cells. Similarly, transient expressions of Ano6 in Caco-2 cells generate a significant increase of HTS-evoked currents when compared to not transfected Caco-2 cells. These HTS-evoked currents display known characteristics of VRAC such as a rapid deactivation at high positive potentials and a block by tamoxifen and mibefradil. Taken together, our results suggest that Ano6 is a putative positive modulator of VRAC function in epithelial cells.

P-04

CHARACTERIZATION OF THE TRAFFICKING OF HUMAN TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (HTRPM8) CHANNEL BY TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) MICROSCOPY

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Transient Receptor Potential (TRP) channels form a superfamily of channels that can be considered as multiple signal integrators. TRP channels play vital roles in perception of all major classes of external stimuli, like thermosensation, chemosensation and mechanosensation. These channels also help individual cells with the ability to sense changes in the local environment, such as alterations in osmolarity. There is very little knowledge of the kinetics and molecular mechanism regarding the intracellular trafficking of TRP channels. Till date, only a handful of proteins are known to interact with TRP channels and to influence their trafficking. However, it is becoming increasingly clear that the dynamic modulation of the number of active TRP channels in the plasma membrane and intracellular organelles represents an important mechanism to regulate channel activity. A better knowledge of the fundamentals of TRP channel trafficking are therefore essential to our understanding of the role of these channels in various physiological/pathophysiological processes. We focused our study on TRPM8 - a cation channel activated by cold and the cooling compounds menthol and icilin. With the help of TIRF Microscopy- a state of art high resolution microscopic system, which allows the detection of individual fluorophores within 100 nm of the cell surface, we present for the first time a detailed depiction of TRPM8-vesicles in the near-membrane field. TRPM8 is present in intracellular structures with diverse morphological characteristics. We observed that a large fraction of TRPM8 resides in highly mobile late endosomal and lysosomal vesicles whereas less than 1% of TRPM8 resides in clathrin- and caveolin- coated vesicles or early endosomal vesicles. We found that TRPM8 vesicle movement is dependent on microtubular tracks while actin filaments could be involved in near membrane movements. Calcium entry as a result of TRPM8 activation alters the dynamics of TRPM8 protein movement, which is perceived as slowing down of the vesicles at the near-membrane TIRF field. Our results fetched significant insights regarding the movement pattern and habitation of TRPM8 within the intracellular compartment and further study need to be persisted to unveil its physiological importance.

P-05

ADENOSINE-5`-TRIPHOSPHATE (ATP) AND PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP_2) ARE POSITIVE REGULATORS OF TRANSIENT RECEPTOR POTENTIAL MELASTATIN 3 (TRPM3) CHANNEL ACTIVITY

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The TRPM3, a member of the melastatin subfamily of transient receptor potential (TRP) ion channels, is a Ca^{2+} permeable non-selective cation channel activated by the neurosteroid pregnenolone sulphate (PS) and by heat. Recent studies reported the expression and functional role of TRPM3 in various tissues including pancreatic β-cells and sensory neurons, but the molecular regulation of the channel remains poorly understood. Intracellular ATP and PIP_2 were shown to modulate several members of TRP family, but we lack any data regarding TRPM3. Therefore, we aimed to investigate the influence of intracellular factors on the activity of TRPM3. We carried out voltage clamp measurements using the cell-attached and inside-out configuration of the patch clamp technique on HEK293T cells overexpressing mouse TRPM3. The channels of the clamped membrane patches were stimulated by 100 μM PS applied to the extracellular side of the membrane via the pipette solution. In cell attached configuration, we measured moderate channel activity, which was dramatically increased in inside-out configuration just after the excision of the membrane patch. Current potentiation after excision was followed by rapid current decay. Application of 2 mM ATP to the cytosolic side of the inside-out membrane patch restored the TRPM3 activity. Kinetic analysis of the effect of cytosolic ATP indicated a dual effect on TRPM3: direct channel inhibition, which may be due to direct binding to the channel, and slow restoration of channel activity, which may represent the action of an ATP-dependent enzyme. Our finding that PIP_2 also caused a partial recovery of TRPM3 current in inside-out patches, suggests that ATP may act, at least partly, by fuelling the restoration of PIP2 levels in the plasma membrane. In our running experiments we are further exploring the molecular basis of the ATP-dependent TRPM3 regulation.

P-06

NEW BENZOPYRAN DERIVATIVES ACTING AS CALCIUM ANTAGONISTS IN VASCULAR SMOOTH MUSCLE

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By exploring a series of 4,6-disubstituted *R*/*S*-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyrans structurally related to (±)-cromakalim, we have recently identified compounds with a relative potency and selectivity for the vascular smooth muscle. These compounds were bearing an alkylsulfonamide moiety at the 6-position as well as, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a *meta* or a *para*-electron-withdrawing group such as Cl or CN. Recent pharmacomodulation generated original compounds characterized by a higher water solubility compared to the previously synthesized compounds. Preliminary biological data indicated that the new molecules barely affected the insulin secretory rate from incubated rat pancreatic islets but exhibited a potent myorelaxant effect on the rat aorta contractile activity. Radioisotopic experiments as well as measurements of contractile activity under different experimental conditions (i.e.: in the absence or presence of glibenclamide; in the presence of different extracellular K+ concentrations) revealed that these new compounds did not act as potassium channel openers. Experiments conducted on rat aorta rings suggested that the drugs behaved as Ca2+ channel blockers. The present results indicated that pharmacomodulation around the benzopyran nucleus can affect the tissue selectivity but also the mechanism of action of the cromakalim analogues.

