BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Friday, October 17th 2014

PROGRAMME

&

ABSTRACTBOOK

Venue

Palace of the Academies
"Espace Roi Baudouin" - Atrium
Rue Ducale / Hertogsstraat 1
1000 Brussels

Local host

Prof. Dr. Chantal DESSY
Faculté de pharmacie et des sciences biomédicales (FASB)
Institut de recherche expérimentale et clinique (IREC)
Pôle de pharmacologie et thérapeutique (FATH)
Université Catholique de Louvain
1200 Woluwé-Saint-Lambert

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Royal Flemish Academy of Belgium for Science and the Arts

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Main Lecture

10.00-10.45 "Role of estrogens in flow mediated vascular mechanotransduction"

Prof. Dr. Daniël HENRION (Université d'Angers, Angers, France)

Oral Communications

10.45-11.00 V. JORIS, E. LEON GOMEZ, I. LOBYSHEVA, D. CATALUCCI, J.L. BALLIGAND, C. DESSY (UCLouvain, Instituto Humanitas, CNR/IRGB/UOS, Rozzano, Milan)
Implication of microRNA 199a-3p in vascular function : modulation of the NOS/NO pathway.

11.00-11.15 E. CATRY, B.D. PACHIKIAN, A.M. NEYRINCK, P.D. CANI, C. DESSY, N.M. DELZENNE (UCLouvain)
Endothelial dysfunction induced by n-3 polyunsaturated fatty acid depletion is improved by a prebiotics supplementation.

11.15-11.30 L. VANDEKERCKHOVE, A.-S. HERVENT, N. HAMDANI, V. SEGERS, W.A. LINKE, W.J. PAULUS, G. DE KEULENAER (UAntwerpen, UMC Amsterdam, The Netherlands, Ruhr Univ., Bochum, Germany)
Effect of neuregulin-1 on left ventricle diastolic function in eccentric and concentric ventricular remodeling.

11.30-11.45 A. VAN STEENBERGEN, M. BALTEAU, J.L. VANOVERSCHELDE, L. HUE, S. HORMAN, L. BERTRAND, C. BEAULOYE (UCLouvain)
Sodium-glucose co-transporters (SGLT) in the heart. Contribution of SGLT-type of transport in hyperglycemia-induced signaling pathway in adult cardiomyocytes.

11.45-12.00 V. MONTIEL, E. LEON GOMEZ , I. LOBYSHEVA, H. ESFAHANI, C. BOUZIN, M. ROMERO PEREZ, O. DEVUYST , C. DESSY, J.L. BALLIGAND (UCLouvain)
Role of AQP1 in the function and cardiovascular remodeling.

12.00-12.15 L. WALRAVE, M. VINKEN, L. LEYBAERT, I. SMOLDERS (VUBrussel, UGent)
The role of connexin43 hemichannels in limbic seizures.

12.15-12.30 L. LAMBOT, A. DE KERCHOVE, S. N. SCHIFFMANN, D. GALL (ULBruxelles)
Striatopallidal NMDAR is implicated in goal directed behavior, habituation and amphetamine sensitization.

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

Posters

(height 120 cm – width 100 cm)

1. M. VERGOUTS, I. MEES, B. BOSIER, E. HERMANS (UCLouvain)
Metabotropic Glutamate receptor 5 desensitization: importance of protein kinase C epsilon.

2. W.J. MALAISSE, G. STANGÉ, I. KOJIMA (ULBruxelles, VUBrussel, Gunma Univ. Japan)
Presence of the sweet taste T1R3 receptor in human and rat pancreatic alpha cells.

3. A. SANCHEZ, Y.A. ALPIZAR, K. DEMYDENKO, P. HOET, T. TALAVERA (KULeuven)
Modulation of TRPV4 by silica nanoparticles and lipopolysaccharides.

4. J. COMHAIR, S.M. MOLCHANOVA, G. MORELLI, D. GALL, B. BRÔNE, S.N. SCHIFFMANN, J-M. RIGO (UHasselt, ULBruxelles)
Role of the glycine receptor alpha 2 subunit in striatal medium spiny neuron development.

5. J.I. STAS, E. BOCKSTEINS, A.J. LABRO, D.J. SNYDERS (UAntwerpen)
Role of the PxP motif in the U-shaped inactivation process of heterotetrameric Kv2.1/Kv6.4 channels.

6. K. DE CLERCQ, K. HELD, R. VAN BREE, T. VOETS, T. D'HOOGHE, J. VRIENS (KULeuven)
Fingerprint of functional Transient Receptor Potential channels in human endometrial stromal cells during the luteal phase of the menstrual cycle.

7. J-F. DE BACKER, S. MONLEZUN, A. GAZAN, M. ZOLI, O. VALVERDE, D. GALL, O. DE BACKER, S.N. SCHIFFMANN, A. DE KERCHOVE D'EXEARDE (ULBruxelles, Univ. Modena Italia, Univ Pompeu Fabra Barcelona, Spain)
MAGED1, a new protein involved in motor behavior and drug addiction.

8. D. BABU, G. LECLERCQ, R. MOTTERLINI, R. A. LEFEBVRE (UGent, INSERM Paris, France)
Influence of the water-soluble carbon monoxide releasing molecule (CORM-A1) on reactive oxygen species (ROS) induced by TNF-α in murine intestinal epithelial MODE-K cells.

9. L. THYRION, R. RAEDT, E. YDENS, S. JANSSENS, P. VAN LOO, J. KIPS, J. PORTELLI, B. LAMBRECHT, A. MEURS, K. VONCK, P. BOON (UGent, UAntwerpen)
Allopurinol reduces seizure severity and the inflammatory response in a mouse model for status epilepticus: a potential role for uric acid in ictogenesis.

10. A. GRIMONPREZ, R. RAEDT, J. DELBEKE, L. DE TAEYE, K. VONCK, P. BOON (UGent)
Dose-dependent laryngeal muscle evoked potentials as an indicator of effective vagus nerve stimulation.

11. A. GALLO, B. MICHOT, J. DAMBLON, E. HERMANS, R. DEUMENS (UCLouvain)
Mirror-image pain after peripheral nerve injury of mice lacking vasoactive intestinal peptide.

12. C. DUFEYS, G. NOPPE, P. BUCHLIN, N. MARQUET, D. CASTANARES-ZAPATERO, N. HERMIDA, C. BOUZIN, B. VIOLLET, L. BERTRAND, J.L. BALLIGAND, J.L. VANOVERSCHELDE, C. BEAULOYE, S. HORMAN (UCL, INSERM, Paris, France)
Reduced scar maturation and contractility lead to exaggerated left ventricular dilation after myocardial infarction in mice lacking AMPKalpha1.

13. F. MAILLEUX, R. GÉLINAS, B. DEMEULDER, A. GINION, L. HUE, J. HAMMOND, J.L. BALLIGAND, J.-L. VANOVERSCHELDE, C. BEAULOYE, S. HORMAN, L. BERTRAND (UCLouvain)
A769662, a specific AMP-activated protein kinase (AMPK) activator, prevents cardiomyocyte hypertrophy independently of the already identified AMPK downstream targets.

14. I. JADOT, A.E. DECLEVES, V. COLOMBARO, B. MARTIN, V. VOISIN, I. HABSCH, E. DE PREZ, J. NORTIER, N. CARON (UNamur, ULBruxelles)
Enhanced nitric oxide production ameliorates acute kidney injury in experimental aristolochic acid nephropathy.

15. B. BASELET, A. AERTS, A. JANSSEN, A. MICHAUX, R. QUINTENS, R. BENOTMANE, D. LOWE, K. RAJ, P. SONVEAUX, S. BAATOUT (SKC Mol, UCLouvain, AGIR, Oxfordshire, United Kingdom).
Endothelial cell reprogramming following exposure to ionizing radiations.

16. D. HOORELBEKE, E. DECROCK, M. DE BOCK, C. VANDEVOORDE, B. DESCAMPS, H. THIERENS, C. VANHOVE, L. LEYBAERT (UGent)
Role of connexin channels, calcium and ROS in radiation induced bystander effects.

17. M. BALTEAU, A. VAN STEENBERGEN, A.D. TIMMERMANS, C. DESSY, G. BEHETS-WYDEMANS, N. TAJEDDINE, D. CASTANARES-ZAPATERO, P. GILON, J.L. VANOVERSCHELDE, S. HORMAN, L. HUE, L. BERTRAND, C. BEAULOYE (UCLouvain)
AMPK activation by glucagon-like peptide-1 prevents NADPH oxidase activation induced by hyperglycemia in adult cardiomyocytes.

18. S. SMOLDERS, S.M. SMOLDERS, N. SWINNEN, P. LEGENDRE, J.-M. RIGO, B. BRÔNE (UHasselt, INSERM, Paris, France)
Can embryonic microglia bridge the gap between maternal immune activation and neuropsychiatric disorders?

19. S.M. SMOLDERS, A. AVILA, N. SWINNEN, T. STRUYS, I. LAMBRICHTS, M. AMELOOT, N. HELLINGS, J-M RIGO, B. BRÔNE (UHasselt, ULiège, UPMC Paris France, KULeuven)
Migration of microglia in the embryonic neocortex: cellular and molecular interactions

20. D. DELUYKER, V. FERFERIEVA, J.-M. RIGO, V. BITO (UHasselt)
sRAGE limits cardiac dysfunction induced by advanced glycation end products in rats.

21. K. PHILIPPAERT, R. VENNEKENS (KULeuven)
New pharmacology exhibits an antihyperglycaemic and insulinotropic effect in mice through potentiation of TRPM5.

22. V. VAN HÉE, J. PÉREZ-ESCUREDO, P. E. PORPORATO, T. COPETTI, P. SONVEAUX (UCLouvain)
Because of their oxidative avidity for NADH, oxidative tumor cells are resistant to lactate-induced NF-κB activation.

23. M. SBOARINA, F. LEFRANC, P. SONVEAUX (UCLouvain, ULBruxelles)
Temozolomide resistance in glioblastoma: a metabolic culprit?

Oral Communications

14.00-14.15 S. NULLENS, D.M. SCHRIJVERS, J.G. DE MAN, P.A. PELCKMANS, B.Y. DE WINTER (UAntwerpen)
In vitro effects of GTS-21, a selective alpha7 nicotinic acetylcholine receptor agonist, on cytokine release in bone marrow derived macrophages and conventional dendritic cells.

14.15-14.30 J. DAMBLON, N. DINJU, A. GALLO, G. TACCOLA, B. MICHOT, B. BOSIER, E. HERMANS, R. DEUMENS (UCLouvain, SISSA).
Does distinct immune signalling dictate a painful outcome after peripheral nerve injury?

14.30-14.45 G. MORELLI, A. AVILA, R.J. HARVEY, B.BRÔNE, L. NGUYEN, J.-M. RIGO (UHasselt, ULiège, UCL School of Pharmacy, London UK)
Disruption of cortical circuitry development in glycine receptor alpha 2 knockout mice.

14.45-15.00 G. JACOBS, M. KECSKES, I. MATHAR, R. VENNEKENS (KULeuven)
Role of TRPM4 in ventricular action potential and conduction through the heart.

