BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Friday, November 6th 2015

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PROGRAMME

&

ABSTRACTBOOK

Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
""Les Ecuries - Ockeghem room" - Atrium"
Rue Ducale / Hertogsstraat 1
1000 Brussels

Local host

Prof. Dr. Thomas Voets
Katholieke Universiteit Leuven
Deparment of Cellular and Molecular Medicine
Laboratory of Ion Channel Research
Campus Gasthuisberg – O&N I Herestraat 49 – Box 802
3000 Leuven

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with support of the

Royal Flemish Academy of Belgium for Science and the Arts

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Main Lecture

10.00-10.45 Prof. Dr. Thomas GUDERMANN (Walter-Straub-Institut für Pharmakologie und Toxikologie – Ludwig-Maximillans-Universität München – Germany)

Kinase-coupled TRP channels: why care about magnesium ?

Oral Communications

10.45-11.00 K. HELD, A. JANSSENS , T. VOETS, J. VRIENS (KULeuven)
Further evidence of an alternative ion permeation pathway in the nociceptor TRPM3.

11.00-11.15 G. JACOBS, W. OOSTERLINCK, S. KERSELAERS, S. PINTO, A. PIRONET, M. KECSKES, P. HERIJGERS, R. VENNEKENS (KULeuven)
Decreased vulnerability for Ca^{2+}-dependent arrhythmia in $Trpm4^{-/-}$ mice.

11.15-11.30 Y.A. ALPIZAR, B. BOONEN, M. GEES, P. UVIN, T. VOETS, D. DE RIDDER, W. EVERAERTS, K. TALAVERA (KULeuven)
TRPV1 contributes to acrolein-induced toxicity.

11.30-11.45 D. ENGEL, V. SEUTIN (ULiège)
High dendritic expression of I_h in the proximity of the axon origin controls the integrative properties of nigral dopamine neurons.

11.45-12.00 T. DEMUYSER, E. BENTEA, L. DENEYER, G. ALBERTINI, J. VAN LIEFFERINGE, E. MERCKX, D. DE BUNDEL, A. MASSIE, I. SMOLDERS (VUBrussel)
The cystine/glutamate antiporter as a potential novel target to modulate the stress response?

12.00-12.15 B. BOSIER, P. DOYEN, A. BROLET, G. MUCCIOLI, E. AHMED, N. DESMET, E. HERMANS, R. DEUMENS (UCLouvain, Suez Canal University, Egypt)
Spinal regulator of G protein signaling 4 as a novel therapeutic target in the treatment of neuropathic pain.

12.15-12.30 G. REGNIER, E. BOCKSTEINS, W.F. MAREI, J.L. LEROY, J.P. TIMMERMANS, D.J. SNYDERS (UAntwerpen)
Targeted deletion of Kv6.4 disrupts mouse spermatogenesis resulting in male infertility.

12.30-14.00 **Lunch – Guided Poster Session – General Assembly**

Posters

(height 120 cm – width 100 cm)

1. K. LEDEGANCK, O. VANDERVEKEN, G. VERPOOTEN, M. PEETERS, J. VAN MEERBEECK, P. SPECENIER, A. JANSSENS, B. DE WINTER (UAntwerpen, UZAntwerpen)
   Urinary EGF predicts renal magnesium loss in cisplatin-treated patients.

2. M. PIERARD, S. CONOTTE, A. TASSIN, K. ZOUAOUI BOUDJELTI, A. LEGRAND (UMons)
   Effect of continuous and intermittent hypoxia on adiponectin multimeric forms and muscle receptors in mice.

3. S. CONOTTE, A. TASSIN, K. ZOUAOUI BOUDJELTI, A. LEGRAND (UMons)
   Cardiovascular alterations associated to intermittent hypoxia in a mouse model of obstructive sleep apnea (OSA).

4. V. COLOMBARO, I. JADOT, A-E. DECLÈVES, V. VOISIN, L. GIORDANO, I. HABSCH, J. MALAISSE, B. FLAMION, N. CARON (UNamur, ULBruxelles)
   Lack of hyaluronidases exacerbates renal post-ischemic injury, inflammation and fibrosis.

5. I. JADOT, A.E. DECLÈVE, V. COLOMBARO, B. MARTIN, I. HABSCH, J. NORTIER, N. CARON (UNamur, ULBruxelles)
   L-arginine supplementation ameliorates chronic kidney injury in experimental aristolochic acid nephropathy.

6. J. VAN DINGENEN, I. VAN COLEN, R.A. LEFEBVRE (UGent)
   Influence of nitrite on intestinal ischemia/reperfusion injury in a murine model.

7. L. VANDEN DAELE, C. BOYDENS, B. PAUWELS, J. VAN DE VOORDE (UGent)
   Vasorelaxing effect of resveratrol on bovine retinal arteries.

8. F. SEGHERS, X. YERNA, P. GAILLY (UCLouvain)
   Role of TRPV4 in pressure-induced inhibition of renin secretion by juxta-glomerular cells.

9. K. PHILIPPAERT, S. KERSELAERS, R. VENNEKENS (KULeuven)
   TRPM5 is a target for the antidiabetic drug glimepiride.

10. W.J. MALAISSE, R. CRUTZEN, R. BEAUWENS (ULB)
    Anoctamin 1 as a volume-sensitive anion channel in insulin-producing cells.

11. K. DE CLERCQ, S. PINTO, R. VENNEKENS, T. VOETS, J. VRIENS (KULeuven)
    The role of TRPV2 in endometrial stromal decidualization.

12. G. REGNIER, E. BOCKSTEINS, G. VAN DE VIJVER, D.J. SNYDERS, P.P. VAN BOGAERT (UAntwerpen)
    Postnatal development of Kv2-mediated K^+ currents in dorsal root ganglion (DRG) neurons.

13. N. ZANOU, P. GAILLY (UCLouvain)
    TRPV2 ion channel controls cell volume changes during the migration of glioblastoma cells.

14. J.B. STARTEK, D. GHOSH, Y.A. ALPIZAR, A. LÓPEZ-REQUENA, N. VAN RANST, T. VOETS, K. TALAVERA (KULeuven)
Localization of the chemosensory TRPA1 channel in the lipid rafts modulates its activity.

15. V. VAN HAVER, M. DE BOCK, D. HOORELBEKE, E. DECROCK, L. LEYBAERT (UGent)
Transcellular transport in the blood brain barrier in inflammatory conditions.

Oral Communications

14.00-14.15 H. SHAKERI, D. M. SCHRIJVERS, V.F. SEGERS, C. VAN HOVE, G.R. DE MEYER, K. LEMMENS (UAntwerpen)
Neuregulin-1 attenuates stress-induced vascular senescence in vitro and in vivo.

14.15-14.30 A. KURDI, M. DE DOCKER, H. NEELS, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen, ZNA Stuivenberg Antwerpen)
Continuous, long-term administration of the mTOR inhibitor everolimus induces drug tolerance and decreases autophagic flux in mice.

14.30-14.45 M.O.J. GROOTAERT, S.H. KIM, H. VAN SPAENDONK, G.R.Y. DE MEYER, D.M. SCHRIJVERS, W. MARTINET (UAntwerpen, LG Life Sciences Daejean, Korea)
NecroX-7 reduces necrosis in atherosclerotic plaques of ApoE knockout mice.

14.45-15.00 D. VAN DER GRAAFF, M. LANDEN, P. FRANSEN, J.G. DE MAN, B.Y. DE WINTER, P.P. MICHIELSEN, W.J. KWANTEN, S.M. FRANCQUE (UAntwerpen, UZAntwerpen)
Rats with non-alcoholic fatty liver disease (NAFLD) demonstrate a hypersensitivity and a reduced time-dependent adaptation to methoxamine.

15.00-15.15 V. PAUWELYN, E. VAN DEYNSE, R.A. LEFEBVRE (UGent)
Facilitation of cholinergic neurotransmission via 5-HT4 receptors in murine stomach, jejunum and colon.

15.15-15.30 H. VAN SPAENDONK, J.G. DE MAN, S. FRANCQUE, B.Y. DE WINTER (UAntwerpen)
Pharmacological validation of the chronic colitis transfer model by using anti-TNFalpha therapy.

15.30-15.45 M. BITTREMIEU, R. LA ROVERE, H. AKL, K. MIKOSHIBA, J.B. PARYS, G. BULTYNCK (KULeuven, Lebanese Univ., Hadath,Lebanon, RIKEN Brain Science Institute, Saitama, Japan)
Cell death induced by antagonizing the BH4 domain of Bcl-2 in malignant B cells is independent of store-operated Ca^{2+} entry.