P-07

INFLUENCE OF ARTERIAL HYPERTENSION ON L-TYPE CA^{2+} CHANNEL-MEDIATED CONTRACTIONS OF CONDUIT ARTERIES

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L-type Ca^{2+} channels of vascular smooth muscle cells (VSMCs) contribute to the mechanical properties of arteries and exhibit a therapeutic target during the pharmacological treatment of arterial hypertension. In this study, we analysed L-type Ca^{2+} channel-mediated contractions of arterial segments in normotensive and hypertensive mice. C57Bl6 mice were treated with saline (control group) or with angiotensin II (Ang II, hypertensive group) via subcutaneous Alzet osmotic minipumps for 4 weeks (1000 ng/kg/day). Vaso-reactive studies were performed on segments of aorta and femoral artery. L-type Ca^{2+} channel-mediated contractions were measured by stepwise depolarisations induced by stepwise increments of extracellular K^+ concentration (K^+ dose-response curves). Treatment with Ang II induced arterial hypertension (mean arterial blood pressure 164±7 mmHg versus 116±3 mmHg, n=7, p<0.001) and left ventricular hypertrophy (heart/body weight 0.0055±0.00036 versus 0.0040±0.0018, n=7, p<0.05). K^+ dose-response curves for L-type Ca^{2+} channel-dependent contractions of aortic segments were shifted to the left in hypertensive mice (EC_{50} decreased from 31.9±0.3 to 25.8±0.8 mM K^+, n=6, P<0.001). This shift was preserved after initially repolarizing VSMCs with 1 µM levcromakalim, ruling out differences in resting membrane potential between normotensive and hypertensive segments as the only cause of sensitization. Furthermore, when vascular tone was set by clamping the membrane potential of VSMCs to depolarized potentials, diltiazem reduced the contractions with higher affinity in hypertensive than in normotensive segments (EC_{50} -6.39±0.06 versus -6.05±0.06, p<0.01). These results suggested that the K^+-sensitization with hypertension is at least caused by altered voltage-dependence of the L-type Ca^{2+} channels. Similar experiments performed in segments of the femoral artery revealed no differences between normotensive and hypertensive animals. Results indicate that Ang II-induced arterial hypertension alters the voltage-dependence of the L-type Ca^{2+} channels in VSMCs of large arteries in an anatomically heterogeneous manner. The observations may have relevance for the pharmacological benefit of L-type Ca^{2+} channel blockers, and for the development of arterial stiffening during chronic arterial hypertension.

P-08

SCREENING FOR PHARMACOLOGICAL TOOLS TO TARGET Ca^{2+} ACTIVATED NON-SELECTIVE CATION CHANNELS.

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TRP proteins form cation channels that are regulated through strikingly diverse mechanisms. Recently TRPM4 and TRPM5 were identified as monovalent cation selective ion channels, which are activated by an increase of the intracellular Ca^{2+} concentration. Physiologically, TRPM5 is a key player in the transduction of taste signals from taste buds in the tongue towards the central nervous system. Furthermore the channel plays a role in the release of insulin from pancreatic ß cells. TRPM4 on the other hand is important for tuning the activation state of mast cells, and might be a novel drug target for allergic diseases. Moreover, data is accumulating that TRPM4 also plays an important role in the regulation of cardiac contraction strength and the development of arrhythmic diseases. To date no pharmacological tools are available to evaluate there potential as drug targets. In this study we present a novel screening method for identifying compounds, which target these channels. We used a fluorescence based high throughput device to visualize intracellular [Na^{+}] dynamics to screen an extensive library of compounds (> 10.000). Furthermore we have characterized a novel activator of TRPM5. This compound is selective for the TRPM5 channel, and has a direct effect on the channel. We present data that this compound influences specifically the Ca^{2+} sensitivity of the channel. Potentially it could play an important role in the development of new taste modulators, and novel treatments for type 2 diabetes.