15.00-15.15 N. ZANOU, L. MONDIN, F. SEGHERS, I. DUFOUR, Y. IWATA, S. WAKABAYASHI, O. SCHAKMAN, M. DE CLIPPELE, N. TAJEDDINE, P. GAILLY (UCLouvain, Univ. Osaka, Japan)
Osmosensation in TRPV2 dominant negative –expressing skeletal muscle fibres.

15.15-15.30 V. L. PAYEN, P. E. PORPORATO, J. PÉREZ-ESCUREDO, C. J. DE SAEDELEER, P. DANHIER, T. COPETTI, S. DHUP, M. TARDY, T. VAZEILLE, C. BOUZIN, O. FERON, C. MICHIELS, B. GALLEZ, P. SONVEAUX (UCLouvain, UNamur)
A mitochondrial switch promotes tumor metastasis.

15.30-15.45 C. DRESSEN, B. SCHWALLER, G. VEGH, P. LEBRUN, P. LYBAERT (ULBruxelles, Univ. Fribourg, Schweiz)
Expression of EF-hand calcium-binding proteins in sperm cells.

15.45-16.00 K. HELD, T. KICHKO, K. DE CLERCQ, H. KLAASSEN, R. VAN BREE, J.C. VANHERCK, A. MARCHAND, P. REEH, P. CHALTIN, T. VOETS, J. VRIENS (KULeuven, Univ. Erlagen-Nuremberg, Germany)
Activation of TRPM3 by a potent synthetic ligand reveals a role in peptide release.

16.00-16.15 B.I. TOTH, J. VRIENS, D. GHOSH,T. VOETS (KULeuven)
Phosphatidylinositols regulate the nociceptor ion channel transient receptor potential melastatin 3 (TRPM3).

Coffee - Tea

16.30 Awarding of prizes for best poster and oral presentation.

ABSTRACTS

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

O-01 (10.45-11.00)

IMPLICATION OF MICRORNA 199A-3P IN VASCULAR FUNCTION : MODULATION OF THE NOS/NO PATHWAY

V. Joris[1], E. Leon Gomez[1], I. Lobysheva[1], D. Catalucci[2], J.L. Balligand[1], C. Dessy[1]

[1] Pole of Therapeutic Pharmacology, Institute of Experimental and Clinical Research (IREC), University of Louvain, Brussels, Belgium. [2] Instituto Humanitas, CNR/IRGB/UOS, Rozzano, Milan, Italy.

MicroRNAs (miR) are highly conserved small non-coding RNAs that regulate gene expression by promoting the degradation of mRNA or by repressing protein translation. MiR 199a-3p has been mainly implicated in proliferation and cell survival but more recent data obtained in our laboratory proposed a role in cardiovascular functions. This study aimed to evaluate how miR199a-3p modulates endothelial function by identifying its specific targets in mice vessels and endothelial cells. Mice were treated with miR199a-3p inhibitor (antagomiRs) or with control (scramble), the contractile profile and endothelial function of aorta and mesenteric arteries were evaluated ex vivo after 30 days. NO release was measured by EPR in venous blood. Bovine Aortic Endothelial Cells (BAEC) were transfected with a miR199a-3p inhibitor (LNA) and then harvested 48h and 72h after treatment. eNOS expression was measured by Western Blotting, eNOS activation was determined by analyzing its phosphorylation on serine1177 (activation site) and on threonine495 (inactivation site). mTOR, p70S6K, and SIRT1 were also analyzed using Western blotting. Vessels from mice treated with antagomirs directed against miR199a-3p showed a larger NO-dependent relaxation (72.6±2.8% vs 37.0±5.6% and 37.4±4.2%; n=8-12, $p<0.001$) compared with vessels from saline or scrambled treated animals. HbNO measured by EPR in venous blood from mice treated miR199a3p antagomirs was significantly increased compared with controls (323±59% vs 100±17% and 73±31%; n=3-11, $p<0.05$) confirming a role of miR199a-3p in endothelial function.In order to identify miR199a-3p specific targets, BAEC were treated with specific LNA. Efficacy of transfection was confirmed by measuring the expression of mTOR, a known target of miR199a-3p. Total expression of eNOS was similar in cells treated or not with the LNA. However, LNA treated cells showed a significant increase of eNOS phosphorylation at serine 1177 and a tendency to decrease the phosphorylation status of threonine 495. SIRT1 expression does not seem to be affected by the treatment. Our results suggest that the inhibition of miR199a-3p improves endothelial function through a modulation of the NOS/NO pathway. miR199-3p inhibition modulates eNOS phosphorylation status without affecting its total expression. Further work is needed to identify miR199a-3 potential targets responsible of this modulation.

O-02 (11.00-11.15)

ENDOTHELIAL DYSFUNCTION INDUCED BY N-3 POLYUNSATURATED FATTY ACID DEPLETION IS IMPROVED BY A PREBIOTICS SUPPLEMENTATION

E. Catry[1], B.D. Pachikian[1], A.M. Neyrinck[1], P.D. Cani[1], C. Dessy[2], N.M. Delzenne[1]

[1] Metabolism and Nutrition Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium, [2] Pole de Pharmacologie and Thérapeutiques, Institut de Recherche Expérimentale et Clinique, Université catholique de Louvain, Brussels, Belgium

Nutritional disorders such as the depletion in n-3 polyunsaturated fatty acids (PUFA) increase the risk to develop cardiovascular diseases, endothelial dysfunction being an early key marker. We have previously demonstrated that metabolic alterations associated with n-3 PUFA depletion in mice are improved by modulation of gut microbiota. The present work focusses on the potential impact of gut microbiota (and its modulation by a supplementation with non-digestible fructans as prebiotics) on the endothelial function in a n-3 PUFA depleted-ApoE knock-out mice model. Nine-weeks-old C57Bl/6J (WT) and ApoE$^{-/-}$ (KO) mice were fed a n-3 PUFA depleted-diet (DEF) for 12 weeks. For the last fifteen days, WT and KO mice were or not supplemented with prebiotics (Orafti®P95, 0.25gr/d/mouse) (PRE). The endothelial function was evaluated on second and third order mesenteric micro-arteries, isolated and mounted on a wire myograph (Danish Myo Technology). After normalization, arteries were preconstricted with high KCl-solution, the endothelial-dependent relaxation was evaluated by an addition of increasing doses of acetylcholine. Analysis of resting parameters showed that supplementation with prebiotics in WT DEF and KO DEF mice (WT KO DEF/KO DEF PRE) is associated with a larger normalized diameter of the mesenteric micro-arteries and that these vessels develop an increased basal tone in comparison to vessels from non-supplemented mice (WT DEF/KO-DEF). The contractile profile is also modified by the PRE supplementation as KO DEF PRE micro-arteries contracted significantly more than other groups, in the presence of high KCl-solution. As expected for a dyslipidemic mice model, micro-arteries from KO DEF mice present an endothelial dysfunction after 12 weeks of n-3 PUFA depletion as attested by a significant decrease of endothelial-dependent relaxation in comparison to WT DEF arteries. Interestingly, PRE supplementation for only fifteen days is able to improve the endothelial function by restoring the endothelial-dependent relaxation to muscarinic stimulation in KO DEF mice. For the first time we highlight the potential link between endothelial function and gut microbiota, and point out fructan-type prebiotics as a potential therapeutic tool. Our results argue in favor of a potential role of gut microbiota in the control of cardiovascular risks. The modified resting parameters suggest a positive effect on muscular remodeling leading to an increased blood perfusion, the results on endothelial function evoked an important involvement of the nitric oxide pathway.

O-03 (11.15-11.30)

EFFECT OF NEUREGULIN-1 ON LEFT VENTRICULAR DIASTOLIC FUNCTION IN ECCENTRIC AND CONCENTRIC VENTRICULAR REMODELING

L. Vandekerckhove[1], A-S. Hervent[1], N. Hamdani[2], V.F. Segers[1], W.A. Linke[3], W.J. Paulus[2], G.W. De Keulenaer[1]

[1]Laboratory of Physiopharmacology, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium, [2]Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center Amsterdam, Van der Boechorststraat 7, 1081 BT Amsterdam, the Netherlands, [3]Department of Cardiovascular Physiology, Institute of Physiology, Ruhr University, D-44780 Bochum, Germany

Neuregulin-1 (NRG-1) is a cardio-protective and cardio-regenerative protein, secreted by cardiac endothelial cells. It acts through activation of ErbB receptor tyrosine kinases on target cells. Together with nitric oxide and other factors, it contributes to the endothelium-controlled autoregulatory system of the heart. Recombinant human NRG-1 (rhNRG-1) is currently under investigation for the treatment of systolic heart failure in several clinical trials. The effects of rhNRG-1 on left ventricular (LV) diastolic function and myocardial stiffness, however, have been poorly studied. In this study, we examined the effect of rhNRG-1 on LV diastolic compliance and its determinants in different settings. RhNRG-1β or vehicle was administrated ($20\mu g.kg^{-1}.day^{-1}$, i.p., 5 days/week) in 3 groups: (i) healthy C57Bl/6 mice (n=56), (ii) C57Bl/6 mice with concentric LV remodeling (n=59), induced by Ang II (osmotic mini-pumps, 1000 $ng.kg^{-1}.min^{-1}$, 4 weeks) and (iii) Apolipoprotein E deficient mice with eccentric LV remodeling (n=66) (streptozotocin (STZ) -induced type 1 diabetes, 14 weeks follow-up). *In vivo*, LV diameters and LV diastolic compliance (slope EDPVR) were quantified by echocardiography and LV pressure-volume recordings. *Ex vivo*, LV collagen volume fraction (CVFR) and cardiomyocyte diameters (MyoDia) were quantified by immuno-histochemistry. Stiffness of isolated skinned cardiomyocytes, titin composition (N2B versus N2BA isoform) and titin phosphorylation was quantified. Activation of cardiac ErbB2 and ErbB4 receptors by rhNRG-1β was verified in each model. In the normal heart, rhNRG-1β did not affect LV diameters, LV diastolic compliance and cardiomyocyte stiffness both in acute (30 min) and in chronic (14 weeks) experiments. In Ang II-treated mice, rhNRG-1β prevented the development of concentric LV hypertrophy, cardiomyocyte hypertrophy (MyoDia 13,17 vs 17,25 µm, $p < 0,01$), cardiomyocyte stiffening, titin dephosphorylation and shift towards the N2BA isoform, LV fibrosis (CVFR 0,056 vs 0,152 FR%, $p < 0,05$) and LV stiffening (slope EDPVR 0, 396 vs 0,586 mmHg/µL, $p < 0,05$). In type 1 diabetic mice, rhNRG-1β prevented LV dilation (LV end diastolic diameter 11,30 vs 13,57 µm, $p < 0,001$), LV hypercompliance (slope EDPVR 0,473 vs 0,176 mmHg/µL, $p < 0,05$), cardiomyocyte stiffening, titin dephosphorylation and titin shift towards the N2BA isoform, but had no effect on LV fibrosis. RhNRG-1 does not change LV structure or LV diastolic compliance of the healthy heart. By contrast, rhNRG-1 prevents LV hypertrophy, myocardial fibrosis and LV stiffening in Ang II -induced hypertension, and LV dilation and impaired LV diastolic function in type 1 diabetes. During both concentric and eccentric LV remodeling, titin shifts towards the N2BA isoform, titin dephoshorylates and cardiomyocytes stiffen. NRG-1 prevents these effects in both types of LV remodeling. In conclusion, rhNRG-1 has, besides its previously recognized effects on LV systolic function, beneficial and protective effects on LV compliance, LV fibrosis and cardiomyocyte stiffness, which attribute to unique therapeutic opportunities of ErbB4 stimulation during LV remodeling.