15.45-16.00 R. LA ROVERE, M. KERKHOFS, H. AKL, M. BITTREMIEUX, T. VERVLOESSEM, T. LUYTEN, K. WELKENHUYZEN, L. MISSIAEN, H. DE SMEDT, J.B. PARYS, G. BULTYNCK (KU Leuven, Lebanese University, Hadath, Lebanon)
The role of the ER-mitochondria Ca^{2+}-mediated cell death in malignant B cells.

16.00-16.15 M. KECSKÉS, G. JACOBS, S. KERSELAERS, T. VOETS, R. VENNEKENS (KULeuven)
The role of TRPM4 ion channel in ventricular remodelling.

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ABSTRACTS

Legend
O = Oral communication numbered and scheduled time
O = Poster numbered

O-01 (10.45-11.00)

FURTHER EVIDENCE OF AN ALTERNATIVE ION PERMEATION PATHWAY IN THE NOCICEPTOR TRPM3

K. Held [1,2], A. Janssens [2], T. Voets [2] & J. Vriens [1]

[1] Laboratory of Obstetrics & Experimental Gynaecology, KU Leuven, 3000 Leuven, Belgium
[2] Laboratory of ion channel research (LICR), KU Leuven, 3000 Leuven, Belgium

TRPM3 is a member of the melastatin family of the transient receptor potential (TRP) channels and was described as a nociceptor. It can be activated by a multitude of stimuli, including chemical compounds such as pregnenolone sulphate (PS) or nifedipine (Nif), as well as by physical signals like temperature. Activation of TRPM3 by all these different type of stimuli leads to the opening of a central pore that is formed by the transmembrane domains five (S5) and six (S6) and the interconnecting pore-loop, resulting in an outward rectifying current-voltage relationship. Recently, we have described the existence of an alternative ion permeation pathway in TRPM3. This alternative pathway could be opened by simultaneous application of PS and the antifungal drug clotrimazole (Clt), resulting in a current shape that showed an in- and outward rectification. Very recently, we have described a novel synthetic agonist of TRPM3, CIM0216, which is remarkable by the fact that it is able to open as single compound both, the central pore and the alternative ion permeation pathway. By the use of CIM0216, we were able to further investigate the biophysical properties of the alternative ion permeation pathway and to achieve further evidence of the existence of an alternative ion permeation pathway in TRPM3. In addition, new mutagenesis studies of TRPM3 provided further evidence for the location of the alternative ion permeation pathway within the region of S4 of the voltage sensing domain. All together, these data provide more evidence for the existence of an alternative ion permeation pathway in TRPM3, that will open upon displacement of the voltage sensing domain S4.

O-02 (11.00-11.15)

DECREASED VULNERABILITY FOR CA^{2+}-DEPENDENT ARRHYTHMIA IN *TRPM4*$^{-/-}$ MICE

G. Jacobs[1], W. Oosterlinck[2], S. Kerselaers[1], S. Pinto[1], A. Pironet[1], M. Kecskes[1], P. Herijgers[2], R. Vennekens[1]

[1]Laboratory of Ion Channel Research, KU Leuven, 3000 Leuven, Belgium; [2]Laboratory of Experimental Cardiac Surgery, KU Leuven, 3000 Leuven, Belgium

TRPM4 is a Ca^{2+}-activated non-selective cation (CAN) channel that belongs to the family of the Transient Receptor Potential (TRP) ion channels. TRPM4 is activated by high intracellular Ca^{2+} concentration and, although it is impermeable for Ca^{2+}, it is an important regulator of Ca^{2+}-dependent cell functions, such as exocytosis and cell death. *Trpm4* is highly expressed in the heart and it is suggested that TRPM4 might be involved in the development of Ca^{2+}-dependent arrhythmias, more specific in triggered activity. Triggered activity is due to delayed afterdepolarisations (DAD) occurring as a result of Ca^{2+} overloading of cardiomyocytes and spontaneous Ca^{2+} release from the sarcoplasmic reticulum. Ca^{2+} release induces membrane depolarization by activation of a transient Ca^{2+}-activated inward current (I_{ti}). The Na^+/Ca^{2+} exchanger (NCX) is known as the main contributor to this inward current, but also CAN channels might be involved. At this moment, TRPM4 is the only molecular candidate for CAN channels identified in the heart. In this study, we investigated if TRPM4 plays a role in the development of Ca^{2+}-dependent arrhythmias in *in vivo* models. Therefore, heart activity was measured in awake animals by use of ECG transmitters. Three distinct protocols, known to induce Ca^{2+}-dependent arrhythmia, were tested in WT and *Trpm4*$^{-/-}$ mice. First, a chemical protocol with injections of the toxin aconitine, known to induce Na^+ and Ca^{2+} overload. Most WT and *Trpm4*$^{-/-}$ animals developed arrhythmia, although *Trpm4*$^{-/-}$ mice developed less ventricular ectopic beats (VEB) and had a lower arrhythmic score. Secondly, a more clinical relevant arrhythmic model was tested, in which ischemia was induced in the heart for 30 minutes by ligation of the left anterior descending (LAD) coronary artery. During ischemia, significantly more WT animals developed arrhythmia compared to *Trpm4*$^{-/-}$ mice. In the last protocol, development of arrhythmias was investigated in a mouse model of CPVT (catecholaminergic polymorphic ventricular tachycardia). CPVT is a genetic disease characterized by development of arrhythmia during physical activity and emotional stress and caused by mutations in the Ryanodine Receptor (RyR2). *RyR*$^{+/R2474S}$ and *RyR*$^{+/R2474S}$-*Trpm4*$^{-/-}$ mice were completely exhausted by means of an exercise test and extra stimulated with isoprenaline. Afterwards, the development of arrhythmia was determined and also here, more WT animals developed arrhythmia compared to *Trpm4*$^{-/-}$ mice. In the 3 independent arrhythmic models, *Trpm4*$^{-/-}$ mice seems to be less vulnerable for Ca^{2+}-dependent arrhythmia.

O-03 (11.15-11.30)

TRPV1 CONTRIBUTES TO ACROLEIN-INDUCED TOXICITY

Y.A. Alpizar[1], B. Boonen[1], M. Gees[1], P. Uvin[2], T. Voets[1], D. De Ridder[2], W. Everaerts[2], K. Talavera[1]

[1]Laboratory of Ion Channel Research and TRP Research Platform Leuven (TRPLe), Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2]Laboratory of Experimental Urology and TRP Research Platform Leuven (TRPLe), Department of Development and Regeneration, KU Leuven, Leuven, Belgium.

Acrolein is a toxic and highly reactive unsaturated aldehyde, often found in cigarette smoke and vehicle exhaust gases. Likewise, acrolein derived from cyclophosphamide-treated patients constitutes the major culprit of bladder irritation during chemotherapy in cancer patient. Although, initially, its toxicity and inflammatory properties have been related to the activation of the transient receptor potential A1 (TRPA1) in nociceptive neurons, recent evidences suggests that other receptor may also play a role in acrolein-induced toxicity. Here we show that, besides activation of TRPA1, acrolein evokes the activation of TRPV1 channel. Ratiometric calcium measurements and patch-clamp suggested that unlike TRPA1 that desensitizes immediately after activation, acrolein-induced activation of TRPV1 is prolonged in time. Furthermore, we identify the N-terminal amino acid residue C157 as key for acrolein-induced TRPV1 activation. Taken together, our results reveal a mechanism underlying the major role of TRPV1 as mediator for the acrolein-induced toxicity, unveiling TRPV1 as a potential therapeutic target in a wide spectrum of noxious conditions, from exposure to smoke to cancer treatment.

O-04 (11.30-11.45)

HIGH DENDRITIC EXPRESSION OF I_H IN THE PROXIMITY OF THE AXON ORIGIN CONTROLS THE INTEGRATIVE PROPERTIES OF NIGRAL DOPAMINE NEURONS

D. Engel, V. Seutin

GIGA-Neurosciences, Neurophysiology Unit, University of Liège, Sart Tilman, B-4000 Liège, Belgium

Dendrites of most neurons express voltage-gated ion channels in their membrane. In combination with passive properties, active currents confer to dendrites a high computational potential. The hyperpolarization-activated cation current I_h present in the dendrites of some pyramidal neurons affects their membrane and integration properties, synaptic plasticity and higher functions such as memory. A gradient of increasing h-channel density towards distal dendrites has been found to be responsible for the location independence of EPSP waveform and temporal summation in cortical and hippocampal pyramidal cells. However, reports on other cell types revealed that smoother gradients or even linear distributions of I_h can achieve homogeneous temporal summation. Although the existence of a robust, slowly activating I_h current has been repeatedly demonstrated in nigral dopamine neurons, its subcellular distribution and precise role in synaptic integration is unknown. Using cell-attached patch-clamp recordings, we find a higher I_h current density in the axon-bearing dendrite than in the soma or in dendrites without axon in nigral dopamine neurons. I_h is mainly concentrated in the dendritic membrane area surrounding the axon origin and decreases with increasing distances from this site. Single EPSPs and temporal summation are similarly affected by blockade of I_h in axon- and nonaxon bearing dendrites. The presence of I_h close to the axon is pivotal to control the integrative functions and the output signal of dopamine neurons and may consequently influence the downstream coding of movement.