P-09

TRPM4 IS A NOVEL REGULATOR OF CONTRACTION STRENGTH IN THE HEART MUSCLE

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We have investigated Ca^{2+} signaling and contractility properties of the cardiac muscle in wild-type and *Trpm4*$^{-/-}$ mice. TRPM4 is a member of the Transient receptor potential super-family of ion channels, and constitutes a Ca^{2+} activated, but Ca^{2+} impermeable, non-selective cation channel. TRPM4 is expressed both in mouse and human cardiomyocytes. Strikingly, paced ventricular myocytes under β-adrenergic stimulation show a significantly greater Ca^{2+} transient in in *Trpm4*$^{-/-}$ compared to WT mice. Measurements of action potential duration in papillary muscle myocytes show that TRPM4 deficient cells display an accelerated repolarisation, which is associated with an increased peak-amplitude of the nifedipine-sensitive L-type Ca^{2+} current in isolated ventricular cardiomyocytes. These findings can be correlated with a stronger increase of contractile force under ß-adrenergic stimulation in isolated papillary muscle, compared to WT. Finally, using in vivo left ventricular pressure volume-measurements in wild-type and TRPM4 deficient mice, we could show that under basal conditions no difference exists between these mouse strains in contractility-dependent properties of the heart, such as cardiac output and stroke work. However, when mice are infused with increasing doses of a ß-adrenergic agonist, *Trpm4*$^{-/-}$ mice display significantly stronger increased cardiac output, stroke work and pre-load recruitable stroke work. Thus, we propose that the increased Ca^{2+} current in TRPM4 deficient cardiomyocytes during the action potential leads to increased Ca^{2+} induced Ca^{2+} release and consequently a stronger increase of contractility in TRPM4 deficient mice under beta-adrenergic stimulation. Interestingly, in a cohort of human aortic stenosis patients we could show that TRPM4 expression correlation with contraction strength of the heart. TRPM4 might therefore be a novel target in the treatment of heart failure.

P-10

GLYCINE RECEPTOR INFLUENCES INTERNEURON CELL MIGRATION IN THE DEVELOPING BRAIN

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The strychnine-sensitive glycine receptor (GlyR) is a member of the ligand-gated ion channel superfamily. In the adult, the GlyR is known to mediate fast inhibitory neurotransmission in the spinal cord and in the brainstem, but the GlyR has also been described in the embryonic cortex at embryonic day 19 where it could participate in developmental processes. Since other neurotransmitter systems, *i.e.* GABA and its receptors, are known to have a developmental role during early corticogenesis, here it was tested whether this could also be the case for glycine and its GlyR. This study shows the analysis of the presence and function of GlyR in the early development of the cortex using slice culture, patch-clamp, two photon imaging and immunocytochemistry. By using whole cell recordings it was found, for the first time, the presence of GlyR mediated currents in migrating interneurons during early stages of development (E13-E15). The concentration-response curve showed an EC50 of 69 ± 12 micro M for glycine and these elicited currents were fully blocked by strychnine. Picrotoxinin and picrotin also blocked the current, but with different potency, remaining 20 % of the current when 10 micro M of picrotin was used. Consequently, immunostainings directed against the alpha 2 subunit of GlyR showed that 29 ± 2 % of migrating cells express GlyR. In search for the possible physiological impact of GlyR, time lapse experiments were performed and they showed that GlyR can actively modulate interneuron cell migration by affecting nuclear translocation and/or branching dynamics. These findings shows that the glycinergic system appears very early during development and it might have an important role during brain development.

P-11

MIGRATION OF MICROGLIA IN THE EMBRYONIC NEOCORTEX

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Microglia, the macrophages of the central nervous system (CNS), are assumed to support the architecture and functional maturation of the developing CNS, through secretion of various factors. During quail CNS development, retinal microglia migrate along retinal radial glia. In addition, they contact radial cells during development of the mouse spinal cord and neocortex. *In vitro*, microglia express receptors for extracellular matrix proteins present in the developing brain. Therefore, this study aims to elucidate the migratory behavior of microglia together with the molecular and cellular basis for their mobilization. Immunohistochemistry was performed on CX3CR1 $^{+/eGFP}$ mice embryos (Embryonic day (E) 12.5-15.5). Cortical microglial location and protrusion morphology were analyzed using a home-made MATLAB® plug-in. Microglial migration was followed in time lapse imaging experiments of CX3CR1 $^{+/eGFP}$ acute brain slices using 2-photon excitation. Our results show that between E12.5 and 15.5, microglia colonize the neocortex from hot spots near the ventricular lining and pia. During this period, the microglial protrusion length gradually increased and these protrusions were equally oriented in radial and tangential directions. On E14.5 and E15.5, these highly dynamic cells migrated saltatory at similar mean velocities (34.8 and 32.6 µm/h). Cellular migration mechanisms closely resembled features of tangentially and radially migrating microglia in the quail retina. Laminin, fibronectin, and their corresponding receptors were present on radial cells. In addition, laminin and fibronectin receptors were expressed by microglial protrusions. In conclusion, these results imply that microglia are able to make contact with radial cells when migrating to their final destinations in the neocortex, through expression of laminin and fibronectin receptors. Their dynamic saltatory behavior suggests a more complex migration than simply following the entire course of radial cells, which we hypothesize to consist of successive jumps between pillars of radial fibers.

