BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Friday October 26 2012

PROGRAMME

&

ABSTRACT BOOK

Venue

Palace of the Academies
Rue Ducale / Hertogsstraat 1
1000 Brussels

Organisation

Prof. Dr. B. Flamion
Unité de Rercherche en Physiologie Moléculaire
Facultés Notre Dames de la Paix Namur
61, rue de Bruxelles
5000 Namur

BELGIAN SOCIETY OF PHYSIOLOGY AND PHARMACOLOGY

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1000 Brussels

Main Lecture

09.45-10.45 Prof. Dr. Maarten KOLE (Netherlands Institute of Neuroscience – Amsterdam, The Netherlands)

Origins of the action potential in myelinated axons.

Oral Communications

10.45-11.00 S. KOULCHITSKY, T. BEEKEN, J. DETHIE, E. BULLINGER, V. SEUTIN (ULiège).
Combined injection of quinpirole and cocaine induces a stereotypic-like behavior and changes in LFP power within the ventral tegmental area of rats.

11.00-11.15 Y.A. ALPIZAR, L. VAN GERVEN, W. EVERAERTS, B. BOONEN, A. MENINGOZ, F. VERMEULEN, D. DE RIDDER, P. HELLINGS, T. VOETS, K. TALAVERA (KULeuven).
Anesthetic effect of the trpa1 specific agonist cinnamaldehyde.

11.15-11.30 K. VERMOESEN, A. MASSIE, I. SMOLDERS, R. CLINCKERS (VUBrussel).
The antidepressants citalopram and reboxetine reduce seizure frequency in chronic epileptic rats.

11.30-11.45 L. ROTH, D. VAN DAM, C. VAN DER DONCKT, D.M. SCHRIJVERS, G. VANHOUTTE, M. VERHOYE, A. VAN DER LINDEN, W. MARTINET, H. BULT, G.R.Y. DE MEYER (UAntwerpen).
Motor coordination in a new mouse model of atherosclerotic plaque rupture.

11.45-12.00 C. MICHIELS, P. FRANSEN, D.M. SCHRIJVERS, H. BULT, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen).
Effect of autophagy deficiency in vascular smooth muscle cells and cardiomyocytes on cardiovascular health.

12.00-12.15 M. DOGNÉ, C. DESSY, G. RATH, N. CARON, B. FLAMION (FUNDPNamur).
Hyal-1 deficiency protects against endothelial dysfunction in diabetic mice.

12.15-12.30 B. COLSOUL, G. JACOBS, K. PHILLIPAERT, G. OWSIANIK, A. SEGAL, F. SCHUIT, R. VENNEKENS (KULeuven).
Gene regulation of *Trpm5* in animal models of type 2 diabetes.

12.30-14.30 **Lunch - Guided Poster Session – General Assembly**

Posters (height 120 cm – width 100 cm)

1. L. MOLLET, R. RAEDT, J. DELBEKE, R. EL TAHRY, V. DE HERDT, A. MEURS, W. WADMAN, K. VONCK, P. BOON (UGent, UCLouvain, Univ. Amsterdam The Netherlands).
Electrophysiological responses to vagus nerve stimulation in rats.

2. A. EL ARFANI, B. AMPE, Y. MICHOTTE, I. SMOLDERS (VUBrussel).
Effect of MK801 on the dopamine and glutamate release in the subthalamic nucleus of intact and hemi-parkinson rats.

3. E. BENTEA, J. VAN LIEFFERINGE, E. MERCKX, Y. MICHOTTE, I. SMOLDERS, A. MASSIE (VUBrussel).
Development and characterization of the nigral lactacystin mouse model of Parkinson's disease.

4. V. PENNEMANS, J-M. RIGO, C. FAES, J. PENDERS, Q. SWENNEN (Univ. Limburg Diepenbeek, Ziekenhuis Oost-Limburg Genk).
Establishment of age and gender dependent reference values for novel urinary biomarkers for renal damage in the healthy population.

5. L. GIORDANO, A. POZDZIK, V. COLOMBARO, TH. BAUDOUX, V. VOISIN, E. DEPREZ, M.H. ANTOINE, S. LEDBETTER, J. NORTIER, N. CARON (FUNDPNamur).
Therapeutic potential of anti-TGFß antibody on acute tubulo-interstitial injury in aristolochic acid nephropathy.

6. V. COLOMBARO, V. VOISIN, L. GIORDANO, B. FLAMION, N. CARON (FUNDPNamur).
Analysis of renal phenotype of hyaluronidase 1 or hyaluronidase 2 knockout mice.

7. C. ONCLINX, B. FLAMION (FUNDPNamur).
Analysis of chronic haemolysis in Hyal2$^{-/-}$ mice.

8. V. BOURGUIGNON, L. JADIN, R. TAMMI, B. FLAMION (FUNDPNamur).
Lymph node alterations in Hyal2 deficient mice.

9. F. CLAINE, L. WIGGERS, B. MUYLKENS, N. KIRSCHVINK (FUNDPNamur).
Evaluation of colostral antibody protection in lambs born from ewes infected with Schmallenberg virus during pregnancy.

10. S. BALOL'EBWAMI, S. ZIGABE, E. BAHIZIRE, E. SHINDANO, R. CHIRIMWAMI, O. NYAMUGABO, O. BATTISTI, K. MUBAGWA (Univ. Bukavu DR Congo, KULeuven).
Body composition in malnourished children in Bukavu, DR Congo. Can electrical impedance be used for edema evaluation and monitoring?

11. H. AKL, R. LA ROVERE, I. VANDECAETSBEEK, A. KAUSKOT, G. MONACO, T. LUYTEN, K. WELKENHUYZEN, L. MISSIAEN, M.F. HOYLAERTS, J.B. PARYS, H. DE SMEDT, G. BULTYNCK (KULeuven).
BH3 mimetic HA14-1, but not ABT-737, potentiates pro-apoptotic Ca^{2+} signaling in normal and transformed cells.

12. G. MONACO, R. PONSAERTS, R. LA ROVERE, E. DECROCK, K. NUYTS, T. LUYTEN, K. WELKENHUYZEN, S. STRELKOV, L. LEYBAERT, W. DE BORGGRAEVE, H. DE SMEDT, J. B. PARYS, G. BULTYNCK (KULeuven, UGent, Univ. Chieti – Italy).
A dicodon mutation in the BH4 domain of Bcl-2 reveals new structural determinants involved in the inhibition of inositol 1,4,5-trisphosphate receptors (IP_3Rs).

13. B. ISTRATE, A. GWANYANYA, R.B. DRIESEN, V. BITO, K. MUBAGWA (KULeuven).
Non-homogenous distribution of TRPM7 in cardiac ventricular myocytes.

14. L. MONDIN, P. GAILLY (UCLouvain).
Osmosensation in TRPV2 dominant negative expressing cells.

15. B. BOUTIN, N. TAJEDDINE, P. GAILLY (UCLouvain).
Androgen deprivation modulates calcium homeostasis and induces autophagy in prostate cancer cells.

16. B. BOONEN, B. DENLINGER, Y.A. ALPIZAR, T. VOETS, V.M. MESEGUER, C. BELMONTE, K. TALAVERA (KULeuven).
Modulation of voltage-dependent sodium currents by the trpa1 agonist cinnamaldehyde.

17. Y.A. ALPIZAR, M. GEES, A. SANCHEZ, A. APETREI, T. VOETS, B. NILIUS, K. TALAVERA (KULeuven.)
Bimodal action of cinnamaldehyde and camphor on mouse trpa1 channels.

18. I. VANDEWAUW, G. OWSIANIK, T. VOETS (KULeuven).
Expression analysis of TRP channel genes at single trigeminal and dorsal root ganglion levels in mouse.

19. C. D'HONDT, J.P. DECUYPERE, K. WELKENHUYZEN, B. HIMPENS, G. BULTYNCK (KULeuven).
Starvation-induced autophagy alters Connexin43 levels and reduces intercellular calcium wave propagation in corneal endothelial cells.

20. E. BOCKSTEINS, E. MAYEUR, G. REGNIER, A. VAN TILBORG, D.J. SNYDERS (UAntwerpen).
The obligatory Kv2.1/Kv6.4 heterotetramerization is determinated by both N- and C-terminmal interactions.

Oral Communications

14.30-14.45 A-S HERVENT, N. HAMDANI, V. MATHEEUSSEN , M. DEMOLDER, I. DE MEESTER, W. PAULUS, G. DE KEULENAER (UAntwerpen, VUAmsterdam, The Netherlands).
Effects of DPP-IV inhibition on left ventricular compliance in mice with type II diabetes.

14.45-15.00 K. PHILIPPAERT, B. COLSOUL, A. SEGAL, T. VOETS, R. VENNEKENS (KULeuven).
Screening and characterization of pharmacological tools to target Ca^{2+} activated non-selective cation channels.

15.00-15.15 N. TAJEDDINE, P. GAILLY (UCLouvain).
TRPC1 channel is a major regulator of EGFR signalling.

15.15-15.30 J.P. DECUYPERE, D. KINDT, L. MISSIAEN, H. DE SMEDT, J.B. PARYS, G. BULTYNCK (KULeuven).
Intracellular Ca^{2+} signaling: a novel player in mTOR-dependent autophagy.

15.30-15.45 T. VERVLIET, E. DECROCK, Z. TOMASKOVA, G. MONACO, S. KIVILUOTO, L. MISSIAEN, K.N. NADIF, K. ONDRIAS, L. LEYBAERT, H. DE SMEDT, J.B. PARYS, G. BULTYNCK (KULeuven, UGent, Bratislava, Slovak Republic; Univ. Nijmegen, The Netherlands).
The ryanodine receptor is a novel target for Bcl-2.