O-04 (11.30-11.45)

SODIUM-GLUCOSE CO-TRANSPORTERS (SGLT) IN THE HEART. CONTRIBUTION OF SGLT-TYPE OF TRANSPORT IN HYPERGLYCEMIA-INDUCED SIGNALING PATHWAY IN ADULT CARDIOMYOCYTES

A. Van Steenbergen, M. Balteau, JL. Vanoverschelde, L. Hue, S. Horman, L. Bertrand, C. Beauloye

Université catholique de Louvain (UCL), Institut de Recherche Expérimentale et Clinique (IREC), Pôle de Recherche Cardiovasculaire, Brussels, Belgium

Exposure to high glucose results in toxic effects in heart and cardiomyocytes. Our group has demonstrated that hyperglycemia (HG) stimulates reactive oxygen species (ROS) production through NOX2 activation, the major isoform of NADPH oxidase in cardiomyocytes. NOX2 activation is independent of glucose metabolism but results from glucose transport through a co-transport sodium-glucose (SGLT). Seven SGLT's isoforms have been described (SGLT1 to 6 and SMIT1) but their expression in heart and especially in cardiomyocytes remains elusive. The aim of this work is to investigate the expression of the major SGLT isoform in heart and cardiomyocytes and identify the isoform responsible for glucotoxicity. Expression of different SGLT's isoforms was assessed by RT-PCR in cardiomyocytes and intact hearts from mouse, rat and human. The study of the contribution of each isoform to glucotoxicity is based on the substrate specifity of all these SGLT isoforms. Whereas glucose and αMDG are substrates for all SGLT, galactose is transported by SGLT1 and SGLT4, mannose by SGLT4, 1DOG by SGLT3b and myo-inositol by SMIT isoforms (SGLT6 and SMIT1). Three isoforms are expressed in heart and cardiomyocytes of mice and rats: SGLT1, SGLT3b and SMIT1. Same isoforms are express in human heart. In all species, SGLT3 expression is marginal. SGLT4 is only express in rat heart. Incubation of adult rat cardiomyocytes with 16mM galactose does not activate NOX2. By contrast, incubation with 16mM of myo-inositol completely reproduces hyperglycemic effects. Indeed, it favors p47phox translocation inducing NOX2 activation and stimulates ROS production. This ROS production is blocked by a NOX2 specific inhibitor (gp91dstat). Similar observation was perfomed in mice cardiomyocytes. In this study, we provide evidence that isolated cardiomyocytes and heart express SGLT1 and SMIT1. Furthermore, myo-inositol, transported by SMIT1 mimicked toxic effects of hyperglycemia, indicating that this transporter is implicated in glucotoxicity.

O-05 (11.45 -12.00)

ROLE OF AQP1 IN THE FUNCTION AND CARDIOVASCULAR REMODELING

V. Montiel, E. Leon Gomez, I. Lobysheva, H. Esfahani, C. Bouzin, M. Romero Perez, O. Devuyst, C. Dessy, J.L. Balligand

Pole de Pharmacologie et Therapeutique, et de Nephrologie, IREC, Université Catholique de Louvain, Bruxelles, Belgique.

Aquaporins (AQPs) are transmembrane proteins that play a key role in the transport of transcellular water, especially in situations of metabolic stress. However, their expression and their role in cardiac and vascular tissue are not characterized. We examined the expression of different AQPs in cardiac and vascular cells and have focused on AQP1, the major isoform involved in the trans endo/epithelial transport of water and analyzed its specific role in the cardiovascular homeostasis in AQP1 knock-out mice (vs WT littermates). We found essentially four aquaporins in heart tissue (AQP1 -4 - 7 - 8) and two in aortic and mesenteric vessels (AQP1 - AQP7) in WT mice. AQP1 was expressed in endothelial as well as cardiac and vascular muscle cells and co-segregated with caveolin-1. AQP1 knockout mice exhibit a significant microcardia with reduction of the transverse dimension of myocytes without modification of the capillary density per myocyte. Compared to the WT group, AQP1 KO mice have a significantly lower blood pressure which is not due to a negative water balance or impairment of the autonomic nervous system. The endothelial EDHF or NO-dependent relaxation were unchanged in vessels (ex vivo analyses). However, AQP1 KO mesenteric vessels exhibited an increase in endothelial prostanoids-dependent relaxation, together with increased expression of COX-2 in the same extracts. This increase was abolished by indomethacin (COX inhibitor), reinforcing the causal role of COX2. COX2 expression is unchanged in the myocardium. Hemodynamic analyses of mice under chronically neuro-hormonal pro-hypertrophic stimulation (Angiotensin II) showed an increase in the pressure profile in both genotypes but a significant difference in terms of left ventricular remodeling in the two groups. The transverse dimension of myocytes of AQP1 KO mice increases under stimulation but so much smaller compared to the WT mice on the same pro hypertrophic stress. We observe the same trend in the study of fibrosis, a marker of left ventricular remodeling. We conclude that AQP1 does not regulate the endothelial EDHF or NO-dependent relaxation ex vivo or in vivo but its deletion decreases baseline blood pressure together with increased prostanoids-dependent relaxation in resistance vessels. This genetic deletion of AQP1 is associated with microcardia with smaller cardiac myocytes unrelated to perturbed angiogenesis. Surprisingly, we observed a significantly lower left ventricular remodeling after chronically neurohormonal stimulation by Angiotensin II in the AQP1 KO mice versus WT. This could generate interest for new inhibitors of AQP1 and their use in the treatment of hypertrophic cardiac remodeling.

O-06 (12.00 -12.15)

THE ROLE OF CONNEXIN43 HEMICHANNELS IN LIMBIC SEIZURES

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Nowadays, the contribution of astrocytic connexin43 (Cx43) hemichannels (HCs) to brain functioning and dysfunctioning hasn't been fully elucidated, mainly because of the paucity of appropriate tools allowing selective targeting of Cx43-HCs. An exception is made for Cx43 mimetic peptides, which inhibit Cx43-HCs but not gap junctions in *in vivo* settings. In this study, we want to screen Cx43-HCs as novel antiepileptic drug targets through the *in vivo* screening of a newly developed and selective Cx43-HC-inhibitor (TAT-Gap19 and analogues) in different acute models of epileptic seizures. In the acute rat pilocarpine model, we found that an intrahippocampal perfusion of 12.5 µM TAT-Gap19 significantly reduced the seizure-related behavioural movements induced by pilocarpine. We still have to determine the concentration of glutamate and D-serine in the dialysate samples which were collected during the previously described experiment in view of the hypothesis that Cx43-HCs might release these gliotransmitters *in vivo*. In the six Hertz (6 Hz) mouse model, an electrical model of therapy-resistant seizures, we found that an intracerebroventricular injection of 1 nmol TAT-Gap19 and an intraperitoneal injection of 25 mg/kg TAT-Gap19 leads to a significant decrease in the total mean seizure duration. These results suggest that Cx43-HCs play a role in seizure generation. It is now of high importance to screen TAT-Gap19 (and its BBB permeable analogues) in chronic models for epilepsy in order to unravel the role these channels may play in epilepsy and epileptogenesis.

O-07 (12.15 -12.30)

STRIATOPALLIDAL NMDAR IS IMPLICATED IN GOAL DIRECTED BEHAVIOR, HABITUATION AND AMPHETAMINE SENSITIZATION

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Basal ganglia - which consist of several different *nuclei* - are critically involved in building sequences of behavior into meaningful, goal-directed repertories. The *striatum* – made up of the *caudate nucleus* and the *putamen* – receives most of the cortical input to the basal ganglia. Reward-based learning studies suggest that neural activity in the *striatum* changes during learning. In addition, the leading model for motor disorders such as Parkinson's and Huntington's diseases shows that the basal ganglia have distinct pathways that compete with each other functionally to release movement (the direct pathway) or to inhibit movement (the indirect pathway). Although NMDA receptor (NMDAR)-dependent long-term potentiation has been observed in the *striatum*, NMDAR involvement in sensitization and learning remains unclear as well as its respective functions in both pathways. In order to examine the role of striatal NMDAR of indirect pathway, we used selective inactivation of Grin1, the gene encoding the obligatory NR1 subunit of NMDARs, in medium spiny neurons (MSN) of the inhibitory indirect pathway (D2n). Our results show that deleting the NR1 subunit of the NMDAR specifically in the D2n, which virtually suppressed NMDAR mediated currents, resulted in reduced basal locomotor activity and object habituation, delayed instrumental learning and attenuated amphetamine sensitization. Those behavioural data were consistent with ours electrophysiological recordings showing alteration of passive membrane parameters and hyperexcitability of D2n. In addition, confocal imaging followed by computer 3D reconstruction reveal dendritic arborisation impairments and reduced spine density. These data indicated that unbalanced loss of NMDAR signalling in D2n alone disrupts orchestrate activities across all basal ganglia nuclei in a cell-type-specific manner.

O-08 (14.00 -14.15)

IN VITRO EFFECTS OF GTS-21, A SELECTIVE ALPHA7 NICOTINIC ACETYL-CHOLINE RECEPTOR AGONIST, ON CYTOKINE RELEASE IN BONE MARROW DERIVED MACROPHAGES AND CONVENTIONAL DENDRITIC CELLS.

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The 'vagal anti-inflammatory pathway', is a recently discovered negative feedback mechanism that subdues inflammation by inhibiting the release of proinflammatory cytokines from macrophages. This pathway is presumably mediated via the efferent vagal nerve, which ultimately results in the binding of acetylcholine on the alpha7 nicotinic acetylcholine receptor (α7nAChR) on macrophages, thus dampening cytokine release. The effect of α7nAChR-agonists on other different types of immune cells is currently unknown. We aimed to study the effects of GTS-21, a selective α7nAChR-agonist, on the *in vitro* release of the proinflammatory cytokines IL-6 and TNF-α, and of the anti-inflammatory IL-10 in bone marrow derived macrophages (BMDM) and conventional dendritic cells (BMcDC). BMDM were obtained by plating triplicates of 0.5 x 10^6 harvested bone marrow cells from three Swiss OF-1 mice in 1 mL of full medium with M-CSF in sterile tissue culture coated plates and incubating them for seven days at 37°C (95% O_2, 5% CO_2). At day 7, cultures were stimulated with 100 ng/mL LPS and 100 U/mL IFN-γ in order to polarize the BMDM towards a proinflammatory state (M1 macrophages), while simultaneously adding GTS-21 (10 µM or 100 µM) or vehicle. BMcDC were obtained in a similar way and cultured in full medium with GM-CSF. At day 7, 1 µg/mL LPS and 1000 U/mL IFN-γ were added as a maturation stimulus in the presence of GTS-21 (10 µM or 100 µM) or vehicle. After 24h supernatants were collected and stored at -80°C until analysis with *Cytometric Bead Array* (CBA) for IL-6, TNF-α and IL-10, and with ELISA for IL-12p70 in case of BMcDC. Incubating M1 macrophages with 10 and 100 µM GTS-21 dose-dependently reduced the release of TNF-α with 59% and 92% respectively ($p<0.001$) compared to vehicle, and reduced the release of IL-6 with respectively 26% and 59% ($p<0.001$). The release of IL-10 was not altered. Increasing concentrations of GTS-21 did also dose-dependently decrease the release of TNF-α from BMcDC with 47% and 57% ($p<0.001$), and of IL-6 with 13% and 37% respectively ($p<0.001$). Surprisingly, the release of IL-10 from BMcDC was significantly upregulated after incubating with 10 µM GTS-21, and once again normalized when adding 100 µM GTS-21 ($p<0.001$). IL-12p70 secretion from BMcDC was significantly inhibited by adding 10 µM or 100 µM GTS-21 by 55% and 32% respectively ($p<0.001$). Our results demonstrate the presence of the α7nAChR not only on the cell surface of macrophages, but also on the cell membrane of bone marrow derived conventional dendritic cells. The release of proinflammatory cytokines from macrophages as well as dendritic cells was inhibited by GTS-21, a selective α7nAChR-agonist, suggesting a role for the *vagal anti-inflammatory pathway* on dendritic cells in inflammatory conditions.