P-12

EXPERIMENTAL EARLY-LIFE FEBRILE SEIZURES INDUCE CHANGES IN $GABA_AR$-MEDIATED NEUROTRANSMISSION IN THE DENTATE GYRUS

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Febrile seizures (FS) are the most frequent type of seizures during childhood. Retrospective studies have linked early-life FS to hippocampal sclerosis-associated temporal lobe epilepsy (TLE) later in life. However, the mechanisms by which FS may lead to TLE are still largely unknown. Altered inhibitory neurotransmission in the dentate gyrus (DG) circuit, which is known as a critical checkpoint regulating excitability in the hippocampus, has been hypothesized to be involved in epileptogenesis. The aim of the present study is to analyze whether experimental FS change inhibitory synaptic input and postsynaptic $GABA_AR$ function in dentate granule cells. To this end we made use of an established model, where FS are elicited in 10-day old rat pups by hyperthermia (HT; core temperature ~41-42.5°C). Normothermia (NT; cor e temperature ~35°C) littermates served as controls. $GABA_AR$-mediated inhibitory neurotransmission was studied using the whole-cell patch-clamp technique applied on dentate granule neurons in hippocampal slices within 6-9 days after HT-treatment. Our data show that the frequencies of spontaneous inhibitory postsynaptic currents (sIPSCs) were reduced in HT rats that had experienced seizures whereas amplitudes of sIPSCs were enhanced. Whole-cell GABA responses revealed an increased $GABA_AR$ sensitivity in dentate granule cells from HT-treated animals, compared to that of NT controls (EC_{50}: ~32 µM and ~65 µM; respectively). Analysis of spontaneous inhibitory events and whole-cell GABA responses showed similar kinetics in postsynaptic $GABA_ARs$ of HT and NT rats. qPCR experiments indicated that these functional alterations were accompanied by changes in DG $GABA_AR$ subunit expression, which was most pronounced for the α3 subunit. These data support the hypothesis that FS persistently alter the neuronal excitability.

P-13

NEUROPROTECTIVE EFFECTS OF THE INSULIN-LIKE GROWTH FACTOR IGF-I AND 17β-ESTRADIOL AFTER TRANSIENT FOCAL CEREBRAL ISCHEMIA IN THE ENDOTHELIN-1 RAT MODEL.

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Stroke is the first cause of morbidity in the European Union and worldwide it is the leading cause of adult disability. To date, thrombolytic therapy using recombinant tissue plasminogen activator (tPA) is the only approved therapy for acute ischemic stroke. However, only 5-10% of all patients benefit from this treatment because of the inclusion criteria. Targeting different processes involved in the ischemic cascade, which include excitotoxicity, oxidative stress, inflammation and cell death, could be promising for the future of stroke treatment. IGF-I is an anti-apoptotic pleiotropic factor exerting effects on different levels of the ischemic cascade. It has been shown in both in vitro and vivo models that the neuroprotective effects of the IGF-IR and ER or interdependent. This interdependency creates the possibility to increase the efficacy of IGF-I by co-treatment with estradiol. Possible synergistic effects of IGF-I and 17β-estradiol (E2) will be studied in the endothelin (Et)-1 rat model for stroke. Effects on both infarct size and motor/sensory deficits will be investigated. Via a guide cannula, implanted using stereotactic surgery, 200 pmol Et-1 was administered in the vicinity of the middle cerebral artery of male albino Wistar Kyoto (WKY) rats. Rats were treated with estradiol (1mg/kg, 0.2 ml s.c.) and/or IGF-I (3mg/kg, 0.2 ml, s.c.) 30 min after the induction of stroke. Motor/sensory functions were determined 24 h after the insult using the neurological deficit score (NDS). Infarct size was assessed using a cresylviolet staining. Treatment with a combination of E2 and IGF-I 30 min after the induction of stroke resulted in a significant reduction in infarct size (22.19 ± 4.35 mm^3) compared to the vehicle treated animals (65.36 ± 5.49 mm^3). Rats treated with E2 (48.89 ± 12.28 mm^3) or IGF-I (40.98 ± 4.21 mm^3) alone showed smaller infarct sizes, however not significant. The stroke significantly reduced the NDS in vehicle-treated animals but not in rats treated with E2 and/or IGF-I. A combination of both estradiol and IGF-I reduces infarct size after a transient focal cerebral ischemia in the Et-1 rat model. It seems that E2 and IGF-I exert additive or synergistic neuroprotective effects. Further experiments to increase the number of rats have to be performed to make further conclusions.