15.45-16.00 H. AKL, G. MONACO, R. LA ROVERE, K. DUBRON, A. KAUSKOT, K. WELKENHUYZEN, C. ERNEUX, L. MISSIAEN, J. MOLGÓ, C. DISTELHORST, M. BAES, M. HOYLAERTS, J.B. PARYS, H. DE SMEDT, G. BULTYNCK (KULeuven, ULBruxelles, CNRS Gif sur Yvette, France, Case Western Univ. Cleveland, USA).
Targeting IP_3R/Bcl-2 complexes in B-cell lymphomas: relevance of IP_3R2 upregulation and chronic IP_3 signaling.

16.00-16.15 D. BABU, G. LECLERCQ, Q. REMIJSEN, R. MOTTERLINI, R.A. LEFEBVRE (UGent, VIB, INSERM U955 Univ. Paris Est, France).
Contribution of mitochondrial ROS to TNF-α-induced oxidative stress in murine intestinal epithelial MODE-K-cells.

ABSTRACTS

Legend
O= Oral communication numbered
P = Poster numbered

O-01 (10.45-11.00)

COMBINED INJECTION OF QUINPIROLE AND COCAINE INDUCES A STEREOTYPIC-LIKE BEHAVIOR AND CHANGES IN LFP POWER WITHIN THE VENTRAL TEGMENTAL AREA OF RATS

S. Koulchitsky[1], T. Beeken[1], J. Dethier[2], E. Bullinger[2], V. Seutin[1]

[1]GIGA Neurosciences and [2]Montefiore Institute, University of Liège, B-4000 Sart Tilman/Liège, Belgium

Using a recent telemetric system, we recorded electrical signals via preimplanted microelectrode arrays from the ventral tegmental area (VTA) of freely moving rats. An actimeter was used to track locomotor activity. Recorded signals consisted of slow and fast oscillations of electric potential. During the subsequent analysis, we extracted slower frequencies (up to 300 Hz), which represent local field potentials (LFPs), and presumably reflect dendritic activity from the nearby tissue, and faster frequencies (from 300 Hz), which consisted of individual spikes. Here, we focused on LFPs. Because we had recently found that addition of a low, autoreceptor-selective dose of the D2 agonist quinpirole qualitatively changed the behavioural effect of cocaine, the goal of this study was to test whether this was reflected in the LFPs. We compared the effect of 1) saline, 2) quinpirole alone (100 µg/kg, i.p.), 3) cocaine alone (10 mg/kg, i.p.), 4) quinpirole and cocaine. When compared to saline, quinpirole alone induced a sedative/anti-locomotory action, as expected, while cocaine led to increase in locomotion without clear signs of stereotypy. When injected together, quinpirole + cocaine induced an increased, rhythmic locomotion with downward sniffing. This behaviour appeared to be similar to locomotor stereotypy induced by D2 agonists. When both drugs were co-injected, analysis of LFPs revealed an increase of theta-rythm power (8-12 Hz) in the VTA throughout entire locomotion periods. In contrast, in all other cases, significant increases in the power of theta oscillations was observed only when animals expressed rearing and/or performed active exploration in some particular areas of the experimental box. Thus, the temporal relationship between changes in LFP power and the locomotor pattern was strikingly different when quinpirole was added to cocaine. Although these results are preliminary, they suggest that LFP analysis may be important to understand qualitative changes in some behaviors.

O-02 (11.00-11.15)

ANESTHETIC EFFECT OF THE TRPA1 SPECIFIC AGONIST CINNAMALDEHYDE

Y.A. Alpizar[1], L. van Gerven[2], W. Everaerts[1,3], B. Boonen[1], A. Meningoz[1], F. Vermeulen[3], D. De Ridder[3], P. Hellings[2], T. Voets[1], K. Talavera[1]

[1]Laboratory of Ion Channel Research, Dept. Molecular and Cellular Medicine. [2]Laboratory of Clinical Immunology, Dept. of Microbiology and Immunology. [3]Laboratory of Experimental Urology, Dept. of Development and Regeneration. KU Leuven, Herestraat 49, 3000 Leuven, Belgium.

Cinnamaldehyde (CA) and mustard oil (MO) are natural compounds that activate TRPA1, a member of the family of transient receptor potential (TRP) cation channel expressed in nociceptive neurons. In human subjects, they are perceived as pungent, inducing a burning and tingling sensation when orally administered. Thus far, most of the evidence on the pathophysiological role of TRPA1 has been gathered using MO and CA under the assumption that they are specific agonists of this channel. However, we have recently reported that multiple noxious effects of MO, including visceral irritation and acute pain, are partly mediated by the capsaicin receptor TRPV1. These findings leave researchers with CA as the best option to induce specific activation of TRPA1. Accordingly, we used this compound in several models of chemosensation in mice, including oral aversion, visceral irritation and acute pain and inflammation. Cystometry experiments revealed that intravesical infusion of 10 mM CA induces a significantly smaller decrease of the intracontractile interval (ICI) than infusion of MO. Similarly, a forced drinking experiment showed that 10 mM CA induced less oral avoidance than 10 mM MO. Unlike for MO, both CA-induced bladder irritation and avoidance were absent in *Trpa1* knockout mice. Finally, CA (10 mM) induced significantly less pain behavior compared to MO when injected in the mouse hind paw. Next, we tested whether the differences in irritation properties of CA and MO found in the mouse are also present in humans. For this, we performed a series of experiments on healthy volunteers to whom CA or MO were applied into the nasal cavity via an aerosol. Irritation and pain sensations were assessed using a Visual Analogue Scale (VAS) and with measurements of nasal mucosal potentials (NMPs). Application of aerosols containing up to 20 mM CA did not induce any irritation or pain responses as determined from the VAS scores nor any significant NMP signals. In contrast, MO induced clear responses at the same concentrations. Thus, in both mice and humans, CA produces only very weak responses when compared to MO, which is in sharp contrast with its powerful agonist action on TRPA1. These data strongly suggests that, at concentrations up to 10 mM, CA fails to trigger neuronal excitatory responses leading to acute irritation and pain. We therefore hypothesized that this compound inhibits neuronal firing. In agreement with this hypothesis, we found that hind paw injection of a mixture of CA and MO (10 mM each) induces significantly less nociceptive behavior than the injection of MO alone. This effect does not seem to be mediated by a mechanism depending on TRPA1, as co-injection of MO with the alcohol analog of CA, which is inactive on TRPA1, also induced a highly attenuated pain response when compared to MO alone. Furthermore, CA was also able to dramatically reduce the nocifensive response elicited by capsaicin, a highly specific TRPV1 agonist. Likewise, we found that, in the presence of CA, MO induced significantly weaker nasal irritation in humans than when applied alone, as determined by VAS scores and NMPs.The structure of CA resembles the general structure of aminoester drugs commonly used as local anesthetics (LA). LAs potently block voltage-gated Na^+ (Na_V) channels, thereby inhibiting the excitability of sensory neurons. In patch-clamp experiments we have found that CA blocks voltage-dependent sodium currents (for details see abstract from Boonen *et al*.). In conclusion, besides activating TRPA1, CA also exerts a blocking effect on Na_V channels, locally impairing action potential initiation and propagation.

O-03 (11.15-11.30)

THE ANTIDEPRESSANTS CITALOPRAM AND REBOXETINE REDUCE SEIZURE FREQUENCY IN CHRONIC EPILEPTIC RATS

K. Vermoesen[1], A. Massie[1,2], I. Smolders[1], R. Clinckers[1]

[1]Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neuroscience, Vrije Universiteit Brussel, Brussels, 1090, Belgium, [2]Department of Pharmaceutical Biotechnology and Molecular Biology, Center for Neuroscience, Vrije Universiteit Brussel, Brussels, 1090, Belgium.

For a long time, antidepressants have been thought to possess proconvulsant properties. This assumption however remains controversial since anticonvulsant effects have been attributed to certain antidepressants. To date, it remains unclear which antidepressants can be used for the treatment of epilepsy patients suffering from depression. In this respect, studies investigating the convulsant liability of antidepressants in a chronic epilepsy model can give valuable information. The present study was designed to determine the seizure liability of citalopram and reboxetine in the kainic acid-induced post-status epilepticus model for temporal lobe epilepsy. Two months after the induction of status epilepticus, chronic epileptic rats (n=16) were video-EEG monitored during 7 consecutive weeks. Weeks 1, 3, 5 and 7 served as sham weeks during which the rats received intraperitoneal saline injections for 4 consecutive days, followed by a 3-day sham washout period during which no injections were given. During weeks 2, 4 and 6 rats received intraperitoneal injections with either citalopram (5, 10 and 15 mg/kg, once daily, n=8) or reboxetine (10, 20 and 30 mg/kg, twice daily, n=8) for 4 days, again followed by a washout period of 3 days. Drugs were administered in a randomly assigned fixed-dose regimen per week. Each rat served as its own control. The drug doses were selected based on the doses reported to have antidepressant effects in rats. Citalopram significantly decreased the spontaneous seizure frequency at the highest dose tested, i.e. the mean number of seizures decreased from 12.8 seizures to 8.8 seizures per week (31%) after treatment with 15 mg/kg citalopram. This dose also significantly decreased the cumulative seizure duration. Administration of 5 mg/kg and 10 mg/kg citalopram did not alter the seizure frequency. The two highest doses of reboxetine significantly decreased the spontaneous seizure frequency, i.e. 20 mg/kg reboxetine decreased the seizure frequency from 14.1 to 7.9 (44%) and 30 mg/kg reboxetine decreased the seizure frequency from 11.8 to 7.2 (39%). Both doses also significantly decreased the cumulative seizure duration. Administration of 10 mg/kg reboxetine did not alter seizure frequency. Citalopram and reboxetine had no effect on seizure severity and seizure duration in any of the doses tested. In general we can conclude that antidepressant doses of citalopram and reboxetine have, depending on the dose, an anticonvulsant effect or no effect on spontaneous seizures in the kainic acid-induced post-status epilepticus rat model.