O-05 (11.45-12.00)

THE CYSTINE/GLUTAMATE ANTIPORTER AS A POTENTIAL NOVEL TARGET TO MODULATE THE STRESS RESPONSE?

T. Demuyser, E. Bentea, L. Deneyer, G. Albertini, J. Van Liefferinge, E. Merckx, D. De Bundel, A. Massie, I. Smolders

Center for Neurosciences, Vrije Universiteit Brussel, Brussels, 1090, Belgium

In modern society, stress is a major causative factor for a variety of psychiatric disorders. Depression, one of the main causes of disability worldwide, is a multimodal disease with chronic stress considered as a 'trigger' for depressive episodes. Depression and comorbid anxiety are usually related to a malfunctioning mono-aminergic system, nowadays however compelling evidence points at an important role of glutamate in the etiology of the 'depressed/anxious brain'. Being the major excitatory neurotransmitter in the central nervous system, glutamate can potentially have important excitotoxic effects. System xc- is the cystine/glutamate antiporter and the major source of extrasynaptic glutamate in some important depression-related brain areas, where it can be an interesting new target for improved psychopharmacological treatment. In this study we investigated the effect of loss of functional system xc- (e.g. deletion of the specific light chain subunit xCT; $xCT^{-/-}$), on chronic stress induced depression and anxiety in a validated animal model. Therefore we subjected $xCT^{-/-}$ and $xCT^{+/+}$ mice, treated with chronic corticosterone injections (excessive chronic stress), to a battery of acute stress-based tests for depressive- and anxiety- like behavior and compared their behavior to vehicle treated and naïve animals. Interestingly we found decreased depressive- and anxiety- like behavior in the naïve $xCT^{-/-}$ mice in all of the tests conducted. Unexpectedly however the decrease in depressive- and anxiety- like behavior faded and disappeared after vehicle and corticosterone treatment. These findings support further research for the role of system xc- in the stress response, since the involvement of the antiporter in regulating the response to acute versus chronic stress seems to differ.

O-06 (12.00-12.15)

SPINAL REGULATOR OF G PROTEIN SIGNALING 4 AS A NOVEL THERAPEUTIC TARGET IN THE TREATMENT OF NEUROPATHIC PAIN

B. Bosier[1], P. Doyen[1], A. Brolet[1], G. Muccioli[2], E. Ahmed[1,3], N. Desmet[1], E. Hermans[1], R. Deumens[1]

[1]Institute of Neuroscience, Université catholique de Louvain (UCL), Brussels, 1200 Belgium; [2]Louvain Drug Research Institute, UCL, Brussels, 1200 Belgium; Department of Clinical Pharmacology, Faculty of Medicine, Suez Canal University, Ismailia, 41522 Egypt

Regulators of G protein signaling (RGS) are strong determinants of metabotropic receptor activity, reducing the lifespan of GTP-bound G protein. Accumulating evidence suggests a key role of site-specific RGS expression in modulation of analgesic properties of both endogenous signaling systems and exogenously delivered pain medicines. The reduced potency of analgesic agents in neuropathic pain may be due to alterations in RGS. In our recent work we show that partial sciatic nerve ligation in rats caused a marked decrease in efficacy of HU210-induced cannabinoid receptor type 1 (CB1) signaling in the lumbar spinal cord, which could be rescued by intrathecal administration of the specific RGS4 inhibitor CCG 63802. After validating a functional RGS4-CB1 interaction and considering the compromised spinal CB1 signaling after nerve injury despite an up-regulation in endocannabinoids, RGS4 inhibition was investigated as a treatment of neuropathic pain. Intrathecal administration of the specific RGS4 inhibitor CCG 63802 during the first week after partial sciatic nerve ligation potently reduced hyperalgesia and the activation of microglia and astrocytes, key players in the development of neuropathic pain. We suggest that spinal RGS4 inhibition restores endogenous analgesic signaling to effectively mitigate neuropathic pain. This report shows that signaling through the CB1 may be involved in this beneficial effect.

O-07 (12.15-12.30)

TARGETED DELETION OF Kv6.4 DISRUPTS MOUSE SPERMATOGENESIS RESULTING IN MALE INFERTILITY

G. Regnier[1], E. Bocksteins[1], W.F. Marei[2], J.L. Leroy[2], J.P. Timmermans[3], D.J. Snyders[1]

[1]Departmentof Biomedical Sciences, University of Antwerp, Antwerp, Belgium, [2]Gamete Research Center, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium, [3]Core Facility for Biomedical Microscopic Imaging, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium

Electrically silent voltage-gated potassium (KvS) channel subunits (i.e. Kv5-Kv6 and Kv8-Kv9) do not form functional homotetrameric Kv channels. These KvS subunits co-assemble with Kv2 subunits generating heterotetrameric Kv2/KvS channel complexes in which the KvS subunits modulate the Kv2 properties. An increasing number of publications demonstrated the contribution of KvS subunits to different (patho)physiological processes in different cells. However, little is known about the physiological role of Kv6.4 subunits. Here, we report that the targeted deletion of Kv6.4 in a transgenic mouse model (*KCNG4*$^{-/-}$) leads to infertile homozygous male *KCNG4*$^{-/-}$ mice; descendants were only obtained from mating homozygous *KCNG4*$^{-/-}$ females with wild type (WT) or heterozygous *KCNG4*$^{-/-}$ males. Sperm isolation demonstrated that this was due to a very low sperm concentration: homozygous *KCNG4*$^{-/-}$ mice possessed a 200-fold lower sperm concentration compared to the WT and heterozygous littermates. Furthermore, sperm cells of homozygous *KCNG4*$^{-/-}$ mice showed an abnormal morphology characterized by a small head and a short tail. Hematoxylin and eosin stainings of testis tissue suggested that this inability to produce (normal) sperm cells is due to a disruption of the spermatogenesis in homozygous *KCNG4*$^{-/-}$ mice. These results suggested that Kv6.4 subunits play an essential role in spermatogenesis.

O-08 (14.00-14.15)

NEUREGULIN-1 ATTENUATES STRESS-INDUCED VASCULAR SENESCENCE IN VITRO AND IN VIVO

H. Shakeri[1], D.M. Schrijvers[2], V.F. Segers[1], C. Van Hove[1], G.R. De Meyer[2], K. Lemmens[1]

[1] University of Antwerp, Department of Medicine, Pharmacology, Universiteitsplein 1, B-2610 Antwerp, Belgium, [2]University of Antwerp, Department of Pharmaceutical Sciences, Physiopharmacology, Universiteitsplein 1, B-2610 Antwerp, Belgium

Cardiovascular ageing is a key process that determines the life expectancy and health of the elderly. Cellular senescence, a state of irreversible cell cycle arrest, is described as an important ageing-contributor due to the accumulation of damaged cells. Targeting cellular senescence can be a new approach to prevent or treat age-related cardiovascular diseases. In this study, we investigated the effect of neuregulin-1 (NRG-1) on cardiovascular cell senescence in vitro and in vivo. NRG-1 is an endothelial growth factor, which has powerful cardioprotective and anti-atherosclerotic effects but its role in ageing remains unexplored. Cultured aortic rat endothelial cells (AECs) and smooth muscle cells (SMCs) were exposed to 30 µM hydrogen peroxide (H2O2) for 2 hours. Cellular senescence was confirmed 72 hours later using SA-β-galactosidase staining and cell surface area as markers of senescence. In addition, western blot analyses of senescence associated pathways (including acetyl-p53, p21) were performed. In the presence of 20 ng /ml NRG-1, H2O2- induced senescence was significantly attenuated, as shown by a decreased number of SA-β-galactosidase positive AECs and SMCs, a decreased surface area of NRG-1 treated cells and also a decreased expression of acetyl-p53 in cells exposed to NRG-1. To strengthen these observations in vivo, C57BL/6 mice were rendered diabetic with streptozotocin and randomized to receive NRG-1 (20 µg/kg) or vehicle. In all diabetic mice, a significant induction of cell senescence in the aorta was observed using the methods mentioned above. Consistent with our in vitro observations, NRG-1 treatment significantly attenuated hyperglycaemia-induced senescence in the aorta. This study is the first to explore the role of the cardioprotective growth factor NRG-1 in vascular senescence. Our data demonstrate that NRG-1 markedly inhibits senescence induced by oxidative stress in vascular cells in vitro and in the aorta of diabetic mice in vivo.