O-09 (14.15 -14.30)

DOES DISTINCT IMMUNE SIGNALLING DICTATE A PAINFUL OUTCOME AFTER PERIPHERAL NERVE INJURY?

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Nerve lesions in man are frequently, but not always associated with neuropathic pain. At variance, widely used animal models of nerve lesions are typically characterized by the development of pain hypersensitivity in each individual animal. We here report on a new rat model in which – despite identical nerve lesions – only about 30% of rats develop mechanical pain hypersensitivity, while the majority of rats does not. The objective of our study was to further our understanding of how these two opposing responses are regulated on a cellular and/or molecular level. Here, attention was focused on microglia and G-protein signalling as these targets are of key importance to neuroinflammation, a process heavily implicated in mediating neuropathic pain. On the basis of immunohisto-chemistry of the lumbar spinal cord, we evidenced that the macrophage/microglial marker Iba-1 was differentially expressed in the dorsal horn of painful and non-painful nerve injured animals. This difference was particularly noteworthy for the deep lateral part of the dorsal horn. Our most recent data further indicate that painful animals show a different expression of regulators of G-protein signalling. We conclude that the painful response to nerve lesions may depend on distinct immune responses that involve differential regulation of G-protein signalling. Our new animal model could be of great value to gaining new insights into the mechanisms surrounding the inception of neuropathic pain, thereby revealing therapeutic targets for clinical treatment.

O-10 (14.30 -14.45)

DISRUPTION OF CORTICAL CIRCUITRY DEVELOPMENT IN GLYCINE RECEPTOR ALPHA 2 KNOCKOUT MICE

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Previous studies in our laboratory have revealed an important role of the strychnine sensitive glycine receptors (GlyRs) in the cerebral cortex during neurogenesis. Specifically, the absence of GlyR alpha 2 subunits in a genetically disrupted mouse model leads to interneuronal migration defects. In order to evaluate the long-term consequences of these early defects, we examined the role of GlyRs during postnatal development of the cerebral cortex. Remarkably, GlyR alpha 2 knockout mice displayed a significant reduction in the number of parvalbumin positive interneurons at postnatal day 14. Moreover, morphological and synaptic defects were assessed in the cerebral cortex of knockout mice by performing 3D reconstruction of single neurons and by whole-cell patch-clamp recordings. Morphological studies of biocytin-filled dlx-GFP expressing interneurons revealed altered growth of dendrites and of spine generation. Furthermore, whole-cell recordings showed a substantial increase in the frequency of spontaneous post-synaptic currents (sPSCs) in cortical interneurons of knockout mice together with a decrease of inhibitory post-synaptic currents (IPSCs) and an increase of excitatory postsynaptic currents (EPSCs). These preliminary findings are consistent with the hypothesis that GlyRs might have an important role during circuit development in the cerebral cortex.

O-11 (14.45 -15.00)

ROLE OF TRPM4 IN VENTRICULAR ACTION POTENTIAL AND CONDUCTION THROUGH THE HEART

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TRPM4 is a calcium-activated, but calcium-impermeable, non-selective cation channel and plays an important role in different calcium-dependent cell functions in many cells including mast cells, chromaffin cells and neurons. Trpm4 expression was also shown in atrial and ventricular cardiac tissue, but the physiological function in the heart is still largely unresolved. Recently, gain-of-function mutations in the Trpm4 gene have been associated with conduction disorders, such as Progressive Familial heart Block Type 1, Right Bundle Branch Block and Brugada Syndrome. In this study, the contribution of TRPM4 to the ventricular action potential was established and its role in cardiac conduction in the whole heart was evaluated by use of Trpm4 knockout (Trpm4KO) mice. Patch-clamp experiments and membrane potential measurements showed that TRPM4 is activated during repolarisation of the ventricular action potential and that the duration of the action potential in cardiomyocytes of Trpm4KO mice was significantly shorter compared to wild-type (WT) cardiomyocytes. Since TRPM4 influences action potential and increased expression of Trpm4 (due to gain-of-function mutations) is associated to conduction disorders, we further investigated the effect of Trpm4 loss on signal conduction through the heart. Therefore, electrocardiographic intervals were determined in WT and Trpm4KO mice. In conscious mice, 2-lead surface ECG was measured via telemetry. RR, PR, QRS and QTc intervals were calculated and no differences were found between the 2 genotypes. To investigate impulse propagation and possible conduction abnormalities more in detail, we performed intracardiac electrophysiological studies on WT and Trpm4KO mice. Atrial, His and ventricular potentials were analyzed in intracardiac electrograms and atrial-His and His-ventricular intervals were equal in WT and Trpm4KO mice. Taken together, we can conclude that loss of Trpm4 results in shorter ventricular action potentials, but that this has no influence on impulse propagation and conduction properties through the heart.

O-12 (15.00 -15.15)

OSMOSENSATION IN TRPV2 DOMINANT NEGATIVE –EXPRESSING SKELETAL MUSCLE FIBRES

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Increased plasma osmolarity induces intracellular water depletion and cell shrinkage (CS) followed by activation of a regulatory volume increase (RVI). In skeletal muscle, the hyperosmotic shock-induced CS is accompanied by transverse tubule (TT) dilatation inducing a small membrane depolarisation and a release of Ca^{2+} from intracellular pools (sarco-endoplasmic reticulum). Here we investigated the possible involvement of TRP ion channels in the RVI response to a hyperosmotic shock. We observed that both hyperosmotic shock-induced Ca^{2+} transients and RVI were inhibited by Gd^{3+}, by ruthenium red and by the *Grammostola spatulata* GsMTx4 toxin, three inhibitors of mechanosensitive channels. The response was also completely absent in muscle fibres overexpressing a dominant negative mutant of TRPV2 ion channel (TRPV2-DN), suggesting the involvement of TRPV2 channel or of a TRP isoform susceptible to heterotetramerize with TRPV2. The release of Ca^{2+} induced by hyperosmotic shock was increased after pretreatment with cannabidiol and decreased after pretreatment with tranilast, suggesting a role for TRPV2 channel itself. The RVI subsequent to CS has been shown to require the sequential activation and phosphorylation of SPAK (STE20/SPS1-related proline/alanine-rich kinase) and of NKCC1, a Na^+, K^+ and Cl^- cotransporter allowing ions entry and osmotic water driving. We observed that in fibres overexpressing TRPV2-DN as well as in fibres in which Ca^{2+} transients were artificially abolished by the presence of BAPTA, the level of P-SPAK^{Ser373} in response to hyperosmotic shock was largely reduced, suggesting a modulation of SPAK phosphorylation by intracellular Ca^{2+}. Surprisingly, muscles treatment with bumetanide, a specific inhibitor of NKCC1 also decreased P-SPAK^{Ser373}, suggesting a positive amplification loop between NKCC1, $[Ca^{2+}]_i$ and P-SPAK^{Ser373}. In summary, our results show that TRPV2 is involved in osmosensation in skeletal muscle fibres, acting in concert with P-SPAK-activated NKCC1.

O-13 (15.15 -15.30)

A MITOCHONDRIAL SWITCH PROMOTES TUMOR METASTASIS

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Cancers evolve a subpopulation of tumor cells that metabolically rely on glycolysis uncoupled from oxidative phosphorylation irrespectively of oxygen availability (aerobic glycolysis). Given that most metastases are abnormally avid for glucose (which is the rationale for their clinical detection using FDG-PET) and because clinical data show a positive correlation between lactate production and tumor metastasis, we reasoned that cells performing aerobic glycolysis could constitute a population of metastatic progenitor cells that would remain glycolytic in the blood stream. We found a different metabolic phenotype, though. Indeed, using serial rounds of in vitro selection of highly invasive tumor cells (starting from wild-type SiHa human cervix adenocarcinoma cells) and in vivo selection of supermetastatic tumor cells (starting from B16-F10 mouse melanoma cells), we identified a mitochondrial switch corresponding to an overload of the TCA cycle with preserved mitochondrial functions (including ATP production) but increased mitochondrial superoxide production. The switch provided a metastatic advantage which was phenocopied by moderate OXPHOS inhibition associated with mild mitochondrial superoxide increase. Thus, two different events, OXPHOS overload or moderate OXPHOS inhibition, promote superoxide-dependent tumor cell migration, invasion, clonogenicity, and metastasis; demonstrating the central role of mitochondrial superoxide generation in the pathogenesis of metastasis. Consequently, we report that mitochondria-specific superoxide scavenging (using mitoTEMPO or mitoQ) inhibits metastatic dissemination from primary mouse and human tumors, which opens a new avenue for the therapeutic prevention of tumor metastasis.

O-14 (15.30 -15.45)

EXPRESSION OF EF-HAND CALCIUM-BINDING PROTEINS IN SPERM CELLS

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Calcium-dependent processes (motility, acrosomal reaction) are known to be involved in the control of sperm fertility. The EF-Hand Calcium-Binding Proteins (EFCaBP) take part in calcium buffering in many cell types but these proteins have been poorly studied in sperm cells. The aim of the present study was to characterize the expression and the possible role(s) of these EFCaBP in murine spermatozoa. Our investigations compared epididymal sperm cells from Wild Type (WT), Calretinin knock-out ($CR^{-/-}$), Calbindin D-28k knock-out ($CB^{-/-}$) and Calretinin/Calbindin D-28k/Parvalbumin knock-out ($CR^{-/-}CB^{-/-}PV^{-/-}$) mice. The labelling of Calretinin (CR) and Calbindin D-28k (CB) was observed by immunofluorescence in the sperm head and flagellum from WT and single knock-out mice. Detection of these proteins was still observed on the head but not on the flagellum of $CR^{-/-}CB^{-/-}PV^{-/-}$ spermatozoa. Western blotting confirmed the expression of CR and CB in WT sperm extract and the absence of CR and/or CB in knock-out extracts. The cerebellum was used as control tissue for the detection of CR and CB by immunofluorescence and Western blotting. Functional studies were performed comparing WT to $CR^{-/-}$ sperm. C.A.S.A. (Computer Assisted Sperm Analysis) measurements of sperm motility showed an increase in the percentage of hyperactivated sperm from WT mice following incubation with NH_4Cl (25mM). This increase appeared more marked in $CR^{-/-}$ sperm. The percentage of induced acrosomal reaction (A-23187 2µM) was also more pronounced in $CR^{-/-}$ spermatozoa. In conclusion, this study documents the presence of Calretinin and Calbindin D-28k in murine spermatozoa. Moreover, preliminary functional studies indicate that EFCaBP might be involved in important sperm physiological processes, such as motility and acrosomal reaction.

