BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Friday, October 21th 2016

PROGRAMME
&
ABSTRACTBOOK

Venue

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

Local host

Prof. Dr. Nathalie Caron
Laboratory of Physiology-Molecular Physiology
Research Unit
Université de Namur
Rue de Bruxelles 61 - 5000 Namur

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Main Lecture

10.00-11.00 **The bright (renal hemodynamics) and the dark (renal disease) face to Notch3.**

Prof. Dr. Christos CHATZIANTONIOU, research director at INSERM (Paris, France).

Oral Communications

11.00-11.15 I. JADOT, V. COLOMBARO, B. MARTIN, I. HABSCH, O. BOTTON, J. NORTIER, A-E. DECLÈVES, N. CARON (UNamur, ULBruxelles, UMons)
L-Arginine supplementation reduces fibrosis in a mouse model of chronic aristolochic acid induced nephropathy.

11.15-11.30 A. GEVAERT, H. SHAKERI, A. LELOUP, C.E. VAN HOVE, G.R.Y. DE MEYER, E.M. VAN CRAENENBROECK, C.J. VRINTS, K. LEMMENS (UAntwerpen)
Vascular senescence contributes to heart failure with preserved ejection fraction in senescence-accelerated mice fed a Western diet.

11.30-11.45 B. VAN DER VEKEN, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen)
Inhibition of VEGFreceptor signalling attenuates intraplaque angiogenesis and plaque destabilization in a mouse model of atherosclerotic plaque rupture.

11.45-12.00 E. VAN RYMENANT, K. BEERENS, C. BOYDENS, B. PAUWELS, L. VANDEN DAELE, C. GROOTAERT, J. VAN CAMP, G. SMAGGHE, A. KERIMI, G. WILLIAMSON, P. BROUCKAERT, J. VAN DE VOORDE (UGent, VIB, Univ. Leeds UK)
Ferulic acid-4-O-sulfate is a strong vasorelaxant of isolated mouse arteries acting through soluble guanylate cyclase and voltage-dependent potassium channels.

12.00-12.15 D. HOORELBEKE , M. DE BOCK, T. DELVAEYE, V. VAN HAVER, H. THIERENS, D.V. KRYSKO , B. DESCAMPS , C. VANHOVE, L. LEYBAERT, E. DECROCK (UGent, VIB)
The role of Connexin43 hemichannels, ATP release and the Ca^{2+}/ROS/NO signaling axis in the radiation-induced bystander effect in brain micro-vascular endothelial cells.

12.15-13.45 **Lunch – Guided Poster Session – General Assembly**

Posters

(height 120 cm – width 100 cm)

1. B. MARTIN, C. WILKIN, I. JADOT, V. COLOMBARO, O. BOTTON, A-E. DECLÈVES, N. CARON (UNamur, UMons)
Involvement of adipokines in obesity-associated renal dysfunction.

2. S. DOGNE, G. RATH, C. DESSY, B. CARON, B. FLAMION (UNamur, UCLouvain)
Hyaluronidase 1 deficiency preserves endothelial function and glycocalyx integrity in early streptozotocin-induced diabetes.

3. A. SANCHEZ, K. DEMYDENKO, C. JUNG, Y. A. ALPIZAR, J. ALVAREZ-COLLAZO, J.L. ALVAREZ, P. HOET, M.A. VALVERDE, K. TALAVERA (KULeuven,Univ. Barcelona Spain, Univ. Havana Cuba)
Silica nanoparticles inhibit the chemical activation of TRPV4.

4. N. SYAM, A. PIRONET, C. VAN DEN HAUTE, T. BUELENS, G. VANDE VELDE, R. GIJSBERS, R. VENNEKENS (KULeuven):
Mice overexpressing AAV9-driven TRPM4 are more prone to cardiac arrhythmias.

5. K. DE CLERCQ, C. VAN DEN EYNDE, S. PINTO, T. VOETS, J. VRIENS (KULeuven)
Subfertile phenotype of TRPV2 knockout mice: the chicken or the egg?

6. A. HENNES, K. HELD, K. DE CLERCQ, T. VOETS, J. VRIENS (KULeuven)
The mechanosensor Piezo1 as important calcium permeable ion channel in endometrial epithelial cells.

7. C. HMAIED, A. SWIJSEN, D. ENGEL, J. SCUVÉE-MOREAU, V. SEUTIN (ULiège)
Preliminary evidence for the involvement of Na^+ activated K^+channels in the fast afterhyperpolarization (fAHP) of rat serotonergic neurons.

8. A.J. LABRO, E. MARTINEZ-MORALES, D.J. SNYDERS (UAntwerpen)
Converting the depolarized-activated Shaker Kv channel into a hyperpolarized-conducting cation channel by two pore mutations.

9. I. BELO DO NASCIMENTO, C. INGELBRECHT, E. HERMANS (UCLouvain)
Glial tumours: putative link between altered metabolism and impaired glutamate transport.

10. V. VAN HAVER, M. DE BOCK, D. HOORELBEKE, E. DECROCK, L. LEYBAERT (UGent)
Transcellular transport in the Blood Brain Barrier (BBB) in inflammatory conditions.

11. L. VANGEEL , A. JANSSENS, A. SOUSA, K. EGGERMONT, B. SCHMIDT, C. VERFAILLIE, T. VOETS (KULeuven)
TRP channel function in iPSC-derived sensory neurons.

12. M. MULIER, A. JANSSENS, J. VRIENS, T. VOETS (KULeuven)
Measuring subcellular activity of TRPM3 channels using tethered calcium sensors.