O-04 (11.30-11.45)

MOTOR COORDINATION IN A NEW MOUSE MODEL OF ATHEROSCLEROTIC PLAQUE RUPTURE

L. Roth[1], D. Van Dam[2], C. Van der Donckt[1], D.M. Schrijvers[1], G. Vanhoutte[3], M. Verhoye[3], A. Van Der Linden[3], W. Martinet[1], H. Bult[1,] G.R.Y. De Meyer[1]

[1]Laboratory of Physiopharmacology, [2]Laboratory of Neurochemistry and Behaviour, Institute Born-Bunge and [3]Bio-Imaging Lab, University of Antwerp, Belgium

Apolipoprotein E deficient ($ApoE^{-/-}$) mice with a heterozygous mutation in the fibrillin-1 gene ($Fbn1^{C1039G+/-}$, Marfan phenotype) show an increase in arterial stiffness due to fragmentation of the elastin fibres. We recently showed that this results in exacerbated atherosclerosis and spontaneous plaque ruptures, accompanied by neurological symptoms (e.g. head tilt) and sudden death. The present study focused on motor coordination of the $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice. Female $ApoE^{-/-}$ (control, n=24) and $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice (n=21) were fed a Western type diet (WD) for up to 20 weeks. Female $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice on normal chow diet (ND, n=21) were also included. Coordination was assessed every two weeks starting at 10 weeks of WD or ND by the following tests: gait analysis, stationary beam, wire suspension and accelerating rotarod. From 12 weeks onward, the gait analysis test revealed a significant increase in track width of the $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ (WD) mice as compared with the $ApoE^{-/-}$ (WD) and $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ (ND) mice (2.78±0.04 mm vs. 2.65±0.03 mm and 2.65±0.04 mm, p=0.018) and this effect remained consistent throughout the experiment. Moreover, the increase in track width was observed at a time point before head tilt occurred, indicating that gait analysis can detect neurological symptoms at an early stage. The stationary beam test also revealed a decrease in motor performance of the $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ (WD) mice at 18 and 20 weeks of diet. In conclusion, gait analysis showed the early development of differences in motor coordination between $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ (WD), $ApoE^{-/-}$ (WD) and $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ (ND) mice and can therefore be of value to assess the effect of potential plaque stabilizing drugs.

O-05 (11.45-12.00)

EFFECT OF AUTOPHAGY DEFICIENCY IN VASCULAR SMOOTH MUSCLE CELLS AND CARDIOMYOCYTES ON CARDIOVASCULAR HEALTH

C. Michiels, P. Fransen, D.M. Schrijvers, H. Bult, G.R.Y. De Meyer, W. Martinet

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Autophagy is a cellular housekeeping mechanism that protects the cell by eliminating damaged and misfolded proteins or organelles. During ageing the degree of autophagy in cells declines, leading to the accumulation of harmful cytosolic material and increasing the susceptibility for age-related diseases such as heart failure. Indeed, recent investigation in the cardiovascular field shows that autophagy protects against the development of heart failure. The present study investigated the cardiac and vascular effects of autophagy in $Atg7^{+/+}$ SM22 Cre^{+} (control) and $Atg7^{F/F}$ SM22 Cre^{+} (autophagy deficient smooth muscle cells and cardiomyocytes) mice. $Atg7^{F/F}$ SM22 Cre^{+} mice prematurely died from heart failure with 100% mortality at 7 months of age. Echocardiography showed that the end systolic and diastolic diameter of the heart progressively increased with age in $Atg7^{F/F}$ SM22 Cre^{+}mice. Fractional shortening progressively deteriorated from 50.9±0.8 % at 2 months (not different from control) to 28.4±0.7 % at 3.5 months and to 15.1±1.3 % at 5 months. Moreover, heart weight (HW), HW/body weight and HW/tibia length also significantly increased with age in $Atg7^{F/F}$ SM22 Cre^{+} mice. Vascular reactivity, as investigated by measuring isometric contractions of thoracic aorta segments in organ baths, revealed that segments of $Atg7^{F/F}$ SM22 Cre^{+} mice were more sensitive to potassium and showed higher inositol triphosphate-mediated transient contractions. These vascular effects were already evident at 2 months, whereas effects on cardiac function and dimensions were completely absent at this age. Overall, our study indicates that autophagy deficiency in smooth muscle cells and cardiomyocytes induces vascular changes preceding cardiac abnormalities and heart failure. This conclusion is in line with the theory that deficiencies in autophagy speed up the development of age-related diseases.

O-06 (12.00-12.15)

HYAL-1 DEFICIENCY MAY PROTECT THE ENDOTHELIUM IN A MODEL OF STREPTOZOTOCIN-INDUCED DIABETES

S. Dogné[1], C. Dessy[2], G. Rath[2], N. Caron[3], B. Flamion[1]

[1]Laboratory of Physiology and Pharmacology- URphyM, NARILIS, University of Namur-FUNDP, Belgium [2]Pole of Pharmacology and Therapeutics, University of Louvain, Brussels, Belgium, [3]Laboratory of Physiology– URphyM, NARILIS, University of Namur – FUNDP, Belgium

Hyaluronic acid (HA) is a major component of the endothelium surface layer (ESL) called glycocalyx. It has been suggested that the HA content of the ESL is implicated in the protection of vessel walls and partly mediates the beneficial effect of shear stress on the endothelium. In diabetes, the size and permeability of the glycocalyx is altered. In addition, type 1-diabetic patients have increased plasma levels of both HA and its main metabolising enzyme, Hyal-1. These patients are prone to atherosclerosis, especially in glycocalyx-poor areas such as carotid bifurcations.We decided to investigate the potential implication of Hyal-1 in the development of endothelial dysfunction and atherosclerosis linked to diabetes. Hyal-1 knock-out (KO) mice and wild-type (WT) mice were rendered diabetic by daily injections of streptozotocin (or buffer only, for controls [Ctr]) during 5 days. HA, hyaluronidase activity and markers of endothelial dysfunction (ICAM-1 and VCAM-1) were measured in the plasma of the different groups of animals, 4 weeks after diabetes induction. Myocardial capillary glycocalyx was observed using transmission electron microscopy following Alcian Blue 8GX injections into the aorta. Furthermore, endothelial dysfunction was studied in mesenteric arteries by measuring vasodilatation in response to acetylcholine through different pathways. Finally, the expression levels of connexins and potassium channels in mesenteric arteries were determined by real time PCR. Plasma analyses revealed a higher concentration of HA in KO mice compared to WT mice and confirmed the expected elevation of HA with diabetes in WT mice. In KO mice, however, no additional increase in plasma HA was observed after streptozotocin injections. As expected, the levels of endothelial adhesion molecule ICAM-1 were significantly up-regulated by diabetes in the two mice strains. However, Hyal-1 KO mice had significantly lower basal levels of ICAM-1 and VCAM-1 compared with Ctr mice. For ICAM-1, this difference disappeared after the diabetic challenge. Glycocalyx observation under electron microscopy revealed a similar baseline thickness in KO vs WT mice but completely opposite reactions of the glycocalyx after 4 weeks of hyperglycemia, i.e., an almost total disappearance in WT mice but a reinforcement in KO mice. Endothelium-dependent vasodilation did not differ between healthy WT and KO mice. However, in WT diabetic mice, EDHF-mediated vasorelaxation vanished whereas KO diabetic mice had a preserved EDHF pathway. Analysis of small conductance potassium channel expression showed an up-regulation of SK3 in KO mice, both at basal level and during prolonged hyperglycemia, but no difference in the expression of various connexins. These results suggest that a decreased level of Hyal-1 or increased plasma levels of HA orient the diabetic endothelial response towards a reinforced glycocalyx, less inflammation (reduced spillover of ICAM-1), and a lesser damage to the EDHF-dependent vasodilation pathway. A higher availability of SK3 may play a role in this protection. Our results open the door to potential therapeutic interventions designed to alleviate diabetic angiopathy.

O-07 (12.15-12.30)

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

B. Colsoul[1], G. Jacobs[1], K. Philippaert[1], G. Owsianik[1], A. Segal, F. Schuit[2], R. Vennekens[1]

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

O-08 (14.30-14.45)

EFFECTS OF DPP-IV INHIBITION ON LEFT VENTRICULAR COMPLIANCE IN MICE WITH TYPE II DIABETES

A-S Hervent[1], N. Hamdani[3], V. Matheeussen[2], M. Demolder[1], I. De Meester[2], W. Paulus[3], G. De Keulenaer[1]

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Diabetes cardiomyopathy (DCMP) is a leading cause of cardiac morbidity and mortality. DCMP is characterized by left ventricular (LV) hypertrophy and fibrosis, and by LV diastolic dysfunction, the latter which has been linked to increased cardiomyocyte stiffness. Inhibitors of dipeptidyl peptidase-IV (DPP-IV) are novel drugs for the treatment of patients with type 2 diabetes, shown to improve myocardial metabolism. This study investigates the effects of sitagliptin (SITA), an orally active inhibitor of DPP-IV, on LV structure and function in diabetic mice. Obese male type 2 diabetic mice ($Lepr^{db/db}$, n=24) were treated with SITA (300 mg/kg/day in drinking water) or vehicle during 8 weeks. SITA inhibited more than 85% of serum DPP-IV activity but had no effect on fasting glucose levels. SITA reduced cardiomyocyte size ($p<0.05$) and minimally affected LV collagen fraction. Invasive pressure-volume recordings in anesthetized mice at varying preloads showed that SITA increased LV stroke volume ($p<0.005$), cardiac output ($p<0.005$), LV stroke work ($p<0.001$) and LV compliance ($p<0.05$), whereas LV end-systolic elastance and preload-recruitable stroke work remained unchanged. Furthermore, in isolated cardiomyocytes, SITA reduced cardiomyocyte resting tension ($F_{passive}$) and increased total titin phosphorylation ($p<0.001$). It is concluded that in obese diabetic mice, in absence of hypoglycaemic effects, DPP-IV inhibition by SITA improves overall LV performance and LV compliance. These effects seem at least partially mediated by effects of SITA on the phosphorylation status of total titin and on the cardiomyocyte stiffness modulus.