P-14

SYSTEM X_C^- IS AN IMPORTANT SOURCE OF EXTRACELLULAR GLUTAMATE IN MOUSE HIPPOCAMPUS

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The cystine/glutamate antiporter or system x_c^- is a membrane-bound Na^+-independent amino acid transporter that is structurally composed of a heavy chain subunit common to all amino acid transporters, 4F2, and a light chain specific subunit, xCT. We here provide the first evidence that system x_c^- is the major source of extracellular hippocampal glutamate in mice. Semi-quantitative western blotting was performed on hippocampal tissue of young and old $xCT^{+/+}$ and $xCT^{-/-}$ mice using guinea pig antibody to VGLUT1 (1:2000; Millipore); mouse antibody to VGLUT2 (1:5000; Millipore); rabbit antibody to VGLUT3 (1/1000; Synaptic Systems); rabbit antibody to GLT-1 (1:30000); rabbit antibody to GLAST (1:4000); rabbit antibody to EAAC1 (1:1000; Alpha Diagnostic); rabbit antibody to xCT (1:10000); mouse antibody to synaptophysine (1:5000; Stressgen); rabbit antibody to GAPDH (1:5000; Santa Cruz); mouse antibody to GAPDH (1:15000; Millipore). *In vivo* microdialysis was performed in mouse hippocampus by implanting a microdialysis guide (CMA/7) 2 mm above final probe membrane location with the following coordinates relative to bregma: L +3.0, AP –2.7 and DV –1.5. Samples were collected at flow rate of 2 µl/min every 20 min. Glutamate and aspartate content were determined using HPLC. We observed significantly lower glutamate concentrations in baseline dialysis samples obtained from young as well as old $xCT^{-/-}$ mice (young: 0.079 ± 0.022 µM; old: 0.084 ± 0.015 µM) compared to their age-matched $xCT^{+/+}$ littermates (young: 0.208 ± 0.036 µM; old: 0.191 ± 0.034 µM). Our findings demonstrate that protein expression levels of the major glial glutamate transporters GLT-1 and GLAST, the neuronal EAAC1 transporter and the vesicular glutamate transporters (VGLUT1-3) are unaffected by genotype and that no compensatory up- or down-regulations are observed due to the loss of xCT protein. These novel findings sustain that system x_c^- is an important source of extracellular glutamate in mouse hippocampus.

P-15

CAVEOLIN-1 PARTICIPATES TO THE REGULATION OF GAP-JUNCTIONS IN ENDOTHELIAL CELLS

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Previous works have demonstrated that caveolin-1 (cav-1), the caveolar structural protein, is implicated in the endothelium derived hyperpolarizing factor (EDHF)-dependent relaxation, a major relaxing pathway. Indeed, caveolin-1 has been shown to colocalize and interact with connexins 37, 40 and 43 (Cx37, Cx40, Cx43) which constitute gap-junctions. These structures ensure the intercellular spread of the membrane hyperpolarization associated with EDHF relaxation. In the absence of cav-1, alterations in connexins expression and gap-junctions have been observed and result in a decrease in the myo-endothelial coupling. To clarify the regulatory role of cav-1 in the EDHF relaxation, we have investigated its involvement in gap-junctions turn-over, in physiological and pathological conditions. Using a bovine brain microvascular endothelial cell line, we have studied the evolution of Cx43 colocalization (immunocytochemistry) and interaction (proximity ligation assay) with cav-1 depending on cell confluence or hypoxic and inflammatory treatments. Total protein expression was evaluated by Western-blotting. First, we have highlighted changes in Cx43 subcellular localization depending on cell confluence. Indeed, Cx43 staining is localized in the Golgi of sparse cells but is predominant in membranes when cells reach confluence. Cx43 colocalization and interaction with cav-1 are enhanced in the cytosol when confluence increases. In connecting cells, when gap-junctions are functional, interactions decrease. Then, confluent cells have been submitted to hypoxic treatments (1% O_2). In these conditions, Cx43 total expression is drastically enhanced ($p<0.001$) and this is confirmed by an increase in Cx43 membrane staining. Moreover, these treatments induce a higher interaction level between Cx43 and cav-1 than in control. Consequently, cav-1 should be involved in the input of Cx43 to membranes during hypoxic treatments. On another hand, incubation with TNFalpha (10 ng/ml, 4h) significantly decreases Cx43 total expression ($p<0.001$) and is accompanied by a disappearance of Cx43 membrane and cytoplasmic staining. A 24h recovery period is associated with the reestablishment of Cx43 total expression at a level significantly higher than control ($p<0.001$) and, in a lesser extend, of Cx43 membrane staining. Cav-1 total expression is also decreased by these treatments (NS and $p<0.001$ respectively). Furthermore, no significant change in the interaction level is induced by TNFalpha treatment. These results suggest that Cx43 is probably internalized and degraded independently of cav-1, after TNFalpha exposure. Our study demonstrates that Cx43 expression and gap-junction formation are dynamically regulated by hypoxic and TNFalpha exposure. Cav-1 is involved in Cx43 trafficking to the membranes but not in internalization or degradation of the connexins.

P-16

CONNEXIN MIMETIC PEPTIDES INHIBIT Cx43 HEMICHANNEL OPENING TRIGGERED BY VOLTAGE AND INTRACELLULAR Ca^{2+} ELEVATION

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Connexin mimetic peptides (CxMPs) like Gap26 and Gap27 are known as inhibitors of gap junction channels but evidence is accruing that these peptides also inhibit unapposed/non-junctional hemichannels residing in the plasma membrane. We used voltage clamp studies to investigate the effect of Gap26/27 at the single channel level in HeLa cells stably transfected with Cx43 and in isolated pig ventricular cardiomyocytes exhibiting strong endogenous expression of Cx43. Such approach allows unequivocal identification of hemichannel currents by their single channel conductance that is typically ~220 pS for Cx43. Gap26/27 peptides inhibited Cx43 hemichannel unitary currents over minutes and shifted the voltage dependence of hemichannel opening to more positive values. By contrast, an elevation of intracellular calcium ($[Ca^{2+}]_i$) to 200-500 nM increased unitary currents and shifted the voltage dependence of hemichannel opening to the left. Gap26/27 inhibited Ca^{2+}-mediated potentiation of hemichannels and prevented the left-shift of voltage dependent hemichannel activation induced by $[Ca^{2+}]_i$ elevation. Our results indicate that under pathological condition, when $[Ca^{2+}]_i$ is elevated, Cx43 hemichannel opening is promoted in cardiomyocytes and CxMPs are able to counteract this effect.