Oral Communications

13.45-14.00 W. VAN LYSEBETTENS, L.E. LARSEN, M. SPRENGERS, L. THYRION, A. GRIMONPREZ, S. DAELEMANS, J. DELBEKE, W.J. WADMAN, K. VONCK, P. BOON, R. RAEDT (UGent, Univ. Amsterdam NL.)
Counteracting Vagus Nerve Stimulation-induced hypothermia reverses effects on hippocampal electrophysiology.

14.00-14.15 V. PAUWELYN, E. VAN DEYNSE, R.A. LEFEBVRE (UGent)
Influence of phosphodiesterases on cholinergic contractility and its 5-HT_4 receptor-mediated modulation in the murine gastrointestinal tract.

14.15-14.30 C. INGELBRECHT, N. DESMET, J. DAMBLON, E. HERMANS (UCLouvain)
Biochemical and pharmacological evidence for the existence of spare glutamate transporters – the concept of transporter reserve.

14.30-14.45 L. LEGAT, S. BROUWERS, I. SMOLDERS, A.G. DUPONT (VUBrussel)
Hypotensive response to angiotensin II type 2 receptor stimulation in the rostral ventrolateral medulla: interaction with gamma-aminobutyric acid.

14.45-15.00 N. EMERIAU, M. DE CLIPPELE, P. GAILLY, N. TAJEDDINE (UCLouvain)
Involvement of receptors tyrosine kinase in the modulation of store-operated Ca^{2+} entry SOCE.

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Coffee - Tea

ABSTRACTS

Legend
O = Oral communication
P = Poster

O-01 (11.00-11.15)

L-ARGININE SUPPLEMENTATION REDUCES FIBROSIS IN A MOUSE MODEL OF CHRONIC ARISTOLOCHIC ACID INDUCED NEPHROPATHY

I. Jadot[1], V. Colombaro[1], B.Martin[1], I. Habsch[1], O. Botton[1], J. Nortier[2], A-E. Declèves[3], N. Caron[1]

[1]Molecular Physiology Research Unit - URPHYM, University of Namur (UNamur), Namur, Belgium, [2]Laboratory of Experimental Nephrology, Faculty of Medicine, Université Libre de Bruxelles (ULB), Brussels, Belgium, [3]Laboratory of Molecular Biology, Faculty of Medicine and Pharmacy, Research Institute for Health Sciences and Technology, University of Mons (UMONS), Mons, Belgium

Aristolochic Acid (AA) nephropathy (AAN) is a rapidly progressive tubulo-interstitial nephritis from toxic origin characterized by two interconnected phases: an early phase of acute kidney injury (AKI) leading to the later phase of chronic kidney disease (CKD) with progressive interstitial fibrosis. A reduced nitric oxide (NO) production in AAN has been demonstrated, which may contribute to renal function impairment. We investigated in the present study the potential benefit of L-Arg supplementation in a chronic mouse model of AAN. To address this point, 8 weeks-old C57BL/6J male mice were subjected to daily i.p. injection of AA for 4 days and L-Arg was supplemented in drinking water 7 days before first day of injection and all along the protocol. Mice were euthanized 20 days after the first day of injection. At day 20, we observed a significant reduction of NO bioavailability in AA-treated animals as measured by reduced urinary nitrate/nitrite and cGMP excretions. AA-treated mice displayed polyuria, proteinuria as well as increased levels of plasma creatinine and blood urea nitrogen. Histological analyses of AA-treated mice revealed numerous foci of tubular atrophy surrounded by severe interstitial fibrosis. Immunohistochemical staining of α-SMA revealed a significant increase of positive area staining. Moreover, mRNA expression of pro-fibrotic mediators like *TGF-β*, *CTGF*, *Periostin*, *ColI* and *ColIII* was also increased. L-Arg supplementation in AA-treated mice significantly decreased tubular atrophy as attested by reduced tubular injury score. L-Arg treatment also significantly limited development of interstitial fibrosis as attested by both reduced α-SMA immunohistological staining and collagens mRNA expressions. Finally, L-Arg treatment also decreased *TGF-β*, *CTGF* and *Periostin* mRNA expressions. These results suggest that a preservation of NO bioavailibity may lead to a morpho-functionnal protection in AA-induced CKD. In the present mouse model of chronic AAN, NO seems to act as a key factor in protection of renal function and fibrosis development. In conclusion, restored NO bioavailability by L-Arg supplementation was demonstrated beneficial in improving renal injury in chronic AAN.

O-02 (11.15-11.30)

VASCULAR SENESCENCE CONTRIBUTES TO HEART FAILURE WITH PRESERVED EJECTION FRACTION IN SENESCENCE-ACCELERATED MICE FED A WESTERN DIET

A. Gevaert[1,2], H. Shakeri[1], A. Leloup[1], C.E. Van Hove[1], G.R.Y. De Meyer[1], E.M. Van Craenenbroeck[1,2], C.J. Vrints[1,2], K. Lemmens[1,2]