O-09 (14.45-15.00)

SCREENING AND CHARACTERIZATION OF PHARMACOLOGICAL TOOLS TO TARGET CA^{2+} ACTIVATED NON-SELECTIVE CATION CHANNELS

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TRP proteins form cation channels that are regulated through strikingly diverse mechanisms. TRPM4 and TRPM5 have been recently indentified as monovalent cation selective channels, activated by a rise in the intracellular calcium concentration. TRPM5 has an important role in the release of insulin from the pancreatic ß-cell, and the signal transduction from taste cells to the central nervous system. TRPM4 is important for the activation state of mast cells, and might be a novel drug target for allergic diseases. Furthermore evidence is accumulating that TRPM4 plays an important role in blood pressure regulation and cardiac contraction. To date there are no pharmacological tools available to evaluate their potential as drug targets. In this study we present a novel screening method for identifying compounds, which target these channels. We used a fluorescence based high throughput device to visualize intracellular [Na^{+}] dynamics to screen an extensive library of compounds. We further characterized initial hit compounds modulating the channels, using patch clamp. The main focus was on an activator of TRPM5 showing potent, reversible and specific activation of TRPM5 over TRPM4 in micromolar concentrations. We observed TRPM5 activity even in resting [Ca^{2+}] and show that the open probability of the channel shifts towards more negative potentials in the presence of the compound. This could potentially play an important role in the development of new taste modulators, and novel treatments for type 2 diabetes.

O-10 (15.00-15.15)

TRPC1 CHANNEL IS A MAJOR REGULATOR OF EGFR SIGNALING

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TRP channels have been associated with cell proliferation and aggressiveness in several cancers. In particular, TRPC1 regulates cell proliferation and motility, two processes underlying cancer progression. We and others have described the mechanisms of TRPC1-dependent cell migration. However, the involvement of TRPC1 in cell proliferation remains unexplained. In this study, we show that siRNA-mediated TRPC1 depletion in non small cell lung carcinoma cell lines induced G0/G1 cell cycle arrest resulting in dramatic decrease in cell growth. The expression of cyclins D1 and D3 was reduced after TRPC1 knock-down, pointing out the role of TRPC1 in G1/S transition. This was associated with a decreased phosphorylation and activation of EGFR and with a subsequent disruption of PI3K/Akt and MAPK downstream pathways. Stimulation of EGFR by its natural ligand, EGF, induced Ca^{2+} release from the endoplasmic reticulum and Ca^{2+} entry through TRPC1. Ca^{2+} entry through TRPC1 conversely activated EGFR, suggesting that TRPC1 is a component of a Ca^{2+}-dependent amplification of EGF-dependent cell proliferation.

O-11 (15.15-15.30)

INTRACELLULAR CA^{2+} SIGNALING: A NOVEL PLAYER IN MTOR-DEPENDENT AUTOPHAGY

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Autophagy, a lysosomal degradation pathway important for cellular homeostasis and survival, is induced via inhibition of the mammalian target of rapamycin (mTOR). Intracellular Ca^{2+} also regulates autophagy, but its role seems to be complex. Basal constitutive Ca^{2+} signals from endoplasmic reticulum (ER) towards mitochondria through the inositol 1,4,5-trisphosphate receptor (IP_3R), an ubiquitous ER Ca^{2+}-release channel, enhance ATP production and hence suppress autophagy. In addition, we have found that IP_3R-mediated Ca^{2+} signals are also essential for autophagy stimulation by inhibitors of mTOR activity as nutrient starvation or rapamycin. Interestingly, during these treatments, a polymodal sensitization of the intracellular Ca^{2+} machinery occurs in order to generate these pro-autophagic Ca^{2+} signals. Our results point to a dual role for IP_3Rs and intracellular Ca^{2+} in autophagy regulation and reveal intracellular Ca^{2+} signals as novel essential players in the canonical mTOR-dependent autophagy pathway.

O-12 (15.30-15.45)

THE RYANODINE RECEPTOR IS A NOVEL TARGET FOR BCL-2

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Ca^{2+} signals control the balance between cell survival and cell death. B-cell lymphoma (Bcl)-2 family members are known regulators of these Ca^{2+} signals. Bcl-2 itself targets the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), a major Ca^{2+}-release channel localized in the endoplasmic reticulum (ER). Bcl-2 suppresses IP_3-induced Ca^{2+} release and protects cells from apoptosis. Until now, it is unknown if this interaction is shared with other intracellular Ca^{2+}-release channels. Therefore, we set out to identify the possible interaction of Bcl-2 with the ryanodine receptor (RyR), another important ER-localized intracellular Ca^{2+}-release channel. First, we found that HEK293 cells up-regulated their endogenous Bcl-2 levels upon ectopic expression of RyR3. A similar up-regulation of Bcl-2 levels with increasing RyR levels was found in C2C12 cells differentiating to myotubes. Second, co-immunoprecipitation experiments demonstrated that both ectopically overexpressed and endogenous Bcl-2 interact with RyR3 in RyR3-overexpressing HEK293 cells. Co-immunoprecipitation of endogenous RyRs and Bcl-2 from differentiated C2C12 cells demonstrated the existence of endogenous RyR/Bcl-2-protein. Third, GST-RyR domains corresponding to a.a. 2274-2690 (on RyR3) were made and purified. Utilizing a biotinylated-BH4 domain of Bcl-2 and these GST-RyR domains in surface plasmon resonance experiments, we found that all these RyR fragments bound to biotin-BH4-Bcl-2. Finally, single cell Ca^{2+} imaging showed that overexpression of Bcl-2 in HEK293 cells overexpressing RyR3 resulted in a reduced caffeine-induced Ca^{2+} release. In conclusion, our data indicate that Bcl-2 interacts and regulates the RyR.

O-13 (15.45-16.00)

TARGETING IP_3R/BCL-2 COMPLEXES IN B-CELL LYMPHOMAS: RELEVANCE OF IP_3R2 UPREGULATION AND CHRONIC IP_3 SIGNALING

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IP_3Rs, which play a critical role in cell survival and death, are tightly regulated by anti-apoptotic Bcl-2 proteins. We developed a novel peptide tool (TAT-IDP^S) that targets the BH4 domain of Bcl-2, reverses Bcl-2's inhibitory action on IP_3Rs and sensitizes cells to apoptotic stimuli. TAT-IDP^S triggered pro-apoptotic Ca^{2+} signaling in cells from chronic lymphocytic leukemia patients. However, it is not clear why some cancer cells are more vulnerable towards TAT-IDP^S than others. Thus, we studied a set of "primed to death" cancer cell lines (KARPAS, TOLEDO, PFEIFFER, SU-DHL-4 and OCI-LY-1) derived from diffuse large B-cell lymphomas (DL-BCL). We found a heterogeneous response of these cell lines to TAT-IDP^S exposure. These responses correlated with the occurrence of TAT-IDP^S-induced aberrant Ca^{2+}-signaling events mediated through the IP_3R. The TAT-IDP^S responsiveness of the different cell lines displayed a positive correlation with the upregulation of IP_3R2, the IP_3R isoform with the highest sensitivity to IP_3. Blocking IP_3R activity or reducing IP_3R2 levels protected against TAT-IDP^S-induced apoptosis. Since most DL-BCL cells display chronic B-cell-receptor activation, the importance of on-going IP_3 signaling for TAT-IDP^S-induced apoptosis was assessed. Pharmacological inhibition of phospholipase-C activity suppressed TAT-IDP^S-induced apoptosis. HepG2 cells, a hepatocyte cell line displaying a natural high level of endogenous IP_3R2 but without stimulated IP_3 signaling, was resistant to TAT-IDP^S. This indicates that the combination of IP_3R2 upregulation with chronic IP_3 signaling renders cells addicted to high levels of Bcl-2 to prevent aberrant IP_3R activity.

O-14 (16.00-16.15)

CONTRIBUTION OF MITOCHONDRIAL ROS TO TNF-A-INDUCED OXIDATIVE STRESS IN MURINE INTESTINAL EPITHELIAL MODE-K CELLS

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In an in-vitro model using the mouse intestinal epithelial cell line, MODE-K, we previously showed that TNF-α/cycloheximide (CHX)-induced apoptosis corresponded with the occurrence of reactive oxygen species (ROS) production, and that resveratrol (75 µM), a polyphenolic antioxidant in red wine, reduced both effects (Babu et al., Curr Pharm Des. 2012). Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and mitochondrial oxidative phosphorylation are considered to be the two major endogenous sources of ROS production in cells. We therefore investigated the contribution of mitochondrial ROS to TNF-α-induced oxidative stress in MODE-K cells. Carboxy-H_2DCFDA is a widely used fluorescent probe to measure total intracellular ROS; DHR123 is a probe that can easily cross cell membranes and react with ROS in mitochondria to generate the positively-charged rhodamine 123 (R123), and thus is a useful probe to measure mitochondrial ROS production. MODE-K cells were exposed to various concentrations of TNF-α/CHX for 6h; simultaneous detection of ROS production and cell death has been performed using either carboxy-H_2DCFDA or DHR123 together with Sytox Red in a single experimental setup using flow cytometric analysis. Treatment of TNF-α/CHX increased mean fluorescence intensity (ΔMFI) of both Carboxy-H_2DCFDA and DHR123-derived fluorescence in a concentration-dependent manner with parallel increase in cell death as measured with Sytox Red. Pretreatment with resveratrol significantly reduced the TNF-α/CHX-induced ΔMFI of both ROS sensitive probes implying that resveratrol reduced both the total ROS and mitochondria-derived ROS; in parallel resveratrol reduced TNF-α/CHX-induced cell death. Mitochondrial ROS thus seems an important contributor to TNF-α-induced oxidative stress in intestinal epithelial cells.