P-17

TRANSFER OF INOSITOL TRISPHOSPHATE THROUGH GAP JUNCTIONS IS CRITICAL, BUT NOT SUFFICIENT, FOR THE SPREAD OF APOPTOSIS

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Decades of research have provided evidence that apoptosis can not be regarded anymore as a 'single cell' process but rather as a 'contagious' phenomenon involving intercellular communication. Gap junctions (GJs; channels which connect the cytoplasm of neighboring cells and are composed of transmembrane connexin (Cx) proteins) have been shown to play a pivotal role by allowing the spread of signals from dying cells to protect or harm the health of their neighbors. Up to now however, the identity of those signals still remains elusive. The Ca^{2+} mobilizing molecule inositol 1,4,5-trisphosphate (IP_3) has been proposed as the responsible molecule conveying the apoptotic message, though conclusive results are still missing. We here aimed to explore the evidence for IP_3 in a model of GJ-mediated spread of apoptosis. Knowledge on the identity of the key messenger involved will improve our insights into how dying cells influence surrounding healthy cells and may pave the way to therapeutically target cell death communication. To address this issue we used a previously optimized *in vitro* model in which apoptosis was induced in a well-defined 'trigger zone' by loading C6 glioma cells heterologously expressing Cx43 or Cx26 with cytochrome C (CytC) making use of *in situ* electroporation. The model allowed a clear distinction between the cell death trigger zone and an adjacent 'communication zone' where apoptosis subsequently developed. We applied targeted loading of high molecular weight agents interfering with the IP_3 signaling cascade in the apoptosis trigger zone and cell death communication zone. Blocking IP_3 receptors or stimulating IP_3 degradation both diminished the propagation of apoptosis. To determine whether IP_3 transfer via GJs is necessary, we transduced C6 cells with cDNA for the mutant Cx26V84L, which forms GJs with a strongly impaired IP_3 permeability. These experiments demonstrated significantly reduced cell death communication in Cx26V84L cells compared to wild-type Cx26 cells. However, IP_3 by itself was not able to induce cell death but only potentiated cell death propagation when the apoptosis trigger was applied. We conclude that the generation of IP_3 in apoptotic cells, its transfer via GJs and its presence in target cells are all necessary events for cell death communication to occur. However, IP_3 is not sufficient by itself and needs to team-up with other yet unidentified signaling partners to become a cell death-provoking messenger.

P-18

NOVEL URINARY BIOMARKERS FOR PROXIMAL TUBULAR DAMAGE: IS AGE AN ISSUE?

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Routinely used biomarkers for kidney disease, such as serum urea nitrogen and creatinine, lack sensitivity and/or specificity. Therefore a majority of recent studies has focused on the discovery and validation of novel renal biomarkers for a wide range of renal pathologies and renal damage after toxic insults and ischemia. Amongst others, KIM-1, NAG and Cystatin C are promising novel biomarkers for early proximal tubule damage in the kidney. To further enhance the clinical usability of these biomarkers, we investigated the effect of age in a healthy population. Urine samples were collected from 272 non-smoking healthy volunteers (age ranging from 0.5 years to 95 years; median 47 years). Written consent was obtained from all participants. Urinary KIM-1 and Cystatin C concentrations were determined by means of an sandwich ELISA method. Urinary NAG is measured colorimetrically and creatinine levels through the kinetic Jaffe method. Linear Regression analyses were conducted to study the possible association between age and the renal biomarkers, both before and after correction for urinary creatinine levels. Regression analyses show that -both before and after the normalization to urinary creatinine levels- age has a significant effect on urinary KIM-1 and on NAG ($p < 0.0001$) but not on urinary Cystatin C ($p = 0.34$). In conclusion we can state that KIM-1 and NAG are age-related in our healthy population and further research is needed in order to establish age-dependant reference values for KIM-1.