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With a globally aging population, the prevalence of heart failure with preserved ejection fraction (HFpEF) continues to rise. Its pathophysiology remains incompletely understood, but endothelial inflammation, induced by risk factors, is stated to play a central role. In this study we use a model of accelerated aging to investigate the impact of a western diet on the development of HFpEF. Senescence-accelerated mice (SAM) were fed a western diet and 1% NaCl drinking water (SAM-WD, n=9) and compared to SAM on normal chow (SAM, n=9) and senescence-resistant controls (CON, n=9). Cardiac and vascular function was assessed by high-resolution ultrasound at 2, 4 and 6 months and invasively at sacrifice. LV structure and vascular inflammation were assessed via immunohisto-chemistry. At 6 months, SAM-WD and SAM displayed similarly impaired relaxation of aortic segments to acetylcholine. Vascular senescence was present in SAM-WD and SAM. However, only SAM-WD mice had diastolic dysfunction: elevated LV end-diastolic pressure, prolonged relaxation constant tau, and elevated E/e' ratio. Systolic function was preserved. Structural cardiac abnormalities in SAM-WD included LV hypertrophy, left atrial dilatation and myocardial fibrosis. Exercise capacity was reduced and lung and liver weights were increased, indicating heart failure. Also, endothelial inflammation, as measured by intercellular adhesion molecule 1 expression, was only present in SAM-WD. Blood pressure, glycaemia and body weight did not differ between groups. In conclusion, senescence-accelerated mice develop endothelial dysfunction regardless of their diet. Adding a high-salt, high-fat diet, however, causes endothelial inflammation and triggers HFpEF, independent of hypertension, diabetes or obesity. This is the first animal HFpEF model integrating the key risk factor aging. Therefore, this novel model will be of value to uncover new therapeutic avenues for HFpEF.

O-03 (11.30-11.45)

INHIBITION OF VEGFRECEPTOR SIGNALLING ATTENUATES INTRAPLAQUE ANGIOGENESIS AND PLAQUE DESTABILIZATION IN A MOUSE MODEL OF ATHEROSCLEROTIC PLAQUE RUPTURE

B. Van der Veken, G.R.Y. De Meyer, W. Martinet

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Atherosclerosis is a chronic inflammatory disease of the large and medium-sized arteries and leads to the development of plaques in the vessel wall. It is generally accepted that not the size but rather the stability of a plaque is determinant for acute clinical complications. In atherosclerosis, it is still unclear whether microvessels have a major causative effect on plaque development. Yet, an increase in microvessel density in ruptured versus non-ruptured human plaques suggests that vascular endothelial growth factor (VEGF) and other angiogenic factors promote atherosclerosis and potentially destabilize plaques. Because apolipoprotein deficient mice with a heterozygous mutation (C1039G+/-) in the fibrillin-1 gene [ApoE-/-Fbn1$^{(C1039G+/-)}$] manifest substantial intraplaque neovascularization, they represent a unique tool to investigate angiogenesis and its role in atherosclerosis. We examined whether administration of a VEGFreceptor-1,-2 and -3 inhibitor attenuates intraplaque neovascularization and improves plaque stability. Results showed that intraplaque neovascularization was decreased alongside improved plaque stability. Cardiac function was monitored to evaluate the effect on complications such as myocardial infarction. Overall cardiac function was improved in treated animals. Moreover, the amount of animals that suffered a myocardial infarction was decreased in the treated group. These data confirm a key role for VEGF in pathological neovascularization in atherosclerosis and emphasizes its therapeutic potential.

O-04 (11.45-12.00)

FERULIC ACID-4-*O*-SULFATE AS A POTENT VASORELAXING COMPOUND: MECHANISTIC INVESTIGATION

E. Van Rymenant[1], J. Van Camp[1]; B. Pauwels[2], C. Boydens2; L. Vanden Daele[2], K. Beerens[1], P. Brouckaert[3], G. Smagghe[4]; A. Kerimi[5], G. Williamson[5]; C. Grootaert[1], J. Van de Voorde2

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Consumption of phenolics such as ferulic acid in while grains and coffee is considered beneficial to health. During absorption, ferulic acid is subjected to phase II metabolism, resulting in the production of ferulic acid-4-*O*-sulfate, the main circulating metabolite. We have investigated the vaso-relaxing potential of both compounds (10^{-7} – 3.10^{-5} M) in isolated mouse arteries in a tissue myograph after contraction with phenylephrin. Mechanistic investigations were performed by removal of endothelium, by incubation with specific inhibitors and by using soluble guanylate cyclase (sGC) alpha 1 subunit ($sGC\alpha_1^{(-/-)}$) knockout mice. While ferulic acid did not induce a significant vasorelaxation, ferulic acid-4-*O*-sulfate caused strong relaxation. The ferulic acid-4-*O*-sulfate mediated relaxation was endothelium independent, but was significantly reduced in the presence of K^+-channel blockers TEA and 4-aminopyridin and more strongly decreased by exposure to ODQ, a sGC inhibitor. In $sGC\alpha_1^{(-/-)}$ mice the response toward ferulic acid-4-*O*-sulfate was significantly decreased. We have shown for the first time the potent vasorelaxant activity of ferulic acid-4-*O*-sulfate, which is endothelium-independent and mediated through sGC and voltage dependent K^+-channels.

O-05 (12.00-12.15)

THE ROLE OF CONNEXIN43 HEMICHANNELS, ATP RELEASE AND THE CA^{2+}/ROS/NO SIGNALING AXIS IN THE RADIATION-INDUCED BYSTANDER EFFECT IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

D. Hoorelbeke[1] , M. De Bock[1] , T. Delvaeye[1,2], V. Van Haver[1] , H. Thierens[3], D.V. Krysko[2] , B. Descamps[4] , C. Vanhove[4] , L. Leybaert[1,#], E. Decrock[1,#]