O-09 (14.15-14.30)

CONTINUOUS, LONG-TERM ADMINISTRATION OF THE mTOR INHIBITOR EVEROLIMUS INDUCES DRUG TOLERANCE AND DECREASES AUTOPHAGIC FLUX IN MICE

A. Kurdi[1], M. De Docker[2], H. Neels[2], G.R.Y. De Meyer[1], W. Martinet[1]

[1]Division of Physiopharmacology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium; [2]Toxicology Laboratory, ZNA Stuivenberg, Lange Beeldekenstraat 267, B2060 Antwerp, Belgium

The rapamycin derivative everolimus is known for its autophagy stimulating properties. In this study, osmotic minipumps were used to treat GFP-LC3 mice continuously for 3 or 28 days with either vehicle or everolimus solution (1.5mg/kg/day). Treatment for 3 days resulted in mTOR inhibition and autophagy stimulation in liver, heart and kidney. Surprisingly, treatment for 28 days resulted in hyperactivation of the AKT-mTOR pathway in the liver coupled with a remarkable decrease in autophagy. In the heart en kidney, however, autophagy was unchanged despite clear mTOR inhibition and high plasma concentrations of the drug (503 ± 58 nM). These findings suggest tolerance and encourage the investigation of intermittent regimens designed to extend the autophagy-inducing properties of everolimus.

O-10 (14.30-14.45)

NECROX-7 REDUCES NECROSIS IN ATHEROSCLEROTIC PLAQUES OF APOE KNOCKOUT MICE

M.O.J. Grootaert[1], S.H. Kim[2], H. Van Spaendonk[1], G.R.Y. De Meyer[1], D.M. Schrijvers[1], W. Martinet[1]

[1]Laboratory of Physiopharmacology, University of Antwerp, Wilrijk, 2610, Belgium
[2]R&D Park, LG Life Sciences Ltd., Daejeon, 305-380, Korea

A large necrotic core is a key player in atherosclerotic plaque instability. Necrotic cellular debris accumulates in the lipid-rich core and promotes inflammation and ultimately plaque rupture. Although the role of necrosis in atherosclerosis is rather clear-cut, not many strategies have been performed up till now to specifically target plaque necrosis. In this study, we evaluated the plaque stabilizing potential of NecroX-7, a novel necrosis inhibitor with anti-oxidative properties. Male ApoE knockout mice were treated with NecroX-7 (30mg/kg) or vehicle 3x/week via intraperitoneal injections for 16 weeks. Simultaneously, mice were fed a western-type diet to induce plaque formation. NecroX-7 lowered circulating oxLDL levels and reduced total lipid burden in the thoracic aorta of ApoE knockout mice when compared to vehicle-treated mice. Atherosclerotic plaques in the aortic root showed a significant decrease in necrosis and iNOS expression in NecroX-7-treated ApoE knockout mice as compared to vehicle-treated mice. Moreover, plaques of NecroX-7-treated mice showed a significant increase in collagen and smooth muscle cell content, indicative of improved plaque stability. *In vitro*, NecroX-7 (10µM) prevented *tert*-butyl hydroperoxide (tBHP)-induced ROS formation, necrosis and high-mobility group box 1 (HMGB1) release in primary macrophages. Interestingly, NecroX-7 preferentially protected M2 macrophages against tBHP-induced necrosis and reduced iNOS mRNA expression in tBHP-treated M1 macrophages. Furthermore, necroptosis and apoptosis were not affected by NecroX-7. We may conclude that NecroX-7, besides its anti-oxidative and anti-necrotic potential, also exerts anti-inflammatory and oxLDL-lowering effects, and could be a new promising multipotent drug for the treatment of atherosclerosis.

O-11 (14.45-15.00)

RATS WITH NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) DEMONSTRATE A HYPERSENSITIVITY AND A REDUCED TIME-DEPENDENT ADAPTATION TO METHOXAMINE

D. Van der graaff[1], M. Landen[1], P. Fransen[2], J.G. De Man[1] , B.Y. De Winter[1] , P.P. Michielsen[1, 3], W.J. Kwanten[1], S.M. Francque[1,3]

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NAFLD causes important intrahepatic vascular alterations and an increased intrahepatic vascular resistance (IHVR). Endothelial dysfunction and a decreased sensitivity to the vasoconstrictive α1-adrenoceptor agonist methoxamine (Mx) were reported. *Aim*. To study the underlying mechanisms of the reported hyporesponsiveness to Mx and potential time-dependent effects. *Methods*. Male Wistar rats were fed a methione-choline deficient diet (MCDD) to induce NAFLD or control diet (CD) diet for 4 weeks (n=4-5/group). IHVR was studied by in situ isolated liver perfusion. Dose-response curves to Mx were constructed evaluating intrahepatic vascular tone. Finally, IHVR was assessed as a function of time at a fixed dose of Mx (at Emax, 10^{-4} M) and flow (30ml/min). *Results*. Basal IHVR didn't differ between MCDD and CD, neither was the response to increasing flow rates. Dose-response curves to Mx showed a significantly increased sensitivity to Mx in MCDD compared to CD (-LogEC50: 5.55 vs. 5.20; $p<0.01$). Initial contraction with Mx was followed by time-dependent reduction of IHVR in CD (5>60min: 19.24>11.25mmHg). MCDD showed a similar initial contraction, while time-dependent decrease was delayed and attenuated (5>60min: 19.03>16.88mmHg). Significant difference was achieved after 20min Mx. C*onclusion* These results show a significant and time-dependent adaptation of IHVR to Mx in CD. This phenomenon should be taken into account when interpreting experiments with Mx pre-constriction. Secondly, although we didn't observe an increased IHVR, response to Mx was significantly altered in MCDD, with a significant hyperresponsiviness and impaired time-dependent adaptation.

O-12 (15.00-15.15)

FACILITATION OF CHOLINERGIC NEUROTRANSMISSION VIA 5-HT$_4$ RECEPTORS IN MURINE STOMACH, JEJUNUM AND COLON

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In the canine, porcine and human gastrointestinal (GI) tract, activation of 5-HT$_4$ receptors present on cholinergic neurons innervating smooth muscle (SM) cells enhances the ongoing acetylcholine release resulting in increased SM contractions. In mice the presence of 5-HT$_4$ receptors on myenteric cholinergic neurons towards resident macrophages was already suggested, but their presence on cholinergic neurons towards GI SM cells remains unexplored and was therefore investigated. Circular SM strips from fundus, jejunum and colon were mounted isometrically in the presence of guanethidine (4 µM), L-NAME (300 µM) and for colon also MRS 2500 (1 µM) to exclude noradrenergic, nitrergic and purinergic influences. Reproducible submaximal neurogenic cholinergic on-contractions (checked with 3 µM tetrodotoxin and 1 µM atropine) were induced by electrical field stimulation (10 s trains, 500 µs pulse duration, submaximal voltage and 4 [fundus] or 8 Hz [jejunum and colon], at 5 [fundus and colon] or 10 min [jejunum] interval). The selective 5-HT$_4$ receptor agonist prucalopride concentration-dependently (0.003 to 0.03 µM) increased the submaximal contractions. The increase by prucalopride (0.03 µM) was 104 ± 11 % in the fundus, 38 ± 5 % in the jejunum and 52 ± 10 % in the colon (n = 7-9). The facilitating effect of prucalopride (0.03 µM) was abolished by the 5-HT$_4$ receptor antagonist GR113808 (0.3 µM), that had no influence per se on the cholinergic contractions. The nicotinic receptor antagonist hexamethonium (5.10^{-4} M) significantly increased the cholinergic contractions in the fundus. In none of the tissues, it influenced the facilitating effect of prucalopride. In conclusion, in murine fundus, jejunum and colon, 5-HT$_4$ receptors are, just as in dog, pig and man, present on postganglionic cholinergic neurons innervating the circular SM layer. Compared to other species, the effective prucalopride concentrations for similar contraction increases are lower in the murine GI tract.