O-15 (15.45 -16.00)

ACTIVATION OF TRPM3 BY A POTENT SYNTHETIC LIGAND REVEALS A ROLE IN PEPTIDE RELEASE

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TRPM3 represents a member of the transient receptor potential (TRP) channel superfamily, which was recently identified as a nociceptor channel in the somatosensory system. It was shown to be involved in the detection of noxious heat and the development of heat hyperalgesia. However, the physiological role of TRPM3 in sensory neurons, as well as in other tissues, is still only partially understood due to a lack of potent and selective agonists. In a recent study, we identified the existence of two distinct ion permeation pathways in wild-type TRPM3: 1) the main channel pore and 2) the alternative cation permeation pathway. The alternative pathway could only be opened through combined application of the canonical TRPM3 agonist pregnenolone sulphate (PS) and the antifungal drug clotrimazole (Clt). Here, we present a novel synthetic TRPM3 activator, CIM0216, whose potency greatly exceeds that of PS and which is able to open on its own the two distinct channel pores. Additionally, we show that CIM0216 is capable of 1) evoking robust calcium influxes in TRPM3-expressing trigeminal ganglia (TG) and dorsal root ganglia (DRG) neurons, 2) inducing TRPM3-dependent nocifensive behavior in mice injected with CIM0216 in the hindpaw, and 3) provoking the release of insulin from pancreatic islets and of calcitonin gene-related peptide (CGRP) from sensory nerve terminals. These experiments identify CIM0216 as a powerful tool to investigate the physiological roles of TRPM3 and link TRPM3 activation in sensory nerve endings to neurogenic inflammation.

O-16 (16.00 -16.15)

PHOSPHATIDYLINOSITOLS REGULATE THE NOCICEPTOR ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MELASTATIN 3 (TRPM3)

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TRPM3 is a thermosensitive nociceptor channel involved in the detection of noxious heat. It is activated by the neurosteroid pregnenolone sulphate (PS) and mediates noxious heat sensation and nocifensive behaviour evoked by PS. Recently, we described the opening of an alternative ion permeation pore on the channel which further exacerbates pain sensation. In the current study, we aimed at describing the control of TRPM3 activity by phosphatidylinositol phosphates (PIPs) of the plasma membrane. We applied a combined pharmacological and molecular biological approach and investigated the channel activity by electrophysiological measurements and functional imaging on HEK-293T cells overexpressing a wild-type variant of mouse TRPM3. In inside-out configuration of patch clamp measurements, we found that TRPM3 activity underwent a rapid desensitization following a sudden increase upon excision of the membrane patch. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and adenosine 5`-trisphosphate (ATP) applied to the cytoplasmic side of the excised membrane patches recovered the TRPM3 current. Scavenging the membrane phospholipids or inhibition of the membrane associated phosphatidylinositol kinases (PI-Ks) inhibited the ATP induced recovery, suggesting that ATP induced TRPM3 current recovery depended on the re-synthesis of PIPs in the plasma membrane. Depleting PI(4,5)P2 by using inducible phosphatidylinositol ‘5-phosphatases caused a partial decrease of TRPM3 activity. Testing various PIPs, we found that other PIP2 forms were also able to restore the TRPM3 activity and phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3) was even more effective than PI(4,5)P2. Our results highlight the role of membrane PIPs in the regulation of TRPM3 activity and related sensory processes.

P-01

METABOTROPIC GLUTAMATE RECEPTOR 5 DESENSITIZATION: IMPORTANCE OF PROTEIN KINASE C EPSILON

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Metabotropic glutamate receptors (mGluRs) constitute an unique subclass of G protein-coupled receptors (GPCRs), playing an essential role in regulating neural development and plasticity. Activation of mGluR5 in astrocytes with (S)-3,5-dihydroxyphenylglycine results in Ca^{2+} release which promotes the activation of protein kinase C epsilon (PKCε). Consecutively to repeated and prolonged exposures to this agonist, mGluR5 exhibits a rapid loss of responsiveness and such desensitization is thought to protect against receptor over-stimulation. For several G-protein coupled receptors, PKC is commonly proposed as a key actor in the desensitization process as it promotes the phosphorylation of the receptor and thereby its uncoupling from G proteins. In our study we show that astrocytes display 2 different patterns of mGluR5-mediated Ca^{2+} response that take place either as a peak-plateau profile or as repeated oscillations. Importantly, astrocytes that respond with a peak-plateau profile systematically show desensitization in a concentration-response manner. At variance, astrocytes showing oscillations maintain their responsiveness to the agonist, suggesting the absence of receptor desensitization. Besides, the selective pharmacological inhibition of PKCε was found to convert the oscillatory to a peak-plateau profile and in these conditions, rapid loss of the response was also observed. While previous reports showed the role of PKCε in the oscillatory profile of mGluR5 signalling, we here show that this PKC isoform also participates in the control of mGluR5 desensitization. Based on these data, we here propose that the unique oscillatory profile typically observed for mGluR5 in astrocytes is required to prevent the rapid desensitization of the receptor. In the context of intense glutamate firing, this would help to preserve the glutamate sensing properties of astrocytic mGluR5 which supports the functional adaptation of these glial cells.

P-02

PRESENCE OF THE SWEET TASTE T1R3 RECEPTOR IN HUMAN AND RAT PANCREATIC ALPHA CELLS

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The sweet taste T1R3 receptor was recently found to be expressed in both mouse pancreatic beta cells and MIN6 cells, a glucose-responsive beta cell line. In the latter cells, the artificial sweetener sucralose reproduced the effect of D-glucose in promoting mitochondrial metabolism, increasing intracellular ATP concentration, gating L-type voltage-dependent calcium channels, releasing calcium from an intracellular pool in response to stimulation of phosphoinositide hydrolysis, increasing cytosolic Ca^{2+} concentration, causing activation of adenylate cyclase with increase of cytoplasmic cyclic AMP concentration, activating protein kinase C and stimulating insulin release. The aim of the present study was to investigate the possible presence of T1R3 in human and rat glucagon-producing alpha cells. Human and rat pancreas, as well as rat dispersed islet cells, were immunostained for either insulin or glucagon and T1R3 (rabbit polyclonal anti-G protein-coupled receptor TAS1R3 antibody from Abcam, Cambridge, UK). Cell nuclei were counterstained with DAPI. Whether in human or rat pancreas and in rat dispersed islet cells, the positivity of beta cells in T1R3 staining was readily evident. T1R3 immunostaining was also positive in alpha cells in these three preparations. These findings were confirmed in histogram profiles of beta and non beta cells stained for either insulin or glucagon (green) and T1R3 (red), whether in pancreatic sections or dispersed islet cells. The present findings document by immunohistochemistry the presence of the sweet taste T1R3 receptor in glucagon-producing alpha cells in both human and rat endocrine pancreas. Further investigations are in progress to assess the functional relevance of these findings.

P-03

MODULATION OF TRPV4 BY SILICA NANOPARTICLES AND LIPOPOLY-SACCHARIDES

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TRPV4 is a Ca^{2+} -permeable non-selective cation channel, functioning as molecular sensor and integrator of multiple physical and chemical stimuli. This channel is highly expressed in airway epithelial cells and is involved in the regulation of the ciliary beating frequency. Alpizar et al. showed that TRPV4 plays a key role in the epithelial barrier function and its activation by LPS has a key protective role in the airways (in preparation). In this study, we determined whether TRPV4 is a target of particulate matter, which is known to cause pulmonary inflammation and oxidative stress. We chose Ludox silica nanoparticles (NPs) due to their extensive use in multiple applications. Using intracellular Ca^{2+} imaging in HEK293T cells over-expressing mouse TRPV4 we found that application of NPs inhibits the response of TRPV4 to the synthetic agonist GSK1016790A and to hypotonic stress, but enhances the response to heat. Notably, we found that pre-application of NPs strongly potentiates the response the cells to E. coli lipopolysaccharides (LPS). Interestingly, this effect was not fully mediated by TRPV4, but also by another, yet unidentified, Ca^{2+}-permeable channel expressed at the plasma membrane. We conclude that TRPV4 function is modulated by NPs and LPS in a complex manner and that this is expected to underlie part of the noxious effect of these environmental contaminants.

P-04

ROLE OF THE GLYCINE RECEPTOR ALPHA 2 SUBUNIT IN STRIATAL MEDIUM SPINY NEURON DEVELOPMENT

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The involvement of the glycine receptor alpha 2 (GlyRa2) subunit has recently been established in neurodevelopment during embryonic and postnatal stages. Previous studies from our lab have shown a reduced neostriatal surface area at postnatal day 0 in the GlyRa2 knock-out mice (GlyRa2KO). Moreover, preliminary data have shown glycinergic transmission in MSNs in adult mice by whole-cell patch-clamp recordings combined with biocytin 3D reconstruction. We hypothesize that the GlyRa2KO associated defects are caused by an altered neurogenesis of neostriatal medium spiny neurons (MSNs). Immunohistochemical (IHC) labelings for *CTIP2* (also known as *Bcl11b*), a specific marker for early postmitotic MSNs, were performed at postnatal day 0 and 14 to investigate developmental defects in this neostriatal neuronal population. The combination of these patch-clamp and IHC data during adult and postnatal stages indicate the involvement of GlyRa2 subunit in neostriatal development extending from neurogenesis to adult neurotransmission.

P-05

ROLE OF THE PXP MOTIF IN THE U-SHAPED INACTIVATION PROCESS OF HETEROTETRAMERIC Kv2.1/Kv6.4 CHANNELS

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Voltage-gated K^+ (Kv) channels exist as tetramers assembled from □-subunits that each consist of 6 transmembrane segments (S1-S6) and cytoplasmic N- and C-termini. The S1-S4 segments form the voltage-sensing domains while the four S5-S6 segments form the central pore. A conserved PxP motif within S6 provides flexibility to the bottom half of the S6 segment and regulates channel gating. Based on sequence homology, the *Shaker*-related Kv family is divided in 8 subfamilies; Kv1-6 and Kv8-9. Members of the Kv5-6 and Kv8-9, also known as silent Kv subfamilies (KvS), require co-assembly with Kv2 subunits to be functionally expressed at the plasma membrane. KvS subunits alter the biophysical properties of these heterotetramers compared to Kv2; e.g. with Kv6.4 the voltage-dependence of inactivation is shifted towards hyperpolarized potentials and the unique U-type inactivation is potentiated. KvS subunits lack the 2nd proline of the PxP motif which has been implicated in the Kv9.3-induced effects on Kv2.1 gating. However, the effect on U-type inactivation remains unknown. In this study, we demonstrate that the lack of a full PxP motif contributes to the Kv6.4-induced potentiation of the Kv2.1 U-type inactivation. Introducing the PAT motif of Kv6.4 into the Kv2.1 background increased the U-type inactivation of these mutant Kv2.1(PAT) channels. Furthermore, changing the Kv6.4 PAT motif with the Kv2.1 PIP motif decreased the U-type inactivation of Kv2.1/Kv6.4(PIP) heterotetramers to a level intermediate between Kv2.1 homotetramers and Kv2.1/Kv6.4 heterotetramers. These results indicate that the absence of a full PxP motif contributes to U-type inactivation.