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Brain microvascular endothelial cells (BMECs), the main constituents of the highly selective blood brain barrier (BBB), are very prone to ionizing radiation (IR)-induced damage. The latter mainly includes cell death, activation and senescence, i.e. processes that affect BBB integrity and are tightly linked to DNA damage. Evidence is furthermore accumulating that intercellular pathways can exacerbate these IRinduced effects by propagating them from directly irradiated to adjacent non-exposed bystander cells, a phenomenon known as the IR-induced bystander effect (RIBE). Channels composed of transmembrane connexin (Cx) proteins offer two parallel signaling pathways for intercellular communication; (i)a direct cell-to-cell transfer of ions and small molecules via gap junction channels (GJs) that connect the cytoplasm of neighboring cells and (ii)the uptake and release of paracrine messenger molecules such as ATP via hemichannels (HCs) that form a non-selective pore in the plasmamembrane. We here aimed to identify the role of these Cx channels and the messengers involved in RIBE. Fundamental knowledge on RIBE can contribute to the development of protective strategies against IR-induced BMEC damage, thereby reducing vascular leakage and brain tissue damage. We optimized a set-up in which a well-delineated zone of an adherent BMEC culture is exposed to X-rays (1 and 20 Gy). The presence of DNA double-strand breaks(γ-H2AX foci) was detected in both the irradiated and adjacent non-irradiated bystander zone, with the number of γ-H2AX-positive cells being reduced by Cx43 knockout strategies and Cx43 HC targeting peptides. Application of HCpermeable dyes as well as measurements of extracellular ATP release demonstrated the acute opening of HCs within 5min post-irradiation. Further experiments using inhibitors of the Ca^{2+}/ROS/NO signaling axis and purinergic receptor blockers as well as intracellular Ca^{2+} measurements revealed the involvement of extracellular ATP and the Ca^{2+}/ROS/NO axis in RIBE. We conclude that intercellular signaling via HCs and presumably also via GJs acts as a feed-forward propagation mechanism of intracellular Ca^{2+}/ROS/NO increases that underlie RIBE in BMECs.

O-06 (13.45-14.00)

COUNTERACTING VAGUS NERVE STIMULATION-INDUCED HYPOTHERMIA REVERSES EFFECTS ON HIPPOCAMPAL ELECTROPHYSIOLOGY

W. Van Lysebettens[1], L.E. Larsen[1], M. Sprengers[1], L. Thyrion[1], A. Grimonprez[1], S. Daelemans[1], J. Delbeke[1], W.J. Wadman[2], K. Vonck[1], P. Boon[1], R. Raedt[1]

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We recently showed that vagus nerve stimulation (VNS) induced modulation of hippocampal electrophysiology is associated with brain and body hypothermia. The aim of this study was to investigate whether the VNS-induced hypothermia is responsible for the effects of VNS on hippocampal electrophysiology. Six male Sprague Dawley rats were implanted with a cuff electrode around the left vagus nerve, a stimulation electrode in the Shaffer collaterals and a recording electrode in the CA1 area for registration of evoked potentials (EPs) and EEG. Rats were treated with a stimulation session consisting of 2h baseline, 2h VNS, 2h VNS combined with external heating to counteract hypothermia and 4 h post-VNS, during which EPs, hippocampal EEG and rectal temperature were continuously recorded. VNS modulated EP's by decreasing the slope of the field excitatory post synaptic potential, and increasing the latency and amplitude of the population spike. VNS further modulated the hippocampal EEG by shifting the theta peak frequency to a lower frequency and decreasing theta (4-12 Hz) and gamma (30-100 Hz) band power. Apart from changes in theta band EEG power, all effects of VNS on hippocampal electrophysiology were prominently attenuated or reversed by normalizing body temperature during VNS through application of external heating. These findings suggest that most electrophysiological alterations observed in the hippocampus during VNS in rats are due to a VNS induced hypothermia.

O-07 (14.00-14.15)

INFLUENCE OF PHOSPHODIESTERASES ON CHOLINERGIC CONTRACTILITY AND ITS 5-HT_4 RECEPTOR-MEDIATED MODULATION IN THE MURINE GASTROINTESTINAL TRACT

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The signal transduction of the adenylyl cyclase linked 5-HT_4 receptors on cholinergic neurons, innervating smooth muscle cells in the porcine gastrointestinal (GI) tract, is controlled by phosphodiesterase (PDE) 4. This location of 5-HT_4 receptors was recently also shown in mice, but the possible control by PDEs was not yet investigated. In circular smooth muscle strips from murine fundus, jejunum and colon submaximal cholinergic contractions were repetitively induced by either electrical field stimulation (EFS) or by carbachol (muscarinic receptor agonist). The influence of the PDE inhibitors IBMX (non-selective PDE), vinpocetine (PDE1), EHNA (PDE2), cilostamide (PDE3) and rolipram (PDE4) was tested on these contractions and on the facilitating effect of the 5-HT_4 receptor agonist prucalopride on EFS-induced contractions. In the 3 tissues, IBMX and cilostamide concentration-dependently inhibited both EFS- and carbachol-induced contractions. Rolipram showed a concentration-dependent effect over more than 2 concentrations on carbachol-induced contractions in fundus and jejunum, and on EFS- and carbachol-induced contractions in the colon. This illustrates that PDE3, able to catalyse the breakdown of both cAMP and cGMP contributes to the cyclic nucleotide turnover in murine GI smooth muscle, while the cAMP specific PDE4 also has some influence. None of the selective PDE inhibitors enhanced the facilitating effect of a submaximal concentration of prucalopride on EFS-induced cholinergic contractions. In the murine GI tract, there is thus no evidence that the signal transduction of 5-HT_4 receptors on myenteric cholinergic neurons is controlled by PDEs.