O-13 (15.15-15.30)

PHARMACOLOGICAL VALIDATION OF THE CHRONIC COLITIS TRANSFER MODEL BY USING ANTI-TNFALPHA THERAPY

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Adoptive transfer of naive T cells into SCID (severe compromised immunodeficiënt) mice is a widely used experimental model of chronic colitis. We previously demonstrated a beneficial effect of worm antigen treatment in this model. Before investigating further potential clinical therapeutic targets, our aim was to further validate this model by studying the effect of a clinically relevant immunotherapy namely anti-TNFα antibodies. Animals were treated twice weekly with vehicle or anti-mouse anti-TNFα antibodies (25mg/kg; i.p.) for 4 weeks starting from the day of the transfer. Clinical disease parameters (body weight, mobility, pilo-erection and stool consistency) were scored weekly. Colonic inflammation was evaluated by endoscopy every 14 days. Gastrointestinal permeability was determined using the FITC-dextran assay 30 days after the transfer procedure. Mice were sacrificed 4 hours later. Inflammation was scored macroscopically on isolated colonic tissue. Colonoscopy confirmed gradually increasing signs of colitis from day 0 to 28 in the adoptive transfer group. The colonoscopic score at day 28 was less pronounced in anti-TNFα-treated mice compared to the saline-treated colitis mice (2.6±1.0 vs 8.4±1.6 respectively). Colitis mice lost 16.2±6.9% of their body weight at day 28 whereas control mice and anti-TNFα-treated mice gained respectively 5.7±4.4% and 2.0±2.5% body weight. Macroscopy showed reduced signs of inflammation in anti-TNFα-treated colitis mice. The positive effect of anti-TNFα treatment on body weight, colonoscopy and macroscopy did not mirror an amelioration of the gastrointestinal permeability: serum FITC-dextran was 204.8±93.8 ng/mL in healthy controls vs 1277.9±1547.5 and 2845.8±2733.6 ng/mL respectively in saline-treated and anti-TNFα-treated colitis mice. We conclude that inflammation induced by the adoptive transfer colitis model is ameliorated by anti-TNFα therapy while the permeability changes are not.

O-14 (15.30-15.45)

CELL DEATH INDUCED BY ANTAGONIZING THE BH4 DOMAIN OF BCL-2 IN MALIGNANT B CELLS IS INDEPENDENT OF STORE-OPERATED CA^{2+} ENTRY

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Cell-fate decisions are regulated at the level of the mitochondria by pro- and anti-apoptotic Bcl-2 family proteins. 'Primed to death' cancer cells circumvent apoptotic cell death by the up-regulation of anti-apoptotic Bcl-2. These cells are usually addicted to Bcl-2 at the mitochondria, where it inhibits apoptosis by neutralizing the pro-apoptotic Bim protein. Recently, we discovered that certain cancer cells depend on large amounts of Bcl-2 at the endoplasmic reticulum (ER) for their survival. There, Bcl-2 targets the inositol 1,4,5-trisphosphate receptors (IP_3Rs), suppressing pro-apoptotic Ca^{2+} release. We developed an IP_3R-derived peptide (Bcl-2-IP_3R Disruptor-2, BIRD-2), which selectively antagonizes the function of Bcl-2 at the ER. BIRD-2 triggered spontaneous pro-apoptotic Ca^{2+} release in cancer cells with high expression levels of IP_3R2, such as a subset of diffuse large B-cell lymphoma (DL-BCL) cell lines, suggesting that especially these types of cancer cells are addicted to Bcl-2 at the ER. However, IP_3R2 up-regulation by itself seems not sufficient to determine the apoptotic sensitivity of cells to BIRD-2. To completely understand how BIRD-2 causes apoptotic cell death, we therefore further characterized DL-BCL cancer cells that are 'primed to death at the ER'. In particular, we examined whether store-operated Ca^{2+}-entry (SOCE) occurring via Stim/Orai signaling, which plays an important role in the biology of immune cells, can contribute to the toxic pro-apoptotic Ca^{2+} signaling downstream of ER Ca^{2+} release and may serve as an amplification factor leading to intracellular Ca^{2+} overload and apoptotic cell death. In BIRD-2-sensitive cells (SU-DHL-4) we found that SOCE does not contribute to BIRD-2-induced cell death, and that Ca^{2+} present in the ER lumen is sufficient to induce apoptosis upon BIRD-2 treatment. Inhibition of SOCE by YM-58483 or by siRNA against Stim1 (siStim1) did not influence or reduce the BIRD-2-triggered cytosolic Ca^{2+} rise in Fura-2-loaded SU-DHL-4 cells, nor BIRD-2-induced cell death, measured via Annexin V and 7-AAD staining. A reduction in the effect of BIRD-2 was observed when SOCE was inhibited by DPB162-AE, although this is probably due to a DPB162-AE-induced decrease of the ER Ca^{2+} content (measured, using 1 µM thapsigargin). On the contrary, YM-58483 and siStim1 did not influence the ER store content. Apoptotic cell death triggered by BIRD-2 was also significantly reduced in SU-DHL-4 cells pretreated with thapsigargin, a SERCA inhibitor that empties the ER Ca^{2+} stores. In summary, these data indicate SOCE does not contribute to the pro-apoptotic effects of BIRD-2 and that the ER luminal Ca^{2+} level is sufficient to provoke apoptosis upon BIRD-2 treatment. Future work will focus on measuring after treatment with BIRD-2 the mitochondrial (with Rhod-2) and the ER (with G-CEPIA1er) Ca^{2+} levels under different conditions to further unravel its working mechanism in 'primed to death at the ER' cancer cells.

O-15 (15.45-16.00)

THE ROLE OF THE ER-MITOCHONDRIA Ca^{2+}-MEDIATED CELL DEATH IN MALIGNANT B CELLS

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There is increasing evidence that B-cell cancers are addicted to high levels of anti-apoptotic Bcl-2 proteins at the endoplasmic reticulum (ER) for their survival. The ability of preventing the occurrence of pro-apoptotic Ca^{2+} signals of Bcl-2 appears to be executed in part by its BH4 domain. This directly targets and inhibits inositol 1,4,5-trisphosphate (IP_3) receptors, a family of ubiquitously expressed Ca^{2+}-release channels. A recently developed peptide tool that counteracts Bcl-2's suppressive role on IP_3 receptors (Bcl-2/IP_3R Disrupter – 2, BIRD-2) by targeting specifically BH4 domain, induces pro-apoptotic Ca^{2+}-signaling events in the cytosol of primary chronic lymphocytic leukemia cells and a subset of diffuse large B-cell lymphoma (DL-BCL) cell lines. Yet, the mechanism by which BIRD-2-induced Ca^{2+} overload leads in these cancer cells to cell death is only partially understood. We therefore focus on the impact of BIRD-2 on Ca^{2+} signalling in mitochondria, the apoptosis-initiator organelle. Using Rhod-2 AM, we found in single cell experiments that BIRD-2 exposure leads to a rapid and sustained mitochondrial Ca^{2+} overload in BIRD-2-sensitive DL-BCL cells (like SU-DHL-4), but not in BIRD-2-resistant DL-BCL cells (like OCI-LY-1). As a consequence, in SU-DHL-4 cells, but not in OCI-LY-1 cells, BIRD-2 triggers the production of mitochondrial reactive oxygen species (ROS), measured after loading the cells with MitoSOX red, and the loss of the mitochondrial potential, measured after loading the cells with Tetramethylrhodamine, Methyl Ester (TMRM). These events are followed by the opening of the mitochondrial permeability transition pore (mPTP), measured via a calcein-cobalt-quenching assay, and the activation of caspase 3. Further work aims to first identify and subsequently to interfere with the molecular mechanisms involved in mitochondrial Ca^{2+} transport and ROS production in order to assess their contribution in BIRD-2-mediated cell death.

O-16 (16.00-16.15)

THE ROLE OF TRPM4 ION CHANNEL IN VENTRICULAR REMODELING

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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 humoral factors like Angiotensin II. Prolonged cardiac hypertrophy can results in heart failure and sudden death, therefore better understanding of the disease has significant importance. TRPM4 a member of the TRP ion channel family is a calcium-activated non-selective cation channel. TRPM4 is expressed in the heart both in the atria and ventricle and activated during the cardiac action potential. Our goal was to study the development of hypertrophy in cardiac specific TRPM4 deficient mice (*Trpm4*^{cKO}). We have not found significant hypertrophy under basal condition in these KO mice. However, chronic AngII treatment resulted in increased heart weight to body weight ratio in *Trpm4*^{cKO} mice compared to WT. Furthermore, we have found an increased expression of several hypertrophy marker genes compared to WT. Histological analysis of the hearts showed increased myocite size in *Trpm4*^{cKO} compared to WT mice. Sarcoplasmic reticulum store depletion experiments showed increased store-operated calcium entry in TRPM4 deficient myocytes compared to WT. Store-operated calcium entry has been recently recognized as an important mechanism in cardiac hypertrophy by activating calcium dependent pathways. In line with that we have found increased activity and expression of calcineurin -a calcium dependent phosphatase in hypertrophy- in the hearts of AngII treated *Trpm4*^{cKO} mice compared to WT. Taken together, we propose that TRPM4 plays a specific role in calcium homeostasis of the ventricular myocytes and functions as a regulator of cardiac hypertrophy.