P-06

FINGERPRINT OF FUNCTIONAL TRANSIENT RECEPTOR POTENTIAL CHANNELS IN HUMAN ENDOMETRIAL STROMAL CELLS DURING THE LUTEAL PHASE OF THE MENSTRUAL CYCLE

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Human endometrial stroma serves an important role in human reproduction in that it is responsible for decidualization, a prerequisite for successful embryo implantation. However, the exact mechanism that allows communication between the epithelium and stroma upon embryo attachment remains to be elucidated. Possible candidates for cellular sensors are transient receptor potential (TRP) channels. However, the distribution of TRP channels in the human endometrium is unknown. Here, we investigate the functional expression pattern of TRP channels in human stromal cells by quantitative real-time PCR (qRT-PCR), immunocytochemistry, calcium imaging, and whole-cell patch-clamp experiments. Starting from frozen endometrial biopsies taken during the luteal phase, qRT-PCR revealed the expression of a large subset of the TRP channels. Thereafter, their expression was assessed in purified primary stromal cell cultures set up from freshly isolated endometrial biopsies, and we identified mRNA from TRPV2, TRPV4, TRPC1, TRPC4, TRPC6, TRPM4, and TRPM7. The lack of specific pharmacology confined our further analysis to TRPV2, TRPV4, TRPC6, and TRPM7. Immunohistochemistry showed clear expression of TRPV2 and TRPM7 in the plasma membrane as well as in the cytoplasm. TRPV4 could be detected in the plasma membrane and showed cytoplasmatic vesicle, whereas TRPC6 protein was more abundantly expressed in the perinuclear area. Further functional characterization was done with calcium imaging and whole-cell patch-clamp techniques using specific agonist and antagonist, and provided evidence for functional expression of TRPV2, TRPV4, TRPC6, and TRPM7. In conclusion, we have demonstrated the functional expression of TRPV2, TRPV4, TRPC6, and TRPM7 in human endometrial stromal cells and these channels may act as important cellular sensors in the epithelial-stromal crosstalk.

P-07

MAGED1, A NEW PROTEIN INVOLVED IN MOTOR BEHAVIOR AND DRUG ADDICTION

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Melanoma antigen-encoding gene D1 (MAGED1) belongs to the MAGE superfamilly, first described as tumor markers. However MAGED1 is also expressed in healthy tissues, including a strong expression in the central nervous system. Here we show that mice lacking MAGED1 display hypolocomotion, deficit in motor coordination and lack of acute and chronic responses to cocaine. We carried out experiments to elucidate the mechanisms underlying those phenotypes and unravel the functions of MAGED1. As dopamine is known to regulate motor functions as well as drug related behaviors, we hypothesize that an impairment of its functions could explain the observed phenotypes. Indeed, *in vivo* microdialysis experiments show a significant diminution of cocaine-induced dopamine release in the striatum. Using cyclic voltammetry in acute striatal slices, we show that the dopamine overflow is increased. However, the re-uptake and the expression of the dopamine transporter (DAT), molecular target of cocaine, are not altered. Then, we developed a strain of mice specifically knockout for MAGED1 in dopaminergic neurons. Those mice display normal motor behavior but show an increase in cocaine locomotor sensitization. Taken together, our data show that MAGED1 is an important gene for the control of dopaminergic transmission. Work is now in progress to study the role of MAGED1 in the activatory and inhibitory synaptic inputs controlling the activity of dopaminergic neurons and motor and behavioral response to drug of abuse.

P-08

INFLUENCE OF THE WATER-SOLUBLE CARBON MONOXIDE RELEASING MOLECULE (CORM-A1) ON REACTIVE OXYGEN SPECIES (ROS) INDUCED BY TNF-α IN MURINE INTESTINAL EPITHELIAL MODE-K CELLS

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Using the mouse intestinal epithelial cell line, MODE-K, as an in-vitro model, we have previously reported that TNF-α/cycloheximide (CHX)-induced apoptosis corresponds with the occurrence of reactive oxygen species (ROS) production, and that CORM-A1 (100 µM), a water-soluble carbon monoxide releasing molecule, partially reduces both effects (Babu et al., Curr Pharm Des. 2012). We now investigated the production sites of TNF-α/CHX-induced ROS and the influence thereupon of CORM-A1 in MODE-K cells, by use of two different ROS detecting fluorescent probes. MODE-K cells were exposed to TNF-α (1 $ng \cdot mL^{-1}$)/CHX (10 $\mu g \cdot mL^{-1}$) for up to 6h; simultaneous detection of ROS production and cell death was performed using either carboxy-H_2DCFDA (for detection of both cytoplasmic and mitochondrial ROS) or MitoSOX Red (for selective detection of mitochondrial superoxide anion) together with Sytox Red in a single experimental setup using flow cytometric analysis. Treatment with TNF-α/CHX time-dependently increased mean fluorescence intensity (ΔMFI) of carboxy-H_2DCFDA or MitoSOX Red-derived fluorescence with parallel increase in cell death as measured with Sytox Red. The selective pan-nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) inhibitor, VAS-2870, partially reduced TNF-α/CHX-induced ROS, assessed via carboxy-H_2DCFDA, and cell death. The data suggest that both mitochondria and NOX enzymes contribute to TNF-α/CHX-induced ROS production in MODE-K cells. Treating the cells with CORM-A1 from 1h before till the end of the 6h exposure to TNF-α/CHX significantly reduced the TNF-α/CHX-induced ΔMFI of carboxy-H_2DCFDA, but not that of MitoSOX Red implying that CORM-A1 did not influence ROS production from mitochondria; however in parallel CORM-A1 reduced TNF-α/CHX-induced cell death. The antioxidant effect of CORM-A1 thus seems to involve exclusively inhibition of the extra-mitochondrial ROS source, i.e., the NOX enzymes during TNF-α/CHX-induced cell death of intestinal epithelial cells.

P-09

ALLOPURINOL REDUCES SEIZURE SEVERITY AND THE INFLAMMATORY RESPONSE IN A MOUSE MODEL FOR STATUS EPILEPTICUS: A POTENTIAL ROLE FOR URIC ACID IN ICTOGENESIS

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A growing body of evidence is pointing to an active role for brain inflammation in various forms of refractory epilepsy. In this regard, proconvulsant effects of pro-inflammatory danger molecules recently gained a lot of interest. The objective of this study was to investigate the involvement of the danger molecule uric acid (UA) in the generation of epileptic seizures and the associated inflammatory response. Mice were injected intraperitoneally with saline or allopurinol, an inhibitor of uric acid production. Subsequently, kainic acid or Ringer's solution was infused intrahippocampally through a microdialysis probe. Dialysates were collected every 50 minutes to measure hippocampal uric acid levels and video-EEG monitoring was done to assess the severity of the induced status epilepticus. Twenty-four hours after the development of seizures, the ipsilateral hippocampi were isolated for RNA-quantification of relevant cytokines using RT-qPCR. Infusion of kainic acid in saline-injected animals increased the hippocampal uric acid concentration compared to the Ringer's infused control group. Treatment with allopurinol prior to kainic acid-infusion significantly suppressed this UA-increase and significantly reduced the total seizure severity score. Moreover, allopurinol treatment attenuated the kainic-acid induced upregulation of TNF mRNA-levels. Our results suggest a contribution of uric acid to ictogenesis and its associated inflammatory response.

P-10

DOSE-DEPENDENT LARYNGEAL MUSCLE EVOKED POTENTIALS AS AN INDICATOR OF EFFECTIVE VAGUS NERVE STIMULATION

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Vagus nerve stimulation (VNS) is a neuromodulatory treatment for refractory epilepsy and depression. The major drawbacks of VNS are 1/ the lack of response in a third of patients and 2/ the fact that the optimal stimulation parameters are unknown. Vagal nerve recordings in both an experimental and clinical setting may be useful to overcome these drawbacks. In this study, VNS-induced laryngeal muscle evoked potentials (LMEPs) were recorded in rats as a biomarker for effective activation of the vagus nerve. Rats (n=28) were implanted with a bipolar stimulation cuff-electrode around the left vagus nerve. After at least two weeks of recovery, VNS-induced LMEPs were recorded under anesthesia using a monopolar EMG needle electrode placed subcutaneously near the laryngeal muscles and a distant reference electrode on the skull of the rat. Biphasic VNS pulses of 250 µs per phase were used. The output current of the pulses was gradually ramped-up from 0.1 mA to 1.0 mA in steps op 0.1 mA. For each intensity, 20 sweeps were averaged to improve the signal to noise ratio. VNS-induced LMEPs were reproducibly recorded in 25/28 rats. The LMEPs were characterized by a negative peak with a latency of 2.6 ms ± 0.9 ms after the onset of the stimulation pulse. The threshold for evoking LMEPs was variable, ranging from 0.1 mA to 0.8 mA (0.4 mA ± 0.2 mA). This minimally invasive technique could be used both in animal studies and in clinical practice 1/ to identify non-responders due to inactivity of the vagus nerve and 2/ to determine individual stimulation parameter requirements for efficient activation of the vagus nerve.

P-11

MIRROR-IMAGE PAIN AFTER PERIPHERAL NERVE INJURY OF MICE LACKING VASOACTIVE INTESTINAL PEPTIDE

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Vasoactive intestinal peptide (VIP) is one of the neuropeptides that show a strong up-regulation after peripheral nerve injury. As this neuropeptide has previously been associated with neuropathic pain, the objective of our study was to investigate whether mechanical hypersensitivity after nerve injury is different between VIP-knock out mice and their wild type controls. Spared nerve injury – a sciatic nerve lesion model in which the sural nerve is spared while the common peroneal and tibial nerves are severed – resulted in an early (within one day) and persistent (four weeks) decrease in the threshold to elicit a withdrawal of the ipsilateral hindpaw after mechanical stimulation with von Frey hair filaments. While this ipsilateral mechanical hypersensitivity occurred in both mice strains, only VIP-knock out mice showed a similar reduction in the contralateral hindpaw withdrawal threshold to mechanical paw stimulation. This contralateral pain hypersensitivity is reminiscent of mirror-image pain, which is frequently observed in clinical chronic pain conditions. We then tested whether the mirror-image pain reflects a segmental spreading of pain hypersensitivity or is rather due to a widespread increase in pain hypersensitivity after nerve injury in VIP-knock out mice. Hereto, we performed a lesion of the median nerve in the forepaw and assessed mechanical withdrawal thresholds in the hindpaws. During a four weeks testing period, neither one of the two mouse strains showed mechanical hypersensitivity in the hind paws after median nerve injury. Our data therefore show that VIP-knock out mice develop bilateral pain hypersensitivity after nerve injury via a segmental mechanism. This new animal model may provide key insights into mechanisms underlying mirror-image pain and thereby generate clues that could improve the treatment of chronic pain conditions.