O-08 (14.15-14.30)

BIOCHEMICAL AND PHARMACOLOGICAL EVIDENCE FOR THE EXISTENCE OF SPARE GLUTAMATE TRANSPORTERS – THE CONCEPT OF TRANSPORTER RESERVE

C. Ingelbrecht, N. Desmet, J. Damblon, E. Hermans

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The efficient uptake of synaptic glutamate through high affinity glutamate transporters (EAATs) is essential to limit the duration and intensity of excitatory neurotransmission. Altered glutamate uptake by astrocytes may lead to excitotoxic insults and the loss of glutamate transporters has been proposed as one of the cellular mechanisms supporting the development or progression of neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson disease and Alzheimer's disease. We have developed a model of transfected HEK cells in which the expression of the EAAT2 (GLT-1 in rodents) can be manipulated thanks to a doxycycline-inducible promoter. Combining Western blot detection of GLT-1 and substrate uptake studies allowed us to examine the correlation between transporter expression and function. Our data showed that beyond a substantial level of induction, increasing in the concentration of doxycycline efficiently promoted enhanced expression, but failed to increase the maximal uptake velocity. Detection of cell surface transporter density using confocal microscopy confirmed the efficient addressing of transporters to the cell membrane. With analogy to the pharmacological concept of receptor reserve, these results suggest the existence of transporter reserve or spare transporters. The concept was further validated using the selective GLT-1 blocker WAY-213613. Thus, the sigmoidal curve depicting the inhibition of uptake by increasing concentrations of WAY-213613 was progressively shifted to the right when tested in cells where the transporter density was robustly induced. As widely documented in the context of cell surface receptors, the existence of spare glutamate transporters could impact on the consequence of physiological or pathological regulation of transporters. Also, tissues where the proportion of spare glutamate transporters is substantial may show some resistance against the effect of pharmacological inhibitors.

O-09 (14.30-14.45)

HYPOTENSIVE RESPONSE TO ANGIOTENSIN II TYPE 2 RECEPTOR STIMULATION IN THE ROSTRAL VENTROLATERAL MEDULLA: INTERACTION WITH GAMMA-AMINOBUTYRIC ACID

L. Legat, S. Brouwers, I. Smolders, A.G. Dupont

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Angiotensin II, glutamate and gamma-aminobutyric acid (GABA) interact within the rostral ventrolateral medulla (RVLM) and the paraventricular nucleus (PVN) modulating the central regulation of blood pressure and sympathetic tone. Our aim was to assess the effects of local angiotensin II type 2 receptor (AT2R) stimulation within the RVLM and PVN on neurotransmitter concentrations and mean arterial pressure (MAP). In vivo microdialysis was used for measurement of extracellular glutamate and GABA levels and for local infusion of the AT2R agonist Compound 21 (C21) in the RVLM and PVN of conscious normotensive Wistar rats. The MAP response to local C21 was monitored with a pressure transducer under anaesthesia. AT2R selectivity was assessed using the AT2R antagonist PD123319; the GABAA receptor antagonist bicuculline was used to assess the involvement of GABAA receptors. Infusion of C21 (0.05µg/µl/h) in the RVLM significantly increased GABA levels and lowered blood pressure. These effects were abolished by co-infusion with PD123319. No changes in neurotransmitter levels or effects on blood pressure were seen with PD123319 infusion alone. Co-infusion of bicuculline abolished the C21 evoked decrease in MAP. Infusion of C21 within the PVN did not change extracellular neurotransmitter levels nor MAP. Selective stimulation of AT2R within the RVLM by local C21 infusion reduces blood pressure and increases local GABA levels in normotensive rats. This hypotensive response requires functional GABAA receptors, suggesting that GABAergic neurons are involved in the sympatho-inhibitory action underlying this hypotensive response.

O-10 (14.45-15.00)

INVOLVEMENT OF RECEPTORS TYROSINE KINASE IN THE MODULATION OF STORE-OPERATED CA^{2+} ENTRY SOCE

N. Emeriau, M. de Clippele, P. Gailly, N. Tajeddine

Université catholique de Louvain, Brussels, 1200, Belgium.

A multitude of studies show that alterations in Ca^{2+} signalling initiate or support the development of hallmarks of cancer. In particular, the role of store-operated Ca^{2+} entry (SOCE) in tumorigenesis and tumour progression has been subject to intense investigation. SOCE is the main mechanism by which external Ca^{2+} enters into the cell. It is initiated by depletion of endoplasmic reticulum (ER) Ca^{2+} stores and mediated by several proteins such as STIM1, an ER transmembrane protein sensing Ca^{2+} within the ER and ORAI1, a plasma membrane Ca^{2+} channel. We recently showed that SOC inhibition interfered with EGFR-dependent signalling in non-small cell lung carcinoma cells. In this study, we observed that STIM1 depletion reduced neuregulin-dependent proliferation of breast cancer cells. Since neuregulin binds to ErbB3 and/or ErbB4 and therefore activates ErbB2, this result prompted us to investigate whether ErbB proteins might modulate SOCE. We observed that lapatinib, a dual inhibitor of EGFR and ErbB2, dramatically inhibited SOCE. As expected, lapatinib also inhibited phosphorylation of EGFR, ErbB2 and downstream pathways of both receptors. Specific inhibition of EGFR by erlotinib or gefitinib had no effect on SOCE. In contrast, specific inhibition of ErbB2 by CP-724714 mimicked the effects of lapatinib. We also investigated the role of downstream pathways of ErbB2 in the modulation of SOCE. Inhibition of the MAPK and the JAK-STAT pathways does not modify the amplitude of SOCE. Contrariwise, LY294002 and MK2206, two inhibitors of the PI3K pathway, dramatically reduced SOCE. Interestingly, *in silico* analysis showed that STIM1 might be phosphorylated by Akt. All these results suggest that SOCE is important in the proper function of ErbB2-dependent signalling. This raises the possibility to target molecular mediators of SOCE in order to interfere with the proliferation of breast cancer cells.