P-01

URINARY EGF PREDICTS RENAL MAGNESIUM LOSS IN CISPLATIN-TREATED PATIENTS

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Cisplatin-induced hypomagnesemia is described in humans and rats, but the underlying mechanisms are still unclear. In cisplatin-treated rats a role for the Mg^{2+} channel TRPM6 localized in the distal convoluted tubule and stimulated by epidermal growth factor (EGF) is suggested. We hypothesize that cisplatin-induced hypomagnesemia in patients is due to a renal Mg^{2+} leak resulting from a downregulation of the renal EGF production, thereby inhibiting the activation of TRPM6. Oncological patients treated with cisplatin were recruited into the study (n=45). Before the treatment with cisplatin was initiated (baseline) and at 6 time-points after the first dose of cisplatinum, blood and urine samples were taken from each patient to determine creatinine, Mg^{2+}, and EGF. Sixty-four percent of the patients developed hypomagnesaemia within 3 months after cisplatin treatment. In these patients, the first 3 weeks after initiating cisplatin therapy, the FE Mg^{2+} decreased resulting in a stable serum Mg^{2+} level. From week 4 to week 12 after cisplatin treatment, the FE Mg^{2+} increased resulting in low serum Mg^{2+} level. Thirty-nine percent of the cisplatin-treated patients showed normal serum Mg^{2+} levels with a decreasing FE Mg^{2+}. The log urinary EGF is a predictor of the FE Mg^{2+}, independent from age, gender and renal function. It is concluded that initially, in all cisplatin-treated patients the kidney was able to raise the Mg^{2+} reabsorption in an attempt to keep the serum Mg^{2+} level within the normal range, but at the long term, nearly two third of the patients developed hypomagnesaemia, due to urinary Mg^{2+} loss. In the whole study population, the urinary EGF excretion is independently related to the FE Mg^{2+}.

P-02

EFFECT OF CONTINUOUS AND INTERMITTENT HYPOXIA ON ADIPONECTIN MULTIMERIC FORMS AND MUSCLE RECEPTORS IN MICE

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Adiponectin (Ad) is an adipocytokine with cardiovascular (CV) protective properties. In patient with Chronic Obstructive Pulmonary Disease (COPD), Ad plasmatic level (Ad_{pl}) was shown to vary in opposite direction depending on the endotype (elevated in the hyperinflated endotype, lowered in the metabolic syndrome associated endotype). Hypoxaemia is frequently observed in severe COPD patient, either permanently or intermittently during exercising and while sleeping. Hypoxia was suggested to modulate Ad pathway on cellular models. We postulated that hypoxaemia could determine a distinct COPD endotype with altered Ad_{pl} and associated to a modified CV risk. Therefore, we performed a pilot study on an animal model comparing the effect of intermittent (IH) and continuous hypoxaemia (CH) on Ad_{pl}, Ad multimeric forms (Ad-mers) and muscle transmembrane receptors (AdR). To this aim, C57BL/6J mice (n=37) were exposed either to IH (FiO_2 6% -21%, 30s/30s) or CH (FiO_2 10%) 8h/day during 35 days. In both conditions, an increased haematocrit consecutive to a decreased PaO_2 was observed. This modification appeared earlier in IH mice but at day 35, haematocrit was significantly higher after CH than after IH exposure. Regarding Ad pathway, we observed 1) no difference in Ad_{pl}; 2) a decreased level of low molecular weight (MW) forms in favour of higher MW multimers; 3) a decreased AdR1 and AdR2 expression as compared to control animals. In conclusion, hypoxaemia alters AdRs muscle expression in mice but is also associated to a modified Ad-mer distribution. Consequently, for a better understanding of COPD pathophysiology, the impact of hypoxaemia on Ad pathway and particularly its consequences on CV system have to be further investigated.

P-03

CARDIOVASCULAR ALTERATIONS ASSOCIATED TO INTERMITTENT HYPOXIA IN A MOUSE MODEL OF OBSTRUCTIVE SLEEP APNEA (OSA)

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Obstructive sleep apnea (OSA) is a complex disease characterized by recurrent episodes of upper airway collapse and increased respiratory effort, hypoxia-reoxygenation cycles and repeated microarousals. OSA is associated to cardiovascular (CV) co-morbidities including endothelial dysfunction, dyslipidaemia, pulmonary and systemic hypertension, cardiac hypertrophy and atherosclerosis. The typical patient also suffers from obesity and metabolic syndrome. To better understand the contribution of chronic intermittent hypoxemia (ChIH) to the pathophysiology, C57BL/6J mice were exposed to ChIH (FIO_2 6%-21%, 30sec/30sec) 8 hours per day during 35 days using a standardized and well-controlled device (Chodzynsky *et al.*, 2013, PLoS One. 8 (4): e59973). As anticipated, ChIH led to polycythaemia from day 8, as well as to a significant cardiac hypertrophy as measured by an increase in heart weight / body weight ratio at day 35. Thickness assessment of the septum and right ventricle realized on 4 cardiac transverse sections revealed a septum enlargement, with a significant increase on L2 sections. These data associated to an increased arterial blood pressure suggested, however, a predominant left ventricular hypertrophy. Lipid deposition in the arterial wall was also investigated in the aortic arch. An increased number of lipid droplets was observed in L5 section in spite of a standard diet without animal fat. In conclusion, ChIH per se is sufficient to the development of high blood pressure, cardiac hypertrophy and atherosclerosis leading to an increased cardiovascular risk.

P-04

LACK OF HYALURONIDASES EXACERBATES RENAL POST-ISCHEMIC INJURY, INFLAMMATION AND FIBROSIS

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Renal ischemia-reperfusion injury (IRI) is a pathological process that may lead to acute renal failure and chronic dysfunction in renal allografts. During IRI, hyaluronan (HA) expression increased dramatically throughout the kidney. This may be explained by a specific modulation of HA synthases and degradation by hyaluronidases HYAL1 and HYAL2. It was previously demonstrated that suppression of HA accumulation during IR protected the kidney from ischemic insults. Therefore, we hypothesized that *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice might display exacerbated renal damages due to a higher HA accumulation in the post-ischemic kidney compared to the wild type (WT) littermates. Male *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice were subjected to an ischemia-reperfusion (IR) procedure. Then, mice were euthanized either 2, 7 or 30 days post-IR. Two days after IR, in WT mice, there was an accumulation of HA in the kidney and an increase in creatininemia. *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice presented higher amounts of HA in the post-ischemic kidney compared to the WT littermates and creatininemia was significantly increased in *Hyal2*$^{-/-}$ mice. Regarding histopathological damages, they were similar 2 days after IR between WT and knockout mice. Seven days after the surgical procedure, WT mice presented a significant decrease in renal damages but *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice still displayed important lesions. Moreover, inflammation was exacerbated in *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice as attested by increased amount of MIP-2 in the post-ischemic kidney and increased macrophage infiltration. Thirty days after IR, HA and CD44 expression, assessed by immunohistochemical staining, were still increased in *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice in comparison to the WT littermates, as well as expressions of α-SMA and collagen types I and III. Our results demonstrate that deficiency in HYAL1 or HYAL2 caused a persistence of renal damages and a higher progression to fibrosis after IR. Therefore, HYAL1 and HYAL2 seem to be protective against IR most likely by reducing HA accumulation in the post-ischemic kidney and so on, decreasing the inflammatory processes leading to acute kidney injury.

P-05

L-ARGININE SUPPLEMENTATION AMELIORATES CHRONIC KIDNEY INJURY IN EXPERIMENTAL ARISTOLOCHIC ACID NEPHROPATHY

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Aristolochic acid (AA) nephropathy (AAN) is a rapidly progressive tubulointerstitial nephritis of toxic origin characterized by early and transient acute tubular necrosis followed by fibrosis resulting in end-stage renal disease. Interestingly, a reduced nitric oxide (NO) production in AAN has been observed, which might lead to renal dysfunction. We already demonstrated that maintaining NO bioavailability using L-Arginine (L-Arg) supplementation is essential to improve the outcome of AA-induced acute kidney injury. Indeed, L-Arg, a substrate for NO synthesis, was able to restore NO bioavailability in acute AAN. Therefore, in this present study, we investigated the potential benefit of L-Arg supplementation in a chronic mouse model of AAN. To do so, C57BL/6J male mice were subjected to daily i.p. injection of AAI (3,5mg/kg) for 4 days and L-Arg was supplemented in drinking water (5%). Mice were euthanized 20 days after the first day of injection. At day 20, we observed a significant reduction of NO bioavailability in AA-treated animals as measured by reduced urinary nitrite/nitrate and cGMP excretions that were normalized with L-Arg treatment. AA-treated mice displayed polyuria, proteinuria and decreased urine creatinine level. Histological analyses of AA-treated mice revealed numerous foci of tubular atrophy surrounded by severe interstitial fibrosis as well as an increased collagen 1 and 3 mRNA expression. L-Arg supplementation in AA-treated mice significantly decreased tubular atrophy as attested by reduced tubular injury score. Moreover, L-Arg treatment also significantly decreased interstitial fibrosis as attested by reduced α-SMA staining and reduced collagen 1 and 3 mRNA expression. These results suggest that a preservation of NO bioavailibity may lead to a morpho-functionnal protection in AAN. In the present mouse model of chronic AAN, NO seems to act as a key factor of renal function and fibrosis development. In conclusion, restored NO bioavailability by L-Arg supplementation was demonstrated beneficial in improving renal injury in chronic AAN.