P-01

ELECTROPHYSIOLOGICAL RESPONSES TO VAGUS NERVE STIMULATION IN RATS

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Vagus nerve stimulation (VNS) for refractory epilepsy requires optimization of stimulation parameters in order to improve clinical outcome. Experimental research showed that VNS exerts its effect by activating afferent, fast-conducting fibers. There is however a clear need for an objective parameter reflecting effective stimulation. We recorded electrophysiological responses to stimulation of the vagus nerve in rats. Rats were implanted with a stimulation electrode around the left cervical vagus nerve. Recordings were made using thin point electrodes placed on the vagus nerve rostral to the stimulating cathode. The vagus nerve was stimulated under anesthesia with a charge-balanced biphasic pulse (5µs/phase). The electrophysiological response recorded from the vagus nerve consisted of an early and a late component, identified as respectively an afferent compound action potential (CAP) and a far field potential of the larynx motor evoked potential (LMEP). The $I_{50\%}$ for the CAP and LMEP (respectively 1.9 ± 0.3 mA and 1.6 ± 0.1 mA) were not significantly different. Mean latency for the CAP and LMEP at 1.3 ± 0.3 mm rostral to the stimulating cathode, were 0.4 ± 0.1 ms and 2.0 ± 0.2 ms respectively. At 3.1 ± 0.6 mm rostral to the stimulating cathode, a difference in response latency was measured for the CAP. Conduction velocity was calculated to be 32.5 ± 2.5 m/s. Based on the measured distance between the cuff electrode and the laryngeal muscles, conduction velocity of the efferent action potentials leading to the LMEP was calculated to be 33.3 ± 1.3 m/s. Mean rheobase and chronaxy for the CAP were respectively 35.0 ± 5.0 µA and 40.0 ± 3.5 µs. Short biphasic pulses with an intensity of 1.5-2.5mA activate fast-conducting vagus nerve fibers. Our set-up can be used to evaluate the effects of different stimulation parameters at the level of the cervical vagus nerve in epilepsy models.

P-02

EFFECT OF MK801 ON DOPAMINE AND GLUTAMATE RELEASE AFTER SYSTEMIC L-DOPA ADMINISTRATION IN THE SUBTHALAMIC NUCLEUS OF INTACT AND HEMI-PARKINSON RATS

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Currently, L-3,4-dihydroxyphenylalanine (L-DOPA) is the most effective treatment for the motor symptoms in Parkinson's disease (PD). However, long term treatment with L-DOPA is associated with several motor complications such as dyskinesia. Clinical improvement of this chronic treatment is therefore needed. Lesions or high frequency stimulation of the subthalamic nucleus (STN), a glutamatergic (GLU) nucleus which has been demonstrated to be hyperactive in PD, alleviate the motor symptoms and reduce dyskinesia, either directly and/or by allowing the reduction of the L-DOPA dose. It has been hypothesized that *N*-methyl-D-aspartate (NMDA) receptor antagonists might have similar actions by reducing the STN output and/or by altering the GLU-ergic input to the STN. However, little is known about the neurochemical changes in the STN induced by co-administration of L-DOPA with a NMDA receptor antagonist. By means of *in vivo* microdialysis, the effect of dizocilpine (MK801), an NMDA receptor antagonist, on the extracellular DA and GLU levels after systemic administration of L-DOPA was investigated in the STN of intact and 6-hydroxydopamine-lesioned rats. Extracellular DA levels in the STN increased after L-DOPA (25 mg/kg i.p. after benserazide 10 mg/kg i.p.) administration in intact and DA-depleted rats. There was a tendency to a higher DA-increase in hemi-parkinson rats compared to controls. MK801 (0.1 mg/kg i.p.) did not influence the L-DOPA induced DA release in intact animals. In contrast, MK801 enhanced the L-DOPA induced DA release in hemi-parkinson rats. The extracellular GLU levels in the STN did not alter in both intact and lesioned rats after L-DOPA or L-DOPA/MK801 administration. The present study does not support the hypothesis that MK801 alters the GLU levels in the STN. However, NMDA receptor antagonists could be used as a beneficial adjuvant treatment of PD by enhancing the therapeutic efficacy of L-DOPA.

P-03

DEVELOPMENT AND CHARACTERIZATION OF THE NIGRAL LACTACYSTIN MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a neurodegenerative disorder with important motor manifestations. Various pathogenic pathways drive disease progression, including protein (α-synuclein) aggregation, oxidative stress, mitochondrial dysfunction, glutamate excitotoxicity, and proteasomal impairment. Different animal models of PD have been developed that simulate one or more aspects of the human pathogenesis. However, most fail to reproduce the accumulation and aggregation of α-synuclein, an event that is ubiquitous in the human pathology. A recent approach in this direction involves the local administration of proteasomal inhibitors, such as lactacystin (LAC), to the rodent nigrostriatal tract, that induces both neurodegeneration, as well as α-synuclein pathology. LAC mouse models of PD have been generated, however, only by local administration of the toxin to the medial forebrain bundle, leading to retrograde transport to the substantia nigra pars compacta (SNc), but possibly also anterograde transport to the striatum. However, the proteasome activity has been found to be decreased in the SNc of PD patients, but not in the striatum. In order to develop a more specific model of proteasomal inhibition, we investigated the effect of the local administration of LAC to the SNc of mice. Our data show that nigral administration of LAC leads to a dose dependent decrease in motor function, as well as to a dose dependent degeneration of the nigrostriatal tract, both at the level of the SNc and the striatum. Therefore, we found that nigral proteasomal inhibition can model PD in mice both at the cellular and behavioural level. Further investigation will also reveal additional features of the model, including the level of α-synuclein pathology, as well as the involvement of other pathogenic pathways, such as glutamate excitotoxicity.

P-04

ESTABLISHMENT OF AGE AND GENDER DEPENDENT REFERENCE VALUES FOR NOVEL URINARY BIOMARKERS FOR RENAL DAMAGE IN THE HEALTHY POPULATION

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Recent research has focused on the discovery of novel urinary biomarkers for kidney damage. Amongst others, urinary KIM-1 and NGAL are promising biomarkers in a wide variety of renal pathologies. However, little is known about the normal biomarker concentrations in urine of healthy subjects. Therefore, the goal of our study was to establish reference values for urinary KIM-1, NGAL, NAG and Cystatin C in a healthy population, taking into account possible effects of age and gender. Urine samples were collected from 338 healthy, non-smoking men and women between 0 and 95 years old. Next to the urinary levels of KIM-1, NGAL, NAG and cystatin C, creatinine values and the specific gravity of the urine samples were determined, in order to correct for urinary dilution. For the absolute urinary concentrations of the biomarkers, age had a significant effect on all the biomarkers, except for cystatin C. Gender had an outspoken effect on all of them, except for NAG. Normalisation of biomarkers for creatinine and specific gravity affected the correlation between the biomarkers on one hand and age and gender on the other. In conclusion, age and gender have distinct effects on KIM-1, NGAL, NAG and cystatin. Based on this knowledge, age and gender specific reference values for KIM-1, NGAL, NAG and Cystatin C were established.

P-05

THERAPEUTIC POTENTIAL OF ANTI-TRANSFORMING GROWTH FACTOR-B ANTIBODY ON ACUTE TUBULO-INTERSTITIAL INJURY IN ARISTOLOCHIC ACID NEPHROPATHY

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Aristolochic acid nephropathy (AAN) characterized by rapidly progressing renal fibrosis of toxic origin is primed by acute injury of proximal tubular epithelial cells (PTEC). Anti-transforming growth factor β (TGFβ) antibody has been shown to improve renal fibrosis in various models of glomerular diseases, but its roles in primary tubulo-interstitial nephropathies are not yet well known. We studied the efficacy of a murine pan-specific anti-TGFβ monoclonal antibody (1D11) in an acute phase of AAN. Weight matched rats were daily sc. injected with AA (15 mg/kg/day) or vehicle (polyethylene glycol-PEG) from day 0 to day 5. Four groups (n=6/group) were randomly assessed: PEG+1D11; AA alone; AA+1D11 and AA+control isotype (13C4). The 1D11 and 13C4 antibodies (5 mg/kg) were administered ip. at days -1, 0, 2 and 4. After 5 days of treatment, renal function and morphology remained normal in the control group PEG+1D11. 1D11 statistically attenuated AA induced acute kidney injury, as attested by less increased creatininemia and urinary excretion of N-acetyl-β-glucosaminidase, less severe necrosis of PTEC from S3 segment and reduced macrophages infiltration in outer medulla. Intrarenal regulatory T cells infiltration and neo-angiogenesis around the injured areas were also reduced by 1D11. The proliferation of PTEC from S1-3 segments was maintained with 1D11. Our results demonstrate that anti-TGFβ antibody significantly attenuates acute PTEC injury, reduces macrophages infiltration and modulates the regulatory T cells immune response. Therefore, 1D11 could be a potential, new renoprotective therapy interfering with early fibrogenesis events after a toxic insult.

P-06

ANALYSIS OF RENAL PHENOTYPE OF HYALURONIDASE-1 OR HYALURONIDASE-2 KNOCKOUT MICE

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Hyaluronan (HA), a polysaccharide presents in the extracellular matrix, can reach a size of 6 to 8 MDa, is involved in many biological processes depending on its molecular size, i.e. cell migration and differentiation during embryogenesis, regulation of extracellular matrix organization, metastasis, wound healing and inflammation. HA is synthesized by hyaluronan synthases (HAS) and degraded by hyaluronidases (HYALs). Hyal1 and Hyal2 are now considered as the major hyaluronidases in somatic tissues, acting synergistically to degrade high molecular weight HA. In the kidney, HA distribution is highly heterogeneous, from a high concentration in the interstitium of the renal inner medulla to only very small amounts of HA found in the cortex and the outer medulla. This high concentration in the inner medulla is believed to play a role in renal water handling but also to provide a support for tubules and blood vessels. In view to these points, the aim of our study was to analyse, in *Hyal1 -/-* and *Hyal2 -/-* mice compared to their reference littermates, the parameters that characterize renal excretory capacities and renal water handling in response to water deprivation or acute water loading. Our results indicate that *Hyal1 -/-* mice are characterized by an impaired ability to concentrate urine after water deprivation. Moreover, these mice demonstrated a significant delay in the diuretic response induced by an acute water loading, as it was also the case for *Hyal2 -/-* mice. Taking together, these observations indicate that *Hyal1-/-* mice and *Hyal2 -/-* mice present some impairment in the urine concentration mechanisms, that could be related to changes in intrarenal HA metabolism that remains to be elucidated.