P-19

CALBINDIN AND CALRETININ IN MOUSE AND RAT SPERM

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Calbindin (CB) and calretinin (CR) are two calcium binding proteins involved in calcium buffering in many cell types such as neurons or insulin-secreting cells. Calcium influx is a major step in the cascade of events regulating important sperm physiological processes like acrosomial reaction and motility. The aim of the study was to evaluate the presence and possible role of these calcium buffering proteins in rodent spermatozoa. Indirect immunofluorescence demonstrated the presence of calbindin and calretinin on mouse and rat spermatozoa, as well as on cerebellar control sections. Anti-calbindin and anti-calretinin antibodies both labelled the acrosomal region of the spermatozoa. This localisation was confirmed by double labelling of the acrosome with PNA-FITC. Anti-calretinin, however, also labelled the acrosome of CR-/- (calretinin) knock-out mouse. Western blot analysis detected calretinin and calbindin proteins in total protein extracts from mouse and rat spermatozoa. Calbindin and calretinin were also detected in total protein extract from control cerebellum. In conclusion, this study documents, for the first time, the presence of calbindin and calretinin in the acrosome of mouse and rat spermatozoa. Their physiological role(s) need(s) to be further determined by quantification of the spontaneous acrosome reaction and by assessment of motility parameters on CB/CR knock-out vs wild-type mice.

P-20

GENE REGULATION OF *TRPM5* IN ANIMAL MODELS OF TYPE 2 DIABETES

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Transient receptor potential melastatin 5 (TRPM5) ion channel is a Ca^{2+}-activated non-selective monovalent cation channel that is expressed in pancreatic islets of Langerhans. Previous studies from our laboratory revealed that TRPM5 is a key player in the glucose-induced electrical activity of the beta cell and positively influences glucose-induced insulin release and glucose homeostasis. Since mutations or altered activity of ion channels are proposed to predispose patients to type 2 diabetes, we investigated a possible link between TRPM5 and type 2 diabetes. Therefore, we examined several animal models of type 2 diabetes. Quantitative PCR experiments revealed an altered expression level of *Trpm5* mRNA in the pancreatic islets of these animal models. This altered expression of *Trpm5* could also be detected at the functional level, as the glucose-induced Ca^{2+}-signaling of these islets correlated well with the established role of TRPM5 during glucose-induced Ca^{2+}-oscillations. Confirmative results were obtained in the insulinoma beta cell line MIN6 and in wild type islets that were incubated with several factors that are changed in type 2 diabetes. These data provide a link between TRPM5 and type 2 diabetes and suggest that the onset of type 2 diabetes causes an altered expression of *Trpm5* in pancreatic islets.

P-21

TRPM4, A NEW PLAYER IN SYNAPTIC PLASTICITY MODULATION IN HIPPOCAMPUS

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Synaptic plasticity is the phenomenon modifying synapse strength according to experience. Long term changes in synaptic plasticity like long term potentiation (LTP) or long term depression (LTD) are considered as neuronal substrates for learning and memory. There is evidence that TRP channels are expressed in the central nervous system. However, little is known about the role of TRP in basic and higher brains functions such as neurotransmitter release, synaptic plasticity and memory formation. So far, the best investigated TRP in central nervous system is TRPV1, which has been shown to be involved in both LTP and LTD. Here, we investigated a possible role for TRPM4 in the hippocampal synaptic plasticity. First we determined the expression pattern of TRPM4 in hippocampus. Western blot and *ISH* showed a restrained expression in granulate cells from dentate gyrus and pyramidal neurons from CA1 but not CA3. To investigate the involvement of TRPM4 in the hippocampal physiology, we studied the synaptic plasticity from Schaffer collaterals (SC) to CA1 in the TRPM4 KO mice. Basal synaptic communication and short term plasticity were not different from the WT and the general morphological organization of the hippocampus was similar to a WT structure. Low frequency stimulation protocols induced LTD in both genotypes; however, 1 and 3 TBS failed to potentiate synaptic communication from SC to CA1 neurons. Even a stronger protocol like High Frequency Stimulation failed to induce in the TRPM4 KO a potentiation to a level similar to WT. the TRPM4 KO rats present the same LTP phenotype. All together, the data suggest a important role for TRPM4 in synaptic plasticity. It seems that in TRPM4 KO the potentiation threshold is shifted to a higher limit and point a possible role for TRPM4 in modulation of the NMDA receptors activation.

P-22

INDUCING VISCERAL HYPERSENSITIVITY IN RATS: STUDY OF THE EFFECTS OF INFLAMMATION, STRESS AND THEIR COMBINATION

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The pathophysiological mechanisms underlying visceral hypersensitivity, a hallmark feature of irritable bowel syndrome, remain unclear. However, inflammation and stress appear to be involved. Therefore we assessed the short- and long-term effects of inflammation and stress on visceral sensitivity in rats. Colitis was induced by intrarectal instillation of trinitrobenzene sulphonic acid (TNBS) in ethanol. Stress-exposure consisted of one (acute) or multiple (chronic) water avoidance stress sessions. Visceral sensitivity was assessed by registering visceromotor responses (VMR) to colorectal distension. Four series of experiments were conducted to assess 1/ the short-term effects of TNBS-colitis, acute stress and their combination in Wistar rats, 2/ the short-term effect of chronic stress in Wistar rats, 3/ the long-term effects of TNBS plus concomitant acute or chronic stress in Wistar rats, assessed after complete resolution of colitis and 4/ the long-term effect of TNBS-colitis after complete resolution of inflammation in Sprague-Dawley rats. 1/ In Wistar rats, acute colitis, acute stress and their combination all induced visceral hypersensitivity (n=7-8). Chronic stress-exposure induced a numeric but non-significant increase in visceral sensitivity (n=8). After acute TNBS-colitis combined with concomitant single or multiple stress sessions, post-inflammatory VMR were not significantly different from controls (n=6-8). In Sprague-Dawley rats, TNBS-treatment resulted in significant visceral hypersensitivity 3 days after endoscopic resolution of inflammation (n=8-10). It is concluded that in the acute phase, TNBS-colitis, acute stress-exposure and their combination resulted in visceral hypersensitivity, whereas in our long-term set-up, only TNBS-treated Sprague-Dawley rats exhibited marked post-inflammatory visceral hypersensitivity.