P-06

INFLUENCE OF NITRITE ON INTESTINAL ISCHEMIA/REPERFUSION INJURY IN A MURINE MODEL

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Intestinal ischemia, or reduced oxygen supply to the intestine, followed by restoration of the blood flow, also called reperfusion, causes severe tissue damage and necrosis and is a potentially life-threatening disease. The main mechanism underlying this ischemia/reperfusion (I/R) injury includes the generation of reactive oxygen species (ROS) and an inflammatory cascade involving the production of cytokines (e.g. interleukin [IL]-6) and recruitment of leukocytes. Exogenous administration of nitrite protects heart, kidney, brain and liver from I/R injury; we now investigated the influence of nitrite on intestinal I/R injury in mice. The superior mesenteric artery was occluded for 45 min followed by 6 h reperfusion. Nitrite (4.8, 48 and 480 nmol) was administered i.v. 5 min before starting the occlusion. Intestinal transit was assessed using fluorescent imaging and inflammatory parameters, and levels of ROS and 3',5'-cyclic guanosine monophosphate (cGMP) were measured in the intestinal muscularis and mucosa. Tissues were examined histologically after hematoxylin and eosin staining. Pre-treatment with nitrite did not improve the delay in intestinal transit due to intestinal I/R injury, but the increased myeloperoxidase activity (index of neutrophil infiltration) and IL-6 level in the muscularis externa and the increased ROS level in the mucosa were significantly reduced in mice pre-treated with 48 nmol nitrite. I/R injury increased the level of intracellular adhesion molecule-1 in the muscularis but this was not influenced by nitrite; neither was the decreased level of cGMP in the mucosa. Histologically I/R injury separated the epithelium from the basal membrane, starting at the villous tips and continuing along the sides of the villi; these lesions were less pronounced in mice treated with nitrite. These results indicate that exogenous nitrite partially protects the intestine from I/R injury and deserves further investigation as possible tool in the treatment of intestinal I/R.

P-07

VASORELAXING EFFECT OF RESVERATROL ON BOVINE RETINAL ARTERIES

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Resveratrol is a red wine polyphenol that causes vasorelaxation. This could be of interest in the treatment or prevention of eye diseases with an impaired blood flow, such as glaucoma, age-related macular degeneration and diabetic retinopathy. In this study, the vasorelaxant capacity of cis- and trans-resveratrol was tested on bovine retinal arteries. Its mechanism was investigated and its influence on the continuously released retinal relaxing factor (RRF) was examined. The investigation was performed by mounting isolated bovine retinal arteries into wire myographs for isometric tension measurements. Concentration-response curves of cis- and trans-resveratrol were constructed to investigate the vasorelaxant capacity. Concentration-response curves of resveratrol in the absence or presence of the endothelium or different inhibitors were constructed to elucidate the mechanism. The influence of resveratrol on the RRF was examined by comparing the relaxations, elicited by RRF, with and without resveratrol incubation. Both resveratrol isomers caused a similar strong concentration-dependent relaxation. Removal of the endothelium or blocking endothelium-dependent pathways did not change the relaxation. Also K^+ channels blockers did not reduce the relaxation, except the 120 mM K^+ Krebs Ringer bicarbonate solution. Phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate blocked the relaxation partially, and so did the inhibition of heme oxygenase-1. Blocking adenylyl cyclase, AMP-activated protein kinase, estrogen receptors, sirtuin 1 or sarco/endoplasmic reticulum Ca^{2+} ATPase did not have an effect. The relaxation caused by the RRF was not altered by resveratrol incubation. It is concluded that cis- and trans-resveratrol relax bovine retinal arteries similarly and concentration-dependently. The main relaxation mechanism remains unclear, but K^+ channels, CO and the myosin phosphatase pathway may be involved. Resveratrol does not have an influence on the RRF.

P-08

ROLE OF TRPV4 IN PRESSURE-INDUCED INHIBITION OF RENIN SECRETION BY JUXTAGLOMERULAR CELLS

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The renin – angiotensin system is a crucial blood pressure regulation system. It consists of a hormonal cascade where the rate-limiting enzyme is renin, which is secreted into circulation by renal juxtaglomerular (JG) cells in response to low pressure in the renal afferent arteriole. In contrast, an increase in blood pressure in the afferent arteriole results in a decrease in renin secretion. This is accompanied by a transitory increase in cytosolic calcium concentration ($[Ca^{2+}]_i$) of JG cells. The inverse relationship between $[Ca^{2+}]_i$ and renin secretion has been called the “calcium paradox” of renin release and is due to a Ca^{2+} -dependent inhibition of the adenylate cyclase (AC5 and AC6) that produces cAMP, which activates PKA and triggers renin vesicles release. How increase in pressure induces a $[Ca^{2+}]_i$ transient in these cells is however unknown. We observed that $[Ca^{2+}]_i$ transients induced by mechanical stimuli in JG As4.1 cells were inhibited by Gd^{3+} and ruthenium red, two non specific inhibitors of mechano-sensitive channels. More specifically, the response was reduced by siRNA-mediated repression of TRPV4 expression, but not after repression of TRPV2, PKD2, TRPC1 or Piezo1 ion channels that are also expressed in As4.1 cells. As expected, Ca^{2+} response was inhibited by HC067047 and RN1734, two inhibitors of TRPV4. Interestingly, the stimulation of renin secretion by the AC activator forskolin was blunted by GSK1016790A and 4α−PDD, two activators of TRPV4. Moreover, in isolated perfused kidneys from $TRPV4^{-/-}$ mice, the pressure - renin relationship was significantly altered. Altogether, our results suggest that TRPV4 is involved in the pressure-induced entry of Ca^{2+} in JG cells, which triggers the inhibition of renin release and allows the negative feedback regulation on blood pressure.

P-09

TRPM5 IS A TARGET FOR THE ANTIDIABETIC DRUG GLIMEPIRIDE

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Glimepiride is an oral insulinotropic drug in the class of the sulfonylureas. Their proposed mechanism of action is blocking K_{ATP} channels in the pancreatic beta-cells and therebye inducing depolarization, calcium influx through calcium channels and initiation of insulin secretion. TRPM5 is a calcium activated monovalent cation channel that is functionally expressed in the pancreatic beta-cell. A high-throughput screening of chemically diverse compounds on their activity on TRPM5 identified glimepiride as a positive modulator of TRPM5. Further experiments confirmed potentiation of TRPM5-activity in patch clamp experiments. Isolated pancreatic islets from WT mice show a higher sensitivity to glimepiride than these of *Trpm5*$^{-/-}$ mice, indicating a TRPM5-dependent action of glimepiride *in vivo*. Glimepiride, as a third generation sulfonylurea had very limited risks on inducing hypoglycaemia compared to earlier sulfonylurea drugs as tolbutamide and glibenclamide. This is inconsistent with an identical mechanism of action of these drugs. The action on TRPM5 is downstream of increases in $[Ca^{2+}]_i$, and as such glucose-dependent. Therefore, our data suggest an explanation for the reduced hypoglycaemic effect of glimepiride. Taken together, the promiscuity of glimepiride leads to synergetic action on TRPM5 and K_{ATP} channels to stimulate insulin secretion from pancreatic beta-cells. This new information confirms the hypothesis that targeting TRPM5 is a valid approach to stimulate insulin secretion and in fact, is unknowingly already widely used.