P-07

ANALYSIS OF CHRONIC HAEMOLYSIS IN HYAL2$^{-/-}$ MICE

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Our laboratory has generated Hyal2-deficient mice through a conditional Cre-lox system to study the function of this hyaluronidase in the catabolism of hyaluronan (HA). Indeed, the role of Hyal2 in the turnover of HA is of major interest because the HA fragments generated by the action of Hyal1 and Hyal2 are endowed with wide-ranging and often opposing biological functions. Smaller polysaccharide fragments, for example, are inflammatory, immuno-stimulatory, angiogenic, and anti-apoptotic. The Hyal2$^{-/-}$ mice display a surprising phenotype mostly comprised of skeletal and haematological abnormalities. The latter include thrombocytopaenia and chronic compensated haemolysis. Moreover, HA concentration in Hyal2$^{-/-}$ plasma is significantly higher than in the control group. By using an anti-murine monoclonal C5 antibody (a kind gift of Alexion Pharmaceuticals), we discovered that the haemolysis is independent of the complement in vivo. Furthermore, Hyal2$^{-/-}$ red blood cells (RBCs) display osmotic fragility. Finally, we demonstrated the presence of Hyal2 in RBC membranes of control mice using Western blots. The aim of this study is to determinate whether chronic haemolysis in Hyal2$^{-/-}$ mice is intrinsic or extrinsic to the RBCs. To this aim we monitored the survival of transfused biotin-positive RBCs in both strains of recipient mice. Our results show that the half-life of Hyal2$^{-/-}$ RBCs is dramatically reduced to 7-8 days (versus ± 22-24 days in control mice). The survival of Hyal2$^{+/-}$ or Hyal2$^{-/-}$ RBCs following transfusion into Hyal2$^{-/-}$ recipient mice is in agreement with an extracorpuscular origin for the observed haemolysis. However, to our surprise, neither splenectomy nor a decrease of plasma HA concentration through 4-methylumbelliferone treatment was able to normalize the clearance of Hyal2$^{-/-}$ RBCs in Hyal2$^{-/-}$ mice. However, during the first week of treatment with liposome-encapsulated clodronate, an agent known to deplete systemic macrophages in vivo, the survival of Hyal2$^{+/-}$ and Hyal2$^{-/-}$ RBCs was almost identical, whereas treatment with liposome-encapsulated PBS (control) had no effect on the shortened survival of Hyal2$^{-/-}$ RBCs. This may indicate that an increased removal by macrophages is responsible for the shortened half-life of Hyal2$^{-/-}$ RBCs. In conclusion, the origin of chronic haemolysis in Hyal2$^{-/-}$ mice is mainly extracorpuscular, suggesting that the Hyal2$^{-/-}$ environment itself plays a crucial role in abnormal RBC turnover, although elevated plasma [HA] does not seem to be the major factor. The role of Hyal2$^{-/-}$ macrophages and endothelium is currently being explored.

P-08

LYMPH NODE ALTERATIONS IN HYAL2 DEFICIENT MICE

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Hyaluronan (HA) is an abundant glycosaminoglycan (GAG) of the extracellular matrix. The hyaluronidases Hyal1 and Hyal2 are the sole enzymes responsible for HA degradation in somatic tissues. Metabolic studies based on labeled HA injections have shown that HA molecules have a short half-life of only 1 or 2 days in most tissues and of 3 to 5 minutes in the blood. However, the processes of HA turnover remain insufficiently studied. There is now clear evidence that a part of HA catabolism occurs within the tissues (Jadin, Bookbinder et al. 2012) and that the remaining HA is driven by afferent lymph to the local lymph nodes where a second part of the degradation takes place (Fraser, Kimpton et al. 1988). The cells responsible for this process in the lymph node appear to be the sinusoidal endothelial cells lining the medullary sinuses. The more likely receptors involved are stabilin-2 (Stab2) and LYVE-1 (Jackson 2009). The objective of this work is to collect and use the precious information provided by $Hyal2^{-/-}$ (knockout) mice to study the function of Hyal2 and the role of the lymph nodes in HA turnover. Hyal2 deficient mice show craniofacial and vertebral abnormalities, 10-fold increase in HA plasma level, chronic haemolysis with splenomegaly, thrombocytopenia, and HA accumulation in liver sinusoids (Jadin et al., 2008). Hyal2 is expressed in most endothelia throughout the body and is particularly abundant in liver sinusoidal endothelial cells. Within the lymph nodes, Hyal2 is present in cells lining the medullary sinuses and in high endothelial venules. While there is no systematic accumulation of HA in $Hyal2^{-/-}$ tissues, we have observed enlargement and gross distortion of the lymph nodes in these mice. Medullary sinuses appear widened and empty while lymphoid follicles are scattered. HA content is increased more than 2-fold in $Hyal2^{-/-}$ lymph nodes compared with those of heterozygous ($Hyal2^{+/-}$) mice. HA accumulates in the lymph and within the nodal tissue. This increased HA content persists with age whereas old $Hyal2^{+/-}$ lymph nodes progressively lose this GAG. We have also observed that the expression of Stab2 is decreased, and HA and LYVE-1 partly colocalize, in Hyal2 KO lymph nodes while there is no colocalization in $Hyal2^{+/-}$ mice. Despite the disorganized appearance of the lymphoid follicles, preliminary studies showed no significant modification of lymphocyte populations. In summary, Hyal2 deficiency induces a large accumulation of HA in the lymph nodes with resulting distortion of their normal architecture in the absence of concomitant excess of peripheral tissue HA. Endothelial Hyal2, perhaps in connection with Stab2, forms the basis of a lymph-node specific HA clearance mechanism.

P-09

EVALUATION OF COLOSTRAL ANTIBODY PROTECTION IN LAMBS BORN FROM EWES INFECTED WITH SCHMALLENBERG VIRUS DURING PREGNANCY

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In autumn 2011, the sheep flock of the University of Namur was affected by Schmallenberg virus (SBV), a novel Orthobunyavirus from the Simbu serogroup causing congenital malformations in newborn lambs. The present study provides information about colostral antibody protection in lambs born from ewes infected with SBV during pregnancy. A total of 13 lambs born in January 2012 from SBV-seropositive ewes were investigated. Serum was isolated from blood collected at birth before colostrum intake (t0), 36h after colostrum intake (t36) and then every 14 days. Serum was used for serum neutralisation test (SNT) with serial dilutions (1/2 to 1/4096). Results of SNT were expressed as the efficient dilution 50% (ED50): the dilution that neutralises 50% of the challenge virus. Among the studied group, 7 lambs were seropositive at t0. There was no significant difference in changes in serum anti-SBV antibodies levels between seropositive and seronegative newborn lambs from t36 until 18 weeks after birth ($p<0.05$). Furthermore, a significant decrease of serum anti-SBV antibodies was observed 14 weeks after birth ($p<0.05$). Further studies are needed to investigate the extent and length of colostral antibody protection in a larger number of animals and with other techniques of antibodies detection such as competitive ELISA. This could be a major point of interest concerning the effect of passive immunity against future vaccinal efficacy.

P-10

BODY COMPOSITION IN MALNOURISHED CHILDREN IN BUKAVU, DR CONGO. CAN ELECTRICAL IMPEDANCE BE USED FOR EDEMA EVALUATION AND MONITORING?

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Changes in body composition including alterations in body fluid compartments occur during malnutrition. Techniques using body electrical impedance measurements are increasingly being preferred in studies of body composition and have been widely validated in various adult populations. Till now, most bio-impedance measurements in children have used a single frequency (50 kHz), which can provide estimates of body composition using empirical equations. We used multifrequency (4-1000 kHz) bio-impedance spectroscopy to compare body composition between 22 malnourished children with clinical edema (kwashiorkor) and 21 malnourished children without edema. Total body water (TBW) was higher in edematous children, due to an increase of both extracellular fluid (ECF) and intracellular fluid (ICF) volumes. Interestingly, the relative contributions of ECF and ICF to total body weight were not different between the two groups. Fatty mass was decreased in kwashiorkor. During treatment, weight gain was observed in both groups. In edematous children, this was associated with a decrease of ECF, ICF, TBW and FFM. The study suggests that edema due to malnutrition in children is due to excessive extracellular as well as intracellular water accumulation. Bio-impedance spectroscopy may provide an accurate method to assess the nutritional state during follow-up of malnourished children.