P1

INVOLVEMENT OF ADIPOKINES IN OBESITY-ASSOCIATED RENAL DYSFUNCTION

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Obesity incidence has dramatically increased during the last few years. This disease, characterized by an excessive fat accumulation, has for consequences an alteration of adipose tissue function and a chronic inflammation status, leading to metabolic disturbances. It is also well described that an excess of fat can be considered as a risk factor for kidney disease development. Moreover, it has been shown that in addition to be a storage organ, adipose tissue plays endocrine functions by the secretion of adipokines, such as adiponectin, leptin and TNF-α. In obese patients, secretion of these adipokines is impaired. Therefore, in the present study, we investigated the role of a recently discovered adipokine, chemerin, in obesity progression and, specifically, its impact on obesity-induced kidney alterations. To do so, C57BL/6 male mice were randomized to a low fat diet (LFD), a high fat diet (HFD) or a high fat diet with fructose supplementation (HFDF) for 16 weeks. We demonstrated that mice fed a HFD and HFDF developed obesity, as illustrated by an increase in body weight, kidney hypertrophy as well as glucose metabolism disorders. Regarding kidney function, we observed that HFD and HFDF mice tend to develop renal functional impairment as they exhibited albuminuria and a slight increase in proteinuria, glomerular filtration rate and H_2O_2 excretion. Diuresis, urine osmolarity and water balance remained unchanged in those mice. Histologically, we highlighted lipid vacuolization in proximal tubular cells as well as the emergence of fibrosis. Plasma concentration of adiponectin and renal mRNA expression of its receptors did not differ between all experimental groups. However, plasma leptin level and renal mRNA expression of leptin receptor were statistically higher in HFD and HFDF groups in comparison with LFD group. Regarding chemerin, we observed significant upregulation of its renal mRNA expression. Moreover, renal mRNA and protein expressions of chemerin receptors were increased in obese mice. Finally, inflammation response was evaluated in the kidney tissue of all experimental groups. We highlighted an enhanced *TNF-α* and *MCP-1* mRNA expression in mice fed a HFD and HFDF. In summary, we demonstrated appearance of obesity as well as associated kidney failure in HFD and HFDF mice. According to our results, chemerin pathway seems to be involved in development of these syndromes but further investigations are still needed for a better understanding of the link between this newly described adipokine, chemerin, and the renal dysfunction associated with obesity.

P2

HYALURONIDASE 1 DEfiCIENCY PRESERVES ENDOTHELIAL FUNCTION AND GLYCOCALYX INTEGRITY IN EARLY STREPTOZOTOCIN INDUCED DIABETES

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Hyaluronic acid (HA) is a major component of the glycocalyx. In diabetes, the size and permeability of the glycocalyx are altered. In addition, type 1 diabetic patients have increased plasma levels of both HA and its somatic hyaluronidase Hyal-1, which is endocytosed by endothelial cells. We decided to investigate the potential implication of Hyal-1 in the development of diabetes-induced endothelium dysfunction. The following measurements were obtained in Hyal-1 deficient (KO) and wild-type (WT) mice 4 weeks after they became diabetic following daily injections of streptozotocin during 5 days: plasma HA and Hyal-1 activity, markers of endothelial dysfunction, glomerular barrier properties (urinary albumin/ creatinine and 70/40-kDa dextran ratio), myocardial arterioles glycocalyx (using transmission electron microscopy), and mesenteric artery endothelium-dependent vasodilation. The expressions of various components of the endothelium-derived hyperpolarizing factor (EDHF) pathway were evaluated by real time PCR. KO mice had higher plasma HA concentration than WT mice. Plasma HA increased with diabetes in WT mice but was unaffected in diabetic KO mice. A slight but significant increase of Hyal-1 activity was detected in WT mice during diabetes. ICAM-1 but not VCAM-1 was significantly up-regulated by diabetes in both KO and WT mice. On the other hand, P-selectin increased during diabetes only in WT mice. The glycocalyx had a greater thickness in KO vs WT mice and this difference persisted after 4 weeks of hyperglycemia. At that time, HA did not accumulate in the aortic wall but glycocalyx HA had almost vanished in WT mice whereas it was not affected in diabetic KO mice. Moreover, the 70/40-kDa dextran ratio, as well as the albumin/creatinine ratio, in urine were increased in diabetic WT mice but not in diabetic KO mice, suggesting better protection of the glomerular barrier. Endothelium-dependent vasodilation of mesenteric arteries did not differ between healthy WT and KO mice. However, in WT diabetic mice, EDHF-mediated vasorelaxation was severely impaired whereas KO diabetic mice had a partially preserved EDHF pathway. Among various components of the EDHF pathway, only SK3 channels were up-regulated in KO mice, both at basal level and after diabetes induction. Activation of SK3 channels using CyPPA confirmed a higher SK3-dependent relaxation in KO vs WT mice, with and without diabetes. In conclusion, decreased levels of Hyal-1 orient the diabetic endothelial response towards a preserved glycocalyx, less inflammation, a preserved glomerular barrier, and lesser damage to the EDHF-dependent vasodilation pathway. The mechanisms of this protection could be partly due to SK3 up-regulation. Links between Hyal-1 deficiency and SK3 regulation, as well as between the glycocalyx and the EDHF pathway, need to be further investigated.