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LOCAL OVEREXPRESSION OF INTERLEUKIN -11 IN THE CENTRAL NERVOUS SYSTEM PREVENTS DEMYELINATION AND ENHANCES REMYELINATION

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Demyelination is one of the clinical hallmarks of multiple sclerosis (MS). Till today no therapy is available which potentiates the endogenous remyelination. Interleukin-11 (IL-11) is a member of the gp130 family cytokines, that also includes leukemia inhibitory factor and oncostatin M. These neuropoietic cytokines are upregulated in MS lesions and several have been shown to control neuroinflammation. Recently, IL-11 was identified to directly provide trophic support to oligodendrocytes by potentiating their survival and maturation *in vitro.* Moreover, IL-11 was shown to improve clinical severity in the EAE model. In this study we aim to elucidate the direct in vivo effect of IL-11 demyelination independent of the immune response. To achieve immune independent demyelination, a cuprizone induced demyelination mouse model was used. 0.2% of cuprizone was mixed with powdered chow and fed to 8 week old mice for 5 weeks to achieve acute demyelination. To allow spontaneous remyelination, mice were returned to normal diet after 5 weeks. CNS directed overexpression of IL-11 was achieved by stereotactical administration of IL-11 encoding lentiviral vectors in the striatum of mouse brain. For the prophylactic study, vectors were administered 2 weeks prior to the start of the cuprizone diet and acute demyelination was studied. For the therapeutic study, the vectors were administered 4 weeks after the start of the cuprizone diet. Mice were allowed to remyelinate for 2 weeks and subsequently sacrificed for immunohistochemical analyses (Luxol Fast Blue, Iba-1, MAC2, NG-2). To study the in vitro effect of IL-11 on myelin phagocytosis, RAW264.7 cells were cultured and treated with 1, 3, 10, 30 and 100 ng/ml of murine rIL-11 for a period of 6 and 12 hrs. The uptake of DI labeled myelin was analysed by flowcytometry. Prophylactic overexpression of IL-11 in mouse brain striatum significantly limited the area of acute demyelination (decrease of 27% as compared to controls). Moreover, the number of $NG2^+$ oligodendrocyte progenitor cells (OPC) and $Iba\text{-}1^+$ & $MAC2^+$ double positive microglia were found to be significantly reduced compared to controls. Remyelination also was found to be significantly enhanced (increase of 19% as compared to controls) when IL-11 was overexpressed therapeutically. *In vitro*, IL-11 dose dependently increased myelin phagocytosis (1, 3, 10, 30 ng/ml) by RAW 264.7 cells. Currently, we are confirming the remyelination effect of IL-11 by electron microscopy and also trying to elucidate the mechanisms by which IL-11 is probably showing these effects. Prophylactic delivery of IL-11 reduces acute demyelination and also it promotes remyelination therapeutically, in the cuprizone model. IL-11 potentiates myelin phagocytosis *in vitro,* which could indicate enhanced clearing of myelin debris *in vivo* thereby creating an environment conducive for repair processes in the demyelinated area. Moreover, the reduced number of OPC may indicate that differentiation into mature myelinating oligodendrocytes has occurred, which then play their putative role in remyelination. Future studies will have to confirm these findings.

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NOVEL GATING MECHANISM OF TRPM3 CHANNEL OPENING

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Transient receptor potential melastatin-3 (TRPM3) is a broadly expressed Ca^{2+}-permeable non-selective cation channel. Previous work has demonstrated robust activation of TRPM3 by the neuroactive steroid pregnenolone sulphate (PS) and nifedipine. Recently, we provide evidence that TRPM3 functions as a chemo- and thermosensor in the somatosensory system. TRPM3 is molecularly and functionally expressed in a large subset of small-diameter sensory neurons from dorsal root and trigeminal ganglia, and mediates the aversive and nocifensive behavioral responses to PS. In a pharmacological screen for TRPM3 modulators, we identified clotrimazole (CLT), the antifungal compound, as a strong potentiator of nocifensive behavioral to PS *in vivo*. Surprisingly, TRPM3 null mice showed no prolonged nocifensive behavioral after coinjection of PS and CLT in the hindpaw, suggesting for specific interaction with TRPM3. Here, we propose a possible mechanism of TRPM3 response to PS potentiation by clotrimazole. These experiments reveal an unanticipated novel mechanism of TRP channel modulation.