P-11

BH3 MIMETIC HA14-1, BUT NOT ABT-737, POTENTIATES PRO-APOPTOTIC CA^{2+} SIGNALING IN NORMAL AND TRANSFORMED CELLS

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Overexpression of the anti-apoptotic Bcl-2-family members is a common feature in cancer. The hydrophobic cleft of these proteins docks and inhibits the Bcl-2 homology (BH) domain 3 of their pro-apoptotic counterparts. Although therapeutic targeting of the anti-apoptotic Bcl-2 proteins with BH3 mimetics (e.g. ABT-737, HA14-1) is a promising new anticancer approach, the latter can also affect Ca^{2+} homeostasis. The potential dysregulative role of ABT-737 on Ca^{2+} homeostasis in normal cells is therefore still a subject of concern. In addition, another facet of the anti-apoptotic Bcl-2 proteins is their inhibitory effect on inositol 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} signaling from the endoplasmic reticulum (ER). For this effect, it is the BH4 domain of anti-apoptotic Bcl-2 proteins that targets a relatively conserved region in the modulatory domain of the IP_3R. We developed a peptide corresponding to this binding site (indicated here as TAT-IDP^S for stable TAT-conjugated IP_3R-derived peptide). This peptide enhanced IP_3R-mediated pro-apoptotic Ca^{2+} signals in cancer B-cells. In present study, we explored the possibility to use BH3 mimetics in a combination regimen with TAT-IDP^S treatment to trigger apoptosis in Bcl-2 overexpressing cells. However, we observed a difference between HA14-1 and ABT-737 in their apoptotic action. While both drugs had an additive effect on TAT-IDP^S-induced apoptotic ER Ca^{2+} release in TAT-IDP^S-sensitive cells, only HA14-1 sensitized TAT-IDP^S-resistant cells to the TAT-IDP^S apoptotic effect. Accordingly, HA14-1, but not ABT-737, did trigger changes in intracellular Ca^{2+} signaling in platelets and HeLa cells. Both BH3 mimetics acted on the SERCA ER Ca^{2+}-uptake activity rather than on the ER Ca^{2+}-efflux pathways with a more potent effect for HA14-1. Our findings pave the way for a more specific anti-cancer therapy using a potential compound targeting the BH4 domain of Bcl-2 in combination with a BH3 mimetic at low concentrations.

P-12

A DICODON MUTATION IN THE BH4 DOMAIN OF BCL-2 REVEALS NEW STRUCTURAL DETERMINANTS INVOLVED IN THE INHIBITION OF INOSITOL 1,4,5-TRISPHOSPHATE RECEPTORS (IP_3RS)

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The anti-apoptotic B-cell lymphoma 2 (Bcl-2) typically protects against mitochondrial outer membrane permeabilization (MOMP) by heterodimerizing with its pro-apoptotic relatives on mitochondria. Additionally, Bcl-2 can directly inhibit the endoplasmic reticulum (ER)–resident inositol 1,4,5-trisphosphate-receptors (IP_3Rs) and consequently hamper a pro-apoptotic Ca^{2+} transfer from ER to mitochondria. Although the hydrophobic cleft of Bcl-2, formed by the BH3-BH1-BH2 domains, is the main region required for its mitochondrial activity, the only Bcl-2 portion sufficient for IP_3Rs inhibition is the N-terminal BH4 domain. Here, we wanted to explore the importance of BH4 domain structural organization for IP_3Rs/Bcl-2 interaction and function taking also into account the central role of the hydrophobic cleft. To accomplish this, we performed suitable molecular dynamic simulations alongside a series of biochemical, biophysical and cell biological experimental approaches. We focus our attention on a dicodon mutation in Bcl-2's BH4 domain (I14G-V15G) which was previously report to abolish Bcl-2's anti-apoptotic function. In this regard, we found that Bcl-2 IV/GG mutation altered BH4 domain's secondary structure disturbed Bcl-2-binding to IP_3R1 and ultimately disrupted the anti-apoptotic Bcl-2-modulation of IP_3–induced Ca^{2+} release (IICR). Furthermore, the simulation of Bcl-2 IV/GG's natural motion revealed re-arrangements in the overall structure of this Bcl-2 mutant, influencing the structural organization of Bcl-2 hydrophobic cleft. This result was underpinned by biochemical experiments showing that Bcl-2 IV/GG failed to bind the pro-apoptotic protein Bax. Therefore, the structural organization of the BH4-domain in the full-length Bcl-2 protein is not only critical for binding and regulating IP_3R channels but also for guaranteeing the proper conformation of the hydrophobic cleft required to counteract pro-apoptotic Bcl-2-family members. All together these results may serve as a platform for additional structural and biological experiments aimed at understanding the fine structural cross-talk between BH4 domain and hydrophobic cleft in the regulation of Ca^{2+}-mediated apoptosis by Bcl-2 protein.

P-13

NON-HOMOGENOUS DISTRIBUTION OF TRPM7 IN CARDIAC VENTRICULAR MYOCYTES

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TRPM7 is a ubiquitous non-selective cation channel, and is thought to be involved in magnesium homeostasis, in cell growth and in diverse signaling processes as well as in disease processes like neuronal ischemia and atrial fibrillation. Our previous studies using electrophysiological methods on cardiac myocytes have identified a magnesium-inhibited cation channel with biophysical and regulation properties identical to those of heterologously-expressed TRPM7 channel. However, although the presence of TRPM7 proteins in cardiac tissue has been demonstrated, direct evidence for their presence in ventricular contractile cells is still lacking. We used immunocytochemistry and confocal microscopy on isolated, single ventricular myocytes to confirm the presence of TRPM7 protein and define its membrane and subcellular localization. By applying a highly specific TRPM7 polyclonal antibody with multiple animal species reactivity, we detected the presence of TRPM7 mainly on the surface membranes of rat, pig and mouse myocytes. TRPM7 was found not to be homogenously distributed in the membrane, with a higher density of expression at the intercalated disks. There was also a preferential localization in the Z zones of the sarcomeres, as well as in a perinuclear domain, suggestive of a presence of newly synthesized channels within the Golgi complex. The inhomogeneous distribution of TRPM7 in the plasma membrane of cardiac myocytes is in contrast with a homogeneous distribution in cardiac fibroblasts, and suggests an interaction of the channel with certain cytoskeletal components present at the points of high expression level, as well as a potential role of the channel in the coupling between cells.

P-14

OSMOSENSATION IN TRPV2 DOMINANT NEGATIVE EXPRESSING CELLS

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Skeletal muscle fibres exposed to increased osmolarity elicit initial cellular shrinkage followed by a regulatory volume increase (RVI). So far, mechanosensing mechanism stays unknown. The process involves the activation of sodium-potassium-chloride cotransporter (NKCC) and is accompanied by a transverse tubular system dilation and a consecutive release of Ca^{2+} from the sarcoplasmic reticulum (SR). We previously showed the involvement of TRPV2 ion channel in eccentric contractions in dystrophic muscle. This channel is Ca^{2+} permeable and seems to be activated by membrane stretch and/or osmotic stress. To investigate the possible role of TPRV2 in muscle RVI, we challenged control muscle fibres and fibres overexpressing a dominant negative mutant of TRPV2 (TRPV2DN) with hyperosmotic medium. We measured cytosolic concentration of Ca^{2+} by using Fura-2 probe and evaluated changes in cell volume by measuring fibres diameter. As expected, C57 fibres incubated in hyperosmotic medium (400 mOsm) elicited a fast cytosolic Ca^{2+} transient followed by a long lasting decay of Ca^{2+} concentration. This was accompanied by a fast cell shrinkage (20 % of initial volume) followed by a late RVI (10% of initial volume). In the absence of external Ca^{2+}, only the first phase stayed present confirming the release of Ca^{2+} from the SR. In the presence of Gd^{3+}, a non specific inhibitor of Ca^{2+} channels, or GsMTx4 toxin, an inhibitor of stretch-activated channels, the first phase was largely reduced and the second was absent. Surprisingly, the use of bumetanide (a specific inhibitor of NKCC) also decreased both phases, suggesting that the release of Ca^{2+} was triggered by membrane depolarization induced by Na^{+}, K^{+} and Cl^{-} entry. Interestingly, we found that in TRPV2DN fibres, hyperosmotic shock induced cell shrinkage but induced neither Ca^{2+} transients nor RVI. These results suggest the role of TRPV2 ion channel in osmosensation and in cell volume regulation.

P-15

CALCIUM HOMEOSTASIS IS MODULATED BY ANDROGEN DEPRIVATION IN PROSTATE CANCER CELLS

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Treatment of advanced prostate cancer relies on pharmacological or surgical androgen deprivation. However, after a few months or years, the tumor relapses despite the absence of androgenic stimulation: a state referred to as hormone-refractory prostate cancer (HRPCa). Studying the mechanisms involved in androgen-independent phenotype development in PCa, we found that androgen depletion induced important modifications in calcium homeostasis : thapsigargin-induced release of calcium from the endoplasmic reticulum was diminished, as well as store-dependent entry of calcium; accordingly, using a CAMD1ER probe, we observed that calcium concentration in the endoplasmic reticulum was strongly reduced; manganese-induced quenching of fura-2 demonstrated that calcium influx through cell membrane was also decreased. In parallel, we measured the expression of 25 genes involved in calcium homeostasis (SERCA pumps, ryanodine and IP3 receptors, ion channels…). We found that, in these conditions, the expression of two TRP ion channels was modified: TRPM8 was ~100 times less expressed and that TRPC1 was ~10 times more expressed. We are currently investigating the possible implications of these modifications in several cellular functions known to be deregulated in HRPCa phenotype, such as proliferation, autophagy and apoptosis.