P3

SILICA NANOPARTICLES INHIBIT THE CHEMICAL ACTIVATION OF TRPV4

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Amorphous silica nanoparticles (SiNPs) are extensively used for their beneficial properties in fields such cosmetics and food industry as an additive and fining agent. However, health hazards concerns about the high exposure to it have recently arisen. SiNPs can penetrate tissues and even cells resulting in health problems. Special attention has to be given to the airways, specifically to the epithelium, which functions as a barrier against pollutants and pathogens and is one of the main entries of SiNPs into the body. In this study we evaluated the effects of SiNPs on TRPV4, a channel that is highly expressed in airway epithelial cells and that regulates the ciliary beat frequency and the mucociliary transport. Using intracellular calcium measurements we found that 9 nm SiNPs (Ludox®) inhibited the activation of recombinant TRPV4 by the synthetic agonist GSK1016790A in HEK293T cells. This result was confirmed in whole-cell patch-clamp experiments. Furthermore, SiNPs inhibited the activation of native TRPV4 in human and mouse airway epithelial cells. Finally, SiNPs abrogated the increase in ciliary beat frequency in mouse tracheal epithelial cells induced by chemical activation of TRPV4. We propose that inhibition of TRPV4 by SiNPs may induce a loss of mucociliary clearance in airways epithelial cells, which could lead to pulmonary infections and pulmonary tissue damages when pathogens, contaminants or allergens enter the airways.

P4

MICE OVER-EXPRESSING AAV9-DRIVEN TRPM4 ARE MORE PRONE TO CARDIAC ARRHYTHMIAS

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Transient Receptor Potential cation channel subfamily Melastatin member 4 has been associated with cardiac arrhythmias, and its KO mice have shown better survival of developing such diseases, while the WT have shown worst phenotypes. With the advancing use of AAV viral vector technology as a mean of targeted delivery, we chose AAV9 to facilitate targeting of TRPM4 over expression in the heart and see whether the mice display more severe arrhythmias as a proof of principle. We first observed by bioluminescence imaging that AAV9, injected via tail vein, was able to transduce the heart. Furthermore, we were also able to see over expression of TRPM4 in the heart at the protein level. Such over expression nor way of delivery affected ECG parameters. While no severe arrhythmic events were observed at the baseline, the mice over-expressing TRPM4 displayed higher number of such events during exercise and upon (induced) higher heart rate. Moreover, we also observed unusually more severe (longer) arrhythmic events. In conclusion, TRPM4 over expression delivered by AAV9 to the heart does not affect ECG parameters but renders such mice more prone to cardiac arrhythmias especially during exercise and at higher heart rate.

P5

SUBFERTILE PHENOTYPE OF TRPV2 KNOCKOUT MICE: THE CHICKEN OR THE EGG?

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Transient Receptor Potential (TRP) channels are known to be involved in a myriad of physiological functions and serve as an important role as cellular sensors. TRPV2, a calcium permeable non-selective channel, has been shown to play pivotal roles in various cellular functions. Recently, its functional expression was found in endometrial stromal cells isolated from human and mouse uteri. However, the function of TRPV2 in reproduction remains unknown. Interestingly, TRPV2$^{-/-}$ mice are born with reduced body weights and are susceptible to perinatal death, suggesting intrauterine growth restriction. Here, we present a more detailed description of the reproductive phenotype of TRPV2$^{-/-}$ and their offspring. Notably, TRPV2$^{-/-}$ mice have a later onset of cyclicity and have a more irregular oestrus cycle compared to wild type mice. Moreover, TRPV2$^{-/-}$ mice have smaller litter size and circa 80% of homozygous (-/- x -/-) breeding pairs fail to produce six litters. Interestingly, residual tissue was found in 50% of TRPV2$^{-/-}$ mice after 6 litters or after failure to produce a litter for 3 months. When sacrificing pregnant females at day 18.5 of pregnancy, we found that TRPV2$^{-/-}$ have significantly more abnormal implantation sites compared to wild type. Furthermore, the bodyweight and placental weight from TRPV2$^{-/-}$ pups at E18.5 is significantly smaller compared to wild type mice. However, the amount of TRPV2$^{-/-}$ mice in litters of heterozygous breeding was smaller than what is expected from the Mendelian pattern. These data suggest that TRPV2 might be important in the establishment of successful pregnancy or for the developing embryo and further evidence is necessary to explain this subfertile phenotype.

P6

THE MECHANOSENSOR PIEZO1 AS IMPORTANT CALCIUM PERMEABLE ION CHANNEL IN ENDOMETRIAL EPITHELIAL CELLS

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During the process of uterine receptivity and embryo implantation, many crucial interactions between the blastocyst and luminal epithelium take place. Nevertheless, it remains unclear how signals can be detected by the endometrial epithelial cells and transmitted towards the stromal bed for enhanced decidualization. Several clinical studies providing local injury to the endometrium via an endometrial biopsy, have been conducted and showed enhanced implantation rates in women undergoing IVF treatment. Moreover, this hypothesis is supported by animal studies were decidualization can be induced via mechanical stimulus. These studies indicate a possible role for mechanosensitive ion channels as signal tranducers. Recently the ENaC ion channel was proposed as important signal mediator required for stromal decidualization. Membrane depolarization via ENaC activation in epithelial cells would induce a Ca^{2+} influx via voltage-dependent calcium channels (VDCC) and thus ultimately trigger the signalling response needed for proper stromal decidualization. However, own qRT-PCR and functional analysis experiments (Ca^{2+} microfluorimetry and whole-cell patch clamp) did not show any evidence for the expression of VDCC in primary human endometrial epithelial cells (HEEC). However, high mRNA expression of the mechanosensititve piezo1 ion channel was identified in HEEC and functional analysis using different piezo1 activators, Yoda1 and mechanical stimulation, provided strong evidence for the functional expression of piezo1 in HEEC. This preliminary data suggest a possible role for piezo1 as key player in the signalling pathway during decidualization and implantation.

P7

PRELIMINARY EVIDENCE FOR THE INVOLVEMENT OF NA^+-ACTIVATED K^+ CHANNELS IN THE FAST AFTERHYPERPOLARIZATION OF RAT SEROTONERGIC NEURONS.