P-12

REDUCED SCAR MATURATION AND CONTRACTILITY LEAD TO EXAGGERATED LEFT VENTRICULAR DILATION AFTER MYOCARDIAL INFARCTION IN MICE LACKING AMPK ALPHA1

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Cardiac fibroblasts (CF) are crucial in left ventricular (LV) remodelling after myocardial infarction (MI). They predominantly express the alpha1 catalytic subunit of AMP-activated protein kinase (AMPKalpha1), while AMPKalpha2 is the major catalytic isoform in cardiomyocytes. AMPKalpha2 is known to protect the heart by preserving the energy charge of cardiac myocytes during injury, but whether AMPKalpha1 interferes with maladaptative heart responses remains unexplored. In this study, we aim at further substantiating the role of this AMPK isoform in the pathogenesis of post-MI LV remodelling and more particularly in the regulation of fibrotic properties of CF. AMPKalpha1 knockout (KO) and wild type (WT) mice were subjected to permanent ligation of the left anterior descending coronary artery to mimic MI. Cardiac fibrosis was monitored using QRT-PCR analysis, histology and immunohistofluorescent staining. LV function and remodelling was assessed by echocardiography. In the absence of AMPKalpha1, the CF proliferative response was increased in infarcted myocardia. It resulted in elevated levels of fibrotic factors but did not lead to excessive matrix deposition or degradation in KO infarcts. While CF proliferation was increased, expression of the myodifferentiation marker alpha-smooth muscle actin was decreased. This faulty maturation of myofibroblasts might derive from down-regulation of the transforming growth factor-beta1/p38 mitogen-activated protein kinase pathway in KO infarcts. Although infarct size was similar in KO and WT hearts subjected to MI, these changes resulted in defective scar collagen maturation. This was associated with an exacerbated adverse remodelling as indicated by increased LV diastolic dimension 30 days after MI. Our data genetically demonstrate the centrality of AMPKalpha1 in post-MI scar formation and highlight the specificity of this catalytic isoform in cardiac fibroblast/myofibroblast biology.

P-13

A769662, A SPECIFIC AMP-ACTIVATED PROTEIN KINASE (AMPK) ACTIVATOR, PREVENTS CARDIOMYOCYTE HYPERTROPHY INDEPENDENTLY OF THE ALREADY IDENTIFIED AMPK DOWNSTREAM TARGETS

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Classical AMPK activators, as resveratrol or metformin, inhibit pathological cardiac hypertrophy. However, this phenomenon is mainly circumstantial. Indeed, those agents induce a rather non-specific AMPK activation by increasing the AMP/ATP ratio. In a previous study, using an AMPK specific activator, A769662, we have shown that this compound inhibits cardiomyocyte hypertrophy in vitro. Surprisingly, at low doses (12.5µM), A769662 is able to block phenylephrine (PE)-induced hypertrophy without affecting the known AMPK-related key regulators of cardiac hypertrophy, namely protein synthesis, nuclear factor of activated T-cells (NFAT) and MAP kinase signaling. Hence, this study was undertaken to i) confirm these results using other AMPK activators, phenformin and AICAR, in cultured neonatal and adult rat ventricular myocytes (NRVM and ARVM); and ii) identify a novel mechanism by which AMPK can regulate cardiac hypertrophy. For the latter, we have focused on a post-translational modification called O-GlcNAcylation (O-GlcNAc) which is known to be modulated during cardiac hypertrophy. Hypertrophy was evaluated by alpha-actinin immunostaining and cell surface area measurement. Radio labelled amino acid incorporation was used to estimate protein synthesis. NFAT activity, protein expression and phosphorylation were also analysed. Total O-GlcNAc levels were also evaluated by western blot. Using dose–response experiments, we show that, similarly to A769662, phenformin and AICAR are able to prevent the development of PE-induced NRVM hypertrophy. When high concentrations of these compounds were used, this correlates with the modification of the AMPK-related key regulators of cardiac hypertrophy including a lower protein synthesis (~50%, $p<0.05$), a decreased NFAT signaling (~90%, $p<0.05$) and lower phosphorylation of the MAP kinase ERK1/2 (~60%, $p<0.05$). However, while low doses of phenformin or AICAR (0.03mM and 0.25mM respectively) still efficiently prevent PE-induced NRVM hypertrophy, none of the known AMPK downstream targets are modified. Similar results were obtained in ARVM with A769662. We also show that treatment of neonatal cardiomyocytes with PUGNAc or glucosamine, both known to increase O-GlcNAc levels, inhibit the anti-hypertrophic effect of AMPK in NRVMs and ARVMs, highlighting an interaction between AMPK and O-GlcNAc signaling in cardiac hypertrophy regulation. Collectively, our results using low dose of AMPK activators suggest that AMPK regulates cardiac hypertrophy by a yet to be identified mechanism, which could include O-GlcNAc signaling.

P-14

ENHANCED NITRIC OXIDE PRODUCTION AMELIORATES ACUTE 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 characterised 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 demonstrated, which might lead to renal dysfunction. Therefore, in our study, we investigated the potential benefit of L-Arginine (L-Arg) supplementation in a mouse model of AAN. Indeed, L-Arg, a substrate for NO synthesis, could restore NO bioavailability in AAN. To do so, C57BL/6J male mice were randomly subjected to daily i.p. injection of control solution or AAI (2,5mg/kg) for 5 days and L-Arg was supplemented in drinking water (5%). At day 5, AA-treated mice displayed polyuria, increased plasma creatinine level and proteinuria. By comparison to control kidneys, histological analyses revealed severe proximal tubular cell necrosis, renal inflammation and increased oxidative stress. These lesions were associated with a significant reduction of NO bioavailability, as measured by a reduced urinary nitrite/nitrate excretion. L-Arg supplementation in AA-treated mice significantly improved kidney function, as reported by a significant reduction in urine volume, plasma creatinine level and proteinuria. Moreover, L-Arg treatment resulted in a significant reduction of tubular cell necrosis score, reduced renal inflammation and oxidative stress along with a normalized NO levels. These results suggest that a preservation of NO bioavailibity may lead to a morpho-functionnal protection in AAN. In the present mouse model of acute AAN, NO seems to act as a strong mediator of renal function. In conclusion, improved NO bioavailability by L-Arg supplementation was demonstrated beneficial in improving renal injury in AAN.

P-15

ENDOTHELIAL CELL REPROGRAMMING FOLLOWING EXPOSURE TO IONIZING RADIATIONS

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The current radiation protection system is based on the assumption that there is a threshold of low dose radiation below which no significant non-cancer effects are observed. In recent years, however, there was a growing epidemiological evidence of excess risk of late occurring cardiovascular disease at much lower doses (< 0.5 Gy) without a clear-cut threshold. It is proposed that the vascular endothelium is a critical target in ionizing radiation-related cardiovascular diseases. In the framework of EU FP7 ProCardio project, immortalized human coronary artery endothelial cells were exposed to low doses of X-rays (0.05 and 0.1 Gy) and compared to isogenic cells either sham-irradiated or treated at higher doses (0.5 and 2 Gy). After irradiation, cells were kept in culture for 1, 7 or 14 days followed by total RNA extraction. Single gene analysis uncovered the induction by ionizing radiation of a gene expression profile resembling a pro-atherosclerotic state in endothelial cells. Pathway analysis indeed showed that X-rays induced pathways involved in atherosclerotic processes, such as inflammation, disruption of the extrinsic coagulation cascade, generation of oxidative stress, initiation of hypertension-related processes, disturbances in cell cycle and loss of homotypic cell adhesion. This profile was more pronounced at higher doses, but was to a degree also present at the low dose range. We are currently investigating these different pro-atherosclerotic events at the genomic, proteomic and functional level.

P-16

ROLE OF CONNEXIN CHANNELS, CALCIUM AND ROS IN RADIATION INDUCED BYSTANDER EFFECTS

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Radiotherapy is often used for the treatment of brain glioma tumors. Although nowadays a more precise delivery of radiation is insured, some exposure of healthy brain tissue is inevitable. The resulting brain injury is characterised by vascular damage, being endothelial cell (EC) activation and death. Evidence is accumulating that intercellular communication pathways can propagate radiation-induced effects from directly irradiated to non-irradiated neighboring cells, thereby exacerbating damage. Both gap junctional and paracrine communication have been implicated in these bystander effects. Gap junctions (GJs) connect the cytoplasm of adjacent cells, allowing the direct exchange of small molecules. GJs are formed by the docking of two hemichannels, which are each composed of 6 trans-membrane connexin (Cx) proteins and contribute to paracrine signaling. We previously demonstrated that both types of Cx channels contribute to the spreading of cytochrome C-induced apoptosis in an in vitro glioma model. Intracellular Ca^{2+} changes were involved in the spreading mechanism. We here explored the role of Cx channels and Ca^{2+} signaling in bystander effects in brain ECs exposed to X-rays (1-20 Gy). To investigate this, we optimized 2 in vitro models in which a well delineated zone of an adherent EC monolayer is exposed to X-rays. We investigated kinetics of radiation-induced DNA damage and cell death in the irradiated and surrounding bystander area. DNA damage was observed in the bystander area, even a few 100 µm from the irradiated zone, early after irradiation, while apoptosis was detected at a later stage. Inhibition of Cx channels and scavenging Ca^{2+} and reactive oxygen species (ROS) reduced the bystander effects. We conclude that Cx channels and Ca^{2+}/ROS signalling play a role in radiation-induced bystander damage of brain ECs.

P-17

AMPK ACTIVATION BY GLUCAGON-LIKE PEPTIDE-1 PREVENTS NADPH OXIDASE ACTIVATION INDUCED BY HYPERGLYCEMIA IN ADULT CARDIOMYOCYTES

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Exposure of cardiomyocytes to high glucose concentrations (HG) stimulates ROS production by NADPH oxidase (NOX2). NOX2 activation is triggered by enhanced glucose transport through a sodium-glucose co-transporter (SGLT) but not by a stimulation of glucose metabolism. The aim of this work was to identify potential therapeutic approaches to counteract this glucotoxicity. In cultured adult rat cardiomyocytes incubated with 21 mM glucose (HG), AMP-activated protein kinase (AMPK) activation by A769662 or phenformin nearly suppressed ROS production. Interestingly, GLP-1, a new anti-diabetic drug, concomitantly induced AMPK activation and prevented the HG-mediated ROS production (maximal effect at 100 nM). α2AMPK, the major isoform expressed in cardiomyocytes (but not α1AMPK), was activated in response to GLP-1. Anti-ROS properties of AMPK activators were not related to changes in glucose uptake or glycolysis. Using *in situ* proximity ligation assay, we demonstrated that AMPK activation prevented the HG-induced p47phox translocation to caveolae, whatever the AMPK activators used. NOX2 activation by either α-methyl-D-glucopyranoside, a glucose analog transported through SGLT, or angiotensin II was also counteracted by GLP-1. The crucial role of AMPK in limiting HG-mediated NOX2 activation was demonstrated by overexpressing a constitutively active form of a2AMPK using adenoviral infection. This overexpression prevented NOX2 activation in response to HG, whereas GLP-1 lost its protective action in a2AMPK deficient mouse cardiomyocytes. Under HG, the GLP-1/AMPK pathway inhibited PKCb2 phosphorylation, a key element mediating p47phox translocation. In conclusion, GLP-1 induces α2AMPK activation and blocks HG-induced p47phox translocation to the plasma membrane, thereby preventing glucotoxicity.

P-18

CAN EMBRYONIC MICROGLIA BRIDGE THE GAP BETWEEN MATERNAL IMMUNE ACTIVATION AND NEUROPSYCHIATRIC DISORDERS?