P-10

ANOCTAMIN 1 ACTS AS VOLUME-SENSITIVE ANION CHANNEL IN INSULIN-PRODUCING CELLS

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Anoctamin 1 (Ano1) inhibitors such as tannic acid (TA) or 2-(5-ethyl-4-hydroxy-6-methylpyrimidin-2-ylthio)-N-(4-methoxyphenyl)thiazol-2-yl (AO1) were recently found to impair the bioelectrical and secretory responses to glucose in murine insulin-producing cells. We have now investigated whether Ano1 represents a volume-sensitive channel. When dispersed rat islet cells were preincubated for 15 min at a low glucose concentration (2.8 mM), a progressive increase in cell volume was observed during incubation at a high glucose concentration (16.7 mM), such an increase being most pronounced ($p<0.01$) between the 10^{th} and 20^{th} min of incubation. Covariance analysis indicated that between the 10^{th} and 90^{th} min of incubation, the slope of the regression line between cell volume and time (logarithmic scale) was significantly steeper ($p<0.05$ or less) in cells exposed to either TA or T-AO1 than in the absence of such Ano1 inhibitors. The inhibitors of Ano1 also suppressed the regulatory volume decrease otherwise observed during exposure to a hypoosmolar extracellular medium. After 2 min exposure to such a medium, the cell value averaged 149±3 percent of paired initial values. In the presence of TA or T-AO1, but not in their absence, the cell volume remained at comparable high values. Assuming a specific inhibitory action of TA and/or T-AO1 on Ano1, these findings document that Ano1 indeed acts as a volume-sensitive channel in insulin-producing cells

P-11

THE ROLE OF TRPV2 IN ENDOMETRIAL STROMAL DECIDUALIZATION

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Decidualization is the progesterone-dependent differentiation of endometrial stromal cells into round decidual cells and is a prerequisite for embryo implantation. In order to achieve successful embryo implantation, an effective communication is required between embryo, endometrial epithelial and stromal cells. However, the exact mechanism that allows communication between the epithelium and stroma upon embryo attachment remains to be elucidated. Possible candidates to govern these downstream events are ion channels that serve as important cellular sensors. Recently, the functional expression of TRP channels was shown in human endometrial stromal cells (hESC). In addition, TRPV2 knockout mice showed a significantly lower survival rate per litter. However, the capability of zygotes to develop into blastocyst in an in vitro culture was not different between wild type and knockout animals. Notably, in vivo decidualization experiments, using the menstruating mouse model revealed successful decidualization in only 20% of TRPV2 knockout animals whereas decidualization was observed in 80% for the wild types. Interestingly, we found that mRNA and functional expression of TRPV2 in hESC decreased significantly after 5 days of in vitro decidualization with 0.5 mM cAMP and 1 µM MPA. Next, blockers of pathways possibly involved in decidualization were used during in vitro decidualization in order to illuminate the upstream pathway of this decidualization-induced TRPV2 downregulation. All together, these results suggest an important role of TRPV2 in the onset of decidualization.

P-12

POSTNATAL DEVELOPMENT OF Kv2-MEDIATED K^+ CURRENTS IN DORSAL ROOT GANGLION (DRG) NEURONS

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Delayed rectifier voltage-gated K^+ (Kv) channels play an important role in the regulation of the electrophysiological properties of neurons. In mouse dorsal root ganglion (DRG) neurons, a large fraction of the delayed rectifier current (I_K) is carried by both homotetrameric Kv2 channels and heterotetrameric channels consisting of Kv2 and silent Kv (KvS) subunits (i.e. Kv5-Kv6 and Kv8-Kv9). However, little is known about the contribution of Kv2-mediated currents during the postnatal development of DRG neurons. Here, we report that the Stromatoxin (ScTx) sensitive component of I_K from mouse DRG neurons gradually decreased (~ 13%, $p<0.05$) during the first month of postnatal development. Because ScTx blocks both Kv2.1- and Kv2.2-mediated currents, this gradual decrease may reflect a decrease in Kv2.1 and/or Kv2.2 containing currents. However, the fraction of Kv2.1 antibody sensitive current that only reflects the Kv2.1-mediated currents remained similar during the first month of postnatal development. These results suggested that the number of Kv2.2 channels may decrease during postnatal development while the number of Kv2.1 channels did not change. Indeed, semi-quantitative RT-PCR analysis demonstrated that the mRNA levels of the Kv2.2 subunit decreases gradually during postnatal development while the Kv2.1 mRNA levels remained unaltered.

P-13

TRPV2 ION CHANNEL CONTROLS CELL VOLUME CHANGES DURING THE MIGRATION OF GLIOBLASTOMA CELLS

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Glioblastoma is the most aggressive brain tumour characterized by a high invasive capacity. Recent reports showed that migration of glioblastoma cells is accompanied not only by shape changes but also by volume changes. However the mechanisms that control volume changes during the process are not completely understood. We previously showed a role of TRPV2 ion channel in the mechanism of the regulatory volume increase (RVI) in skeletal muscle cell. Here, we aim at investigating the potential role of TRPV2 during migration and invasion of glioblastoma cells. To this end, we used U251 glioblastoma cells, transfected by si-RNA against TRPV2 or si-RNA control. Stimulation of transfected cells with cannabidiol, an activator of TRPV2/1, induced Ca^{2+} transients in control cells that were decreased by twice in si-TRPV2 treated cells. In these cells, we still conserved Ca^{2+} response to capsaicin, a specific activator of TRPV1. We then challenged transfected cells by osmotic changes. Our results showed a clear inhibition of Ca^{2+} transients in si-TRPV2 cells in response to both hypoosmotic and hyperosmotic shocks. Hyperosmotic shock induced volume decrease to a similar extent in control and si-TRPV2 cells. Interestingly, only control cells recovered their volume, emphasizing the involvement of TRPV2 in the process of RVI in U251 glioblastoma cells. Moreover, chelating extracellular Ca^{2+} during hyperosmotic stress impaired the RVI process. We then measured the rate of migration and invasion in si-TRPV2 and control cells by using a modified Boyden chamber. Our results showed a dramatic inhibition of both migration and invasion in si-TRPV2 cells. Taken together, our results show for the first time a control of glioblastoma cell volume regulation and migration by TRPV2 ion channel.

P-14

LOCALIZATION OF THE CHEMOSENSORY TRPA1 CHANNEL IN THE LIPID RAFTS MODULATES ITS ACTIVITY

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Lipid rafts constitute distinctive and greatly specialized ordered regions of the plasma membrane, characterized by a high content of cholesterol and sphingolipids. These domains accommodate various receptors and channels involved in many cellular processes. It has been recently shown that several members of TRP superfamily (TRPCs, TRPM8, and TRPV1) locate mainly in the lipid raft and that this compartmentalization modulates their activation properties. So far not much is known about the localization of TRPA1 in the plasma membrane. In this study we provide evidence for localization of this channel in lipid rafts. Using TIRF microscopy in HEK293T cells transfected with a mouse TRPA1 channel carrying a C-terminal mCherry tag we found high co-localization rate between this channel and the lipid raft maker cholera toxin subunit B. Ultracentrifugation of Triton-X insoluble fractions confirmed co-expression of TRPA1 and the lipid raft marker flotillin-2 in the low density membrane fractions. Lipid raft disruption experiments further confirmed the cholesterol-related localization of TRPA1, with the major channel population found in higher density gradients. Modification of lipid rafts with the cholesterol-depleting agent methyl-□-cyclodextrin or the sphingolipid hydrolase sphingomyelinase decreased the responses of TRPA1 to lipopolysaccharides, thymol and allyl isothiocyanate and cold. Taken together, these data indicate that TRPA1 is present in lipid rafts and that this location is required for normal channel activation by different agonists.

P-15

TRANSCELLULAR TRANSPORT IN THE BLOOD-BRAIN BARRIER IN INFLAMMATORY CONDITIONS

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Unique features of blood-brain barrier (BBB) endothelial cells (ECs) include highly restrictive tight junctions that prevent paracellular diffusion, and low prevalence of non-specific transcytotic events. The BBB is compromised in pathologic conditions associated with neuroinflammation. Previous work from our lab demonstrated that the EC cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important factor determining BBB function and that connexin hemichannels (CxHCs), small pores in the plasma membrane, contribute to $[Ca^{2+}]_i$ dynamics and BBB alterations. BBB compromise may involve paracellular leak or increased transcytosis. This study investigates transcellular transport in early BBB breakdown in inflammatory conditions. We hypothesize that CxHCs may contribute to transcellular transport by providing a direct diffusion pathway for small MW molecules (<1kDa), or indirectly, by exerting control over $[Ca^{2+}]_i$ that is involved in the transcellular pathway. We used IP injection of lipopolysaccharide (LPS) to trigger an increase in BBB permeability in mice. This LPS-induced BBB permeability increase could be prevented by IV injection of the Ca^{2+} chelator BAPTA-AM and of the CxHC blocking peptide (Gap27). Dye uptake studies were further performed on immortalized bEnd3 cells and primary mouse brain capillary ECs. To distinguish endocytosis from CxHC-mediated dye uptake, fluorescein (376Da) and 10kDa dextran Texas Red were added 1-3-6-24h after inflammatory stimulation. Immunostaining of early endosome antigen 1 and caveolin-1 was used to identify vesicles in ECs. All experiments were performed in combination with inhibitors of vesicular transport (BrefeldinA), CxHCs (Gap27), or $[Ca^{2+}]_i$ changes (BAPTA-AM). The results suggest that transcytosis may play a role in increased BBB permeability in inflammatory conditions and that Ca^{2+} signaling and CxHCs are also involved in this transcellular transport.