P-16

MODULATION OF TETRODOTOXIN-SENSITIVE SODIUM CURRENTS BY THE TRPA1 AGONIST CINNAMALDEHYDE

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Cinnamaldehyde (CA) and mustard oil are highly reactive compounds that have been widely used in experimental models of neurogenic inflammation and have been identified as potent agonists of the chemonociceptor TRPA1. We have recently used CA to study the role of TRPA1 in several models of chemonociception in mice and humans, and surprisingly, we have found that, compared to MO, CA only produces very weak avoidance, pain or visceral irritation responses. Furthermore, we have found that co-injection of CA with MO or capsaicin induced pain responses that are significantly smaller than those evoked by these compounds alone (Alpizar *et al.*, in preparation). Noting the structural similarities between CA and classical local anesthetics, we investigated whether CA blocks voltage-gated Na^+ channels in sensory neurons. Voltage-dependent Na^+ currents were recorded in trigeminal neurons of *Trpa1* knockout mice using whole-cell patch-clamp. Application of CA induced a concentration-dependent decrease of total Na^+ current with an EC_{50} of 1.37 ± 0.11 mM at +10 mV. This inhibitory effect was largely and quickly reversible, suggesting that the underlying mechanism is not based on covalent modification of cysteine residues as described for the activation of TRPA1. In another series of experiments we characterized the effects of CA on TTX-s Na^+ currents in immortalized dorsal root ganglion neuron-derived F11 cells. Quantitative PCR analysis confirmed the presence of a subset of TTX-s Na^+ channels (Nav1.2, Nav1.6 and Nav1.7) in this cell line. In whole-cell patch-clamp experiments we found that the effects of CA on the gating properties of TTX-s Na^+ channels are similar to those of local anesthetics (LAs) such as lidocaine. Indeed, CA induces a concentration-dependent shift of the activation curve to depolarized voltages and a shift of the availability curve to more hyperpolarized voltages. In the presence of CA, the corresponding slope factors, s_{act} and s_{inact}, increase significantly. Furthermore, CA-induced inhibition is more pronounced at a physiological holdingpotential (-75 mV) than at a hyperpolarized holding potential (-100 mV). However, in contrast to LAs, CA does not inhibit TTX-s Na^+ currents in frequency-dependent manner and can occur when the channels are in a resting/closed state(s). In conclusion, our data demonstrate that besides activating TRPA1, CA is an inhibitor of TTX-sensitive Na^+ channels in F11 cells and voltage-gated Na^+ channels in neurons, which may lead to the inhibition of action potential firing and conduction. The identification of this anesthetic-like property of CA is essential for a full understanding of the effects of this compound when used to study the patho-physiological roles of TRPA1.

P-17

BIMODAL ACTION OF CINNAMALDEHYDE AND CAMPHOR ON MOUSE TRPA1 CHANNELS

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TRPA1 is a calcium-permeable nonselective cation channel that functions as an excitatory ionotropic receptor in nociceptive neurons. TRPA1 is activated by cooling and by a myriad of chemical irritants, and serves as a broadly tuned chemonociceptor triggering pain and local inflammatory responses in visceral and peripheral tissues. One of the most intriguing properties of TRPA1 is the bimodal effect of several of its chemical modulators. Originally described either as antagonists or agonists of mouse TRPA1, several compounds were later shown to induce activation at low (µM) concentrations and inhibition at higher concentrations. Such is the case of menthol and related compounds, citral, nicotine and mustard oil. Here we report the bimodal action of two other well-known modulators of TRPA1, namely cinnamaldehyde and camphor, which are thus far known to be agonist and antagonist, respectively. Whole-cell patch-clamp experiments in TRPA1-expressing CHO cells revealed that, as previously reported, extracellular application of 100 µM CA induced a powerful stimulation of TRPA1 currents. However, subsequent application of 3 mM CA induced a fast and reversible current inhibition. Application of 3 mM CA had more complex effects on basal currents, inducing a rather small current increase, followed by current inhibition and a dramatic overshoot of current amplitude upon washout. These observations are indeed reminiscent of the effects of TRPA1 modulators having bimodal effects on this channel. The bimodal effects of CA could be also documented using fotometric measurements of intracellular Ca^{2+} in intact TRPA1-expressing CHO cells and in primary cultures of mouse dorsal root ganglion (DRG) neurons. The agonist action of camphor on TRPA1 was readily observed in patch-clamp experiments performed in CHO cells over-expressing this channel. As previously reported extracellular application of 1 mM camphor induced a decrease of basal currents, but the current amplitude showed a significant overshoot upon washout. On the other hand, application of 100 µM camphor induced a 3-fold increase of the basal current amplitude measured at -75 mV. Intracellular Ca^{2+}-imaging experiments in TRPA1-expressing CHO cells yielded that application of camphor induces only marginal increase in the intracellular Ca^{2+} concentration, probably due to the low stimulatory potency of this compound at micromolar concentrations and its inhibitory effect at higher concentrations. However, washout of camphor triggered robust Ca^{2+} rebound signals, demonstrating therefore its agonist action. Similar results were obtained in cultured mouse DRG neurons. These results highlight once more the complexity of TRPA1 pharmacology and the need for careful characterization of the effects of TRPA1 channel modulators over wide concentration ranges.

P-18

EXPRESSION ANALYSIS OF TRP CHANNEL GENES AT SINGLE TRIGEMINAL AND DORSAL ROOT GANGLION LEVELS IN MOUSE

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Somatosensory nerve fibres arising from cell bodies within the trigeminal ganglia (TG) in the head and from a string of dorsal root ganglia (DRG) located lateral to the spinal cord convey endogenous and environmental stimuli to the central nervous system. Although several members of the transient receptor potential (TRP) superfamily of cation channels have been implicated in somatosensation, the expression levels of TRP channel genes in the individual sensory ganglia have never been systematically studied. Here, we used quantitative real-time PCR to analyse and compare mRNA expression of all TRP channels in TG and individual DRGs from 27 anatomically defined segments of the spinal cord of the mouse. At the mRNA level, 17 of the 28 TRP channel genes, TRPA1, TRPC1, TRPC3, TRPC4, TRPC5, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, TRPV1, TRPV2, TRPV4, TRPML1 and TRPP2, were detectable in every tested ganglion. Notably, four TRP channels, TRPC4, TRPM4, TRPM8 and TRPV1, showed statistically significant variation in mRNA levels between DRGs from different segments, suggesting ganglion-specific regulation of TRP channel gene expression. These ganglion-to-ganglion differences in TRP channel transcript levels may contribute to the variability in sensory responses in functional studies. In conclusion, we developed, compared and refined techniques to quantitatively analyse the relative mRNA expression of all TRP channel genes at the single ganglion level. This study also provides for the first time a comparative mRNA distribution profile in TG and DRG along the entire vertebral column for the mammalian TRP channel family.

P-19

STARVATION-INDUCED AUTOPHAGY ALTERS CONNEXIN43 LEVELS AND REDUCES INTERCELLULAR CALCIUM WAVE PROPAGATION IN CORNEAL ENDOTHELIAL CELLS

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Connexin (Cx) proteins form large conductance channels, mediating communication between neighboring cells via gap junctions (GJs) and hemichannels. Intercellular communication (IC) coordinates cellular responses in tissues and organs, controlling signaling, survival and cell death spreading. Cxs have half-lives of only a few hours due to their rapid degradation. Autophagy, a degradation pathway essential for cell homeostasis and induced by cellular stress like starvation, contributes to the degradation of Cx channels. However, the influence of autophagy on hemichannel-mediated signaling has not been characterized. Therefore, we studied the role of starvation-induced autophagy on Cx level and IC in bovine corneal endothelial cells (BCEC), a model system for hemichannel-mediated signaling. Starvation caused a time-dependent decrease of the Cx43 protein and a concomitant reduction of the active area of the Ca^{2+} wave, correlating with the increase of LC3-II, an autophagic marker. These changes were alleviated by bafilomycin, an inhibitor of autophagic flux. Cells pretreated with TAT-L2, a selective Cx43-hemichannel blocker, did not display this phenomenon. Pretreatment with Gap27, which inhibits Cx43 GJs, decreased the effect of starvation on the active area. Starvation markedly reduced the enhancement of the Ca^{2+} wave propagation by the ecto-nucleotidase inhibitor ARL-67156. The effect of the starvation on the Ca^{2+} wave was not detectable in cells pretreated with exogenous apyrases. To assess whether starvation inhibits hemichannel-mediated PIC, we examined the effects of starvation on LY uptake and ATP release. Starvation of BCEC inhibited LY uptake and ATP release. In conclusion, starvation-induced autophagy of BCECs reduces Cx43 hemichannel-mediated intercellular signaling by decreasing Cx43-protein levels.

P-20

THE OBLIGATORY KV2.1/KV6.4 HETEROTETRAMERIZATION IS DETERMINED BY BOTH N- AND C-TERMINAL INTERACTIONS

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Fully assembled voltage-gated potassium (Kv) channel are tetramers of α-subunits. The silent (KvS) channel subunits (Kv6-9) do not form functional channels in homotetrameric configuration due to retention in the endoplasmic reticulum (ER). This ER retention is elicited by assembly with Kv2 subunits generating functional Kv2/KvS heterotetramers. In case of the Kv1-4 subfamilies, it has been demonstrated that the N-terminal T1 domain determines the subfamily specific tetramerization by preventing interactions between subunits that belong to different subfamilies. We demonstrate that the subfamily specific Kv2.1/Kv6.4 heterotetramerization is determined by interactions between the Kv2.1 and Kv6.4 N- and C-termini. Förster Resonance Energy Transfer (FRET) and co-immunoprecipitation (co-IP) experiments using N- and C-terminal Kv6.4 and Kv3.1 fragments as well as N- and C-terminal truncated Kv6.4 and Kv3.1 subunits indicated that both the Kv6.4 N-terminus and S1-S6 segments interact with the corresponding Kv3.1 segments. However, these interactions did not lead to the production of functional heterotetrameric Kv3.1/Kv6.4 channels since co-expression of Kv6.4 with Kv3.1 did not affect the Kv6.4 localization nor the biophysical Kv3.1 properties, and no interaction between Kv3.1 and Kv6.4 could be detected with co-IP experiments using the full length α-subunits. Furthermore, co-expression of Kv3.1 with the (NKv3.1)Kv6.4 chimera – in which the Kv3.1 N-terminus has been introduced in a Kv6.4 background – did also not lead to the production of functional heterotetramers. These results together suggest that the inability of the Kv6.4 C-terminus to associate with its interaction partner inhibits the formation of functional Kv3.1/Kv6.4 heterotetramers. Indeed, FRET and co-IP experiments using N- and C-terminal fragments demonstrate that the C-terminus of Kv6.4 physically interacts with the N-terminus of Kv2.1 but not with the Kv3.1 N-terminus suggesting that the subfamily specific Kv2/Kv6 heterotetramerization is determined by the specific interaction between the Kv2.1 and Kv6.4 N- and C-termini.