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Several studies have indicated that inflammation during pregnancy increases the risk for the development of neuropsychiatric disorders like autism and schizophrenia in the offspring. Morphological brain abnormalities and deviations in immunity can be observed in patients of both disorders. It has been suggested that the acute infection induces changes in maternal cytokine levels which in turn affects the fetal brain and results in the development of both neuropsychiatric disorders in the offspring. In this study, the poly (I:C) model was used to mimic viral immune activation in pregnant mice in order to study the fetal microglial response to the maternal infection. We injected pregnant mice with poly (I:C) (i.p., 20 mg/kg) on either E11.5 or E15.5. The concentration of IL-6 in the maternal serum was used as a measure for systemic inflammation in the mother. Afterwards, the microglial cell density and activation level (Mac-2, iNOS and IL1β immunostainings) in the cortex and hippocampus of CX3CR1-eGFP +/- embryos was determined. Additionally, to test the possibility that microglia are primed by a first injection and activated only upon a secondary stimulation, pregnant mice were injected with poly (I:C) on both E11.5 and E15.5. Despite the presence of a systemic inflammation in the pregnant mice, we found no significant difference in fetal microglial expression of activation markers Mac-2, iNOS and IL1β between the control and inflammation group. These results suggest that fetal microglia are not ‘classically’ activated by the maternal immune activation.

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MIGRATION OF MICROGLIA IN THE EMBRYONIC NEOCORTEX: CELLULAR AND MOLECULAR INTERACTIONS

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Microglia, the macrophages of the central nervous system (CNS), influence synaptic development and connectivity in the normal and developing CNS. After invading the embryonic CNS, microglia migrate extensively to occupy their final positions. However, the cellular and molecular mechanisms of microglial migration are unknown. Therefore, this study aims to elucidate the microglial migration behavior, cellular interaction partners and ECM-integrin interactions essential for migration in the developing neocortex. Immunostaining and immunogold labeling for TEM were performed on brains of mice embryos (Embryonic day (E) 12.5-17.5). Flow cytometric analyses were performed on CX3CR1$^{+/eGFP}$ embryonic cortici. Microglial migration was recorded in CX3CR1$^{+/eGFP}$-hGFAP-CFP acute brain slices using multiphoton excitation time-lapse microscopy and analyzed using MTrackJ in ImageJ. Laminin (LM) is expressed as punctuate dots near the pia and in the ventricular zone at E10.5 and disappears at E15.5 to remain only in blood vessels and the pia. Fibronectin (FN) is present as aggregates from E12.5-E17.5. Both proteins follow the course of radial cells. More than 90% of the cortical blood vessels is ICAM-1 positive from E13.5-E17.5. The percentage of microglia expressing the FN receptor and ICAM-1 receptor, as well as the expression level of these proteins decreases significantly from E13.5-E17.5. All microglia express the LM receptor and this expression remains stabile. Time-lapse recordings show that embryonic microglia are dynamic cells that migrate in random patterns. These cells seem to make brief contacts with the processes of radial glia. Knowledge about ECM-integrin interactions during microglial migration could reveal targets for intervention in pathological settings, such as Multiple Sclerosis.

P-20

sRAGE LIMITS CARDIAC DYSFUNCTION INDUCED BY ADVANCED GLYCATION END PRODUCTS IN RATS

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Advanced glycation end products (AGEs) are proteins and lipids that become glycated and oxidized after persistent contact with aldose sugar and/or a high degree of oxidative stress. AGEs and their receptor RAGE are known to play a key role in the development and progression of cardiovascular diseases but the precise underlying mechanism remains elusive, in particular in the non-diabetic setting. In this study, adult Sprague-Dawley rats underwent a daily injection of BSA-modified AGEs (20 mg/kg ip, n=9) or control-BSA (n=4) for 6 weeks. Cardiac function was evaluated with echocardiography. Plasma levels of glucose, AGE and sRAGE were measured. Tissue AGE and RAGE expression were determined by Western blot. As expected, after 6 weeks, circulating AGEs levels were higher in the treated animals. AGEs injection led to a modest increase in plasma glucose levels (p=0.07) which remained however within the normal physiological range. Ejection fraction, as a measure of global cardiac function was not altered by AGEs injection while circumferential strain, as a measure of cardiac deformation, was reduced (-16.40 ± 1.89% *vs* -21.65 ± 2.79% at baseline). AGEs-injected animals displayed cardiac hypertrophy characterized by an increase AWTd and PWTd (1.83 ± 0.17 mm and 2.01 ± 0.16 mm *vs* 1.27 ± 0.09 mm and 1.54 ± 0.12 mm at baseline, respectively). Unexpectedly, tissue AGEs and RAGE expression were not different between the groups. However, sRAGE, the soluble form of RAGE, was 3 times higher in the AGEs-injected group compared to the control group (3.90 ng/ml *vs* 1.80 ng/ml). It is known that increased AGEs levels are associated with diabetes. Our model mimics the increased AGEs without the additional effects related to the elevated glucose levels. Data demonstrate that an increased circulating AGEs *per se* plays a role in cardiac remodeling and dysfunction. The high sRAGE levels seem to have a protective role which limits the deleterious cardiac effects related to the increased circulating AGEs.

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NEW PHARMACOLOGY EXHIBITS AN ANTIHYPERGLYCAEMIC AND INSULINOTROPIC EFFECT IN MICE THROUGH POTENTIATION OF TRPM5

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Diabetes is a pandemic that poses a huge challenge for medical research worldwide. Its incidence is still rising and causes tremendous costs to the health care system and a vastly reduced life quality to the patients. There is a high need for new therapies and improved pharmacology. We show that Transient Receptor Potential cation channel Melastatin subfamily member 5 (TRPM5) is an interesting new target for potentiating the glucose induced insulin release from the pancreatic beta-cells. We identified a new family of compounds as potentiators of TRPM5, both in a heterologous overexpression system and a murine animal model. This activation leads to increased glucose induced insulin release in the pancreatic beta-cells and consequently reduced postprandial blood glucose. Mice given a high fat diet (HFD) gradually develop impaired glucose tolerance (IGT) due to insulin resistance which ultimately leads to the development of type II diabetes. Our results show that daily administration of this drug to mice receiving a HFD attenuates the onset of glucose intolerance. These mice have a normal glycaemic profile during a glucose tolerance test compared to mice not being treated with stevioside. Moreover we show that withdrawal of the compound quickly leads to IGT. On the other hand we gave daily treatments to mice that have already a diabetic phenotype after 20 weeks of HFD. There was a gradual decrease in the glucose intolerance, improving the diabetic phenotype. This improvement is seen indifferent of the diet the mice get, either normal food or HFD. These changes in glucose metabolism are only observed in $TRPM5^{+/+}$ mice. The knockout mice show no response to the treatments with our compound. Taken together, our data shows the identification of a new drug acting through TRPM5 has an antihyperglycaemic effect and attenuates the IGT in wild-type mice.

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BECAUSE OF THEIR OXIDATIVE AVIDITY FOR NADH, OXIDATIVE TUMOR CELLS ARE RESISTANT TO LACTATE-INDUCED NF-kB ACTIVATION

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Glycolytic end-product lactate was recently identified as a tumor growth-promoting factor. Importantly, high lactate levels correlate with tumor aggressiveness and metastasis. In endothelial cells, we recently demonstrated that lactate, after its intracellular conversion to pyruvate, can activate transcription factor NF-κB. While pyruvate blocks prolylhydroxylase (PHD) activity, byproduct NADH stimulates NAD(P)H oxidases (Nox) to produce reactive oxygen species (ROS), and both account for NF-κB activation. Here, we tested whether lactate could also activate NF-κB in oxidative tumor cells known to take up lactate. Intriguingly, HeLa and SiHa human cervix cancer cells turned out to be resistant to lactate-induced NF-κB activation, determined by quantifying the p65 phosphorylation and with a luciferase reporter assay. Treatment times ranged from 5 minutes to 6 hours. Still, TNFα successfully activated NF-κB, indicating a specific resistance of these cells to lactate signaling to NF-κB. On another hand, lactate efficiently activates HIF-1 in these cells *via* pyruvate-mediate PHD inhibition. Therefore, resistance could occur from a failure to activate NADH-Nox-ROS signaling. Basal activation of the pathway was excluded by showing that N-acetyl-*L*-cysteine did not modify basal p65 phosphorylation. Conversely, H_2O_2 successfully increased p65 phosphorylation. For this reason, we focused on NADH. Interestingly, treatment of the cells with lactate did not increase NADH/NAD and cytosolic ROS (measured with CM-H_2DCFDA). These results suggest that NADH fuels OXPHOS rather than Nox in oxidative tumor cells. Unexpectedly however, mitochondrial complex 1 inhibitor rotenone did not restore lactate induced NF-κB activation: it increased the NADH/NAD but not ROS production. Combination of rotenone with lactate did not further increase NADH/NAD nor p65 phosphorylation and activity. Collectively, our data demonstrate that lactate is not sufficient to activate NF-κB in oxidative tumor cells because of a failure to activate Nox.

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TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA: A METABOLIC CULPRIT?

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Gliomas account for more than 50% of all brain tumors in humans, with glioblastoma (GBM) representing the ultimate grade of malignancy. The current standardized clinical protocol includes maximal surgical resection followed by radiotherapy, and concomitant temozolomide (TMZ) chemotherapy. TMZ is an orally available alkylating prodrug, the conversion/activation of which is entirely pH dependent and does not require enzymatic activation. Indeed, TMZ is stable/inactive at acidic pH but decomposes to monomethyl-triazeno-imidazole-carboxamide (MTIC) at pH > 7. MTIC is stable/inactive at alkaline pH but fragments to produce methyldiazonium ion, the active compound, at pH < 7. However, most aggressive tumor cells are usually glycolytic, with alkaline intracellular pH owing to a high level of expression and activity of proton transporters at the cell membrane, further causing extracellular acidification. This would cause resistance to TMZ. Therefore, this study aims to investigate whether and which differences in the metabolism of resistant *versus* sensitive GBM cells can be modulated to preclinically improve the response to TMZ. We used human GBM (U373, T98G) and oligodendro-glioma (Hs683) cell lines that were treated with increasing doses of TMZ. Resistant and sensitive cells were metabolically profiled for glucose metabolism (CMA600 enzymatic assays), oxygen consumption (Seahorse XF96 bioanalyzer), and intra- and extra-cellular pH (SNARF-AM fluorescence and Seahorse technology). The expression of pH transporters was determined using Western blotting. We first identified that T98G and U373 cells are more resistant to TMZ than Hs683 cells. From a metabolic standpoint, we found that TMZ-resistant tumor cells consume more glucose and release more lactic acid compared to sensitive cells, indicating that a glycolytic metabolism can confer resistance to TMZ chemotherapy. There was no difference in intracellular pH. We further found that resistant GBM cells do express the lactic acid transporter monocarboxylate transporter 1 (MCT1) preferentially to MCT4, whereas there was no difference in the expression of the sodium-proton exchanger NHE1. These results prompt for a translational evaluation of different proton transporter inhibitors in combination with TMZ using our cell models, orthotopic human gliomas in immunodeficient mice, and fresh biopsies collected from GBM patients at the time of surgery.