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Serotonergic neurons located in the ventro-medial part of the dorsal raphe (DR) display broad action potentials and a prominent medium duration afterhyperpolarization (mAHP) which is blocked by apamin, indicating that it is mediated by small-conductance Ca^{2+}-activated K^+ channels (SK). A residual component of the AHP, the fast afterhyperpolarization (fAHP), which is insensitive to apamin, can be isolated in these neurons. The mechanism of this fAHP, which may be at least as important as the mAHP in regulating firing frequency, is unknown. The purpose of this study was to characterize the currents mediating the fAHP. Intracellular recordings of DR neurons were performed in rat brain slices. Using specific blockers, we were able to rule out a role for large conductance Ca^{2+}-activated K^+ channels (BK), as well as for a variety of voltage-dependent K^+ channels, in particular I_A–type channels, in the fAHP. .We next tested whether K_{Na+} channels could be involved. When Na^+ was substituted with Li^+, the fAHP was abolished but this procedure induced aspecific effects, thus making the interpretation of this experiment difficult. We subsequently performed patch-clamp recordings to investigate this further. In these voltage-clamp recordings, Ca^{2+} currents were blocked by Cd^{2+} (100 µM) and Ni^{2+} (200µM). Application of depolarizing pulses from -60 mV to +20 mV induced a transient tetrodotoxin (TTX)-sensitive inward current followed by a large outward K^+ current. The maximal amplitude of the outward current (at ~ 2 ms after the onset of the pulse) was reduced by 72 $\pm$ 2 % by 0.5µM TTX (n= 24), suggesting that part of this current is carried by K_{Na+} channels. We are currently characterizing the biophysical properties of this current and investigating whether the TTX-sensitive K^+ current can be potentiated when pulses are delivered repeatedly at high frequency.

P8

CONVERTING THE DEPOLARIZED-ACTIVATED *SHAKER* KV CHANNEL INTO A HYPERPOLARIZED-CONDUCTING CATION CHANNEL BY TWO PORE MUTATIONS

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The hyperpolarized cyclic-nucleotide activated (HCN) cation channels underlie *in vivo* the pacemaker (If) current, displaying a unique voltage dependence of activation and selectivity for both K^+ and Na^+ ions. The structural topology of HCN channels is very similar to that of voltage-dependent K^+ (Kv) channels. The voltage-sensing domain (VSD) of both HCN and Kv channels responds in a similar manner to changes in membrane potential. However, Kv channels are activated upon membrane depolarization and whereas the outward movement of the VSD opens the gate of the ion conducting pore in Kv channels, it results in gate closure in HCN. Besides their opposite voltage dependence of ion conduction, Kv channels are, in contrast to HCN, highly selective for K^+ over Na^+. Due to their high structural similarity, the question remains which differences result in the different behavior of both channels. Substituting in the *Shaker* Kv channel the pore residue W434 by a phenylalanine (W434F) accelerates the channel´s inactivation process, which involves the collapse of the selectivity filter (SF), such that it precedes gate opening. Consequently, no ionic currents are recorded from this *Shaker*-W434F mutant. By combining this W434F mutation with a pore mutation that prevents the gate of closing completely, P475D, we hypothesized to record ionic currents of the double *Shaker*-W434F-P475D mutant at hyperpolarized potentials when the channel closes and the SF recovers from its inactivated state. Indeed, *Shaker*-W434F-P475D resulted in functional voltage-dependent channels that are conducting at hyperpolarized potentials and cease conducting at depolarized potentials due to SF inactivation. Most interestingly, the conductive state of *Shaker*-W434F-P475D is Na^+ and K^+ permeable, i.e. this channel lost its high K^+ selectivity. The mutant remained, however, sensitive to external TEA block. The identification of only two pore mutations to convert the voltage dependence of activation and changing the K^+ selectivity of *Shaker* Kv channels is, to our knowledge, the minimal interference reported thus far to make a Kv channel behave as a HCN channel, albeit without the cyclic-nucleotide sensitivity.

P9

GLIAL TUMOURS: PUTATIVE LINK BETWEEN ALTERED METABOLISM AND IMPAIRED GLUTAMATE TRANSPORT

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Glutamate is the major excitatory neurotransmitter in the nervous system. Tight regulation of extracellular glutamate concentrations is ensured by glial cells and is critical for normal brain function. However, glutamate homeostasis is disrupted in glial tumours, and this was correlated with a reduced expression of glial glutamate transporters. Therefore, glioma cells are unable to efficiently clear glutamate from the synaptic cleft, leading to excitotoxicity and neuronal death in the vicinity of the tumour, thus potentially favoring tumour progression. The mechanisms causing the loss of glutamate transporters in glioma cells remain unknown. However, ongoing investigations in the laboratory suggest that the cellular energy sensor AMP-activated protein kinase (AMPK) is able to regulate astrocytic glutamate transporters. Given that AMPK has been implicated in the progression of several tumours and considering that brain tumours share many common features with neural stem cells - which reflect their undifferentiated status - we exposed C6 glioma cells to a validated differentiation protocol (dibutyryl-cAMP) and monitored the expression and activity of AMPK and glutamate transporters. Consistent with the recent literature, C6 cells presented an increased constitutive activity of AMPK compared with healthy rodent astrocytes. Our data also showed that driving differentiation of C6 cells into an astrocytic phenotype induces a decrease in AMPK expression and activity and promotes their glutamate uptake capacity. Further experiments including pharmacological and genetic inhibition of AMPK should consolidate our hypothesis for a functional link between the high constitutive AMPK activity found in glioma cells and their impaired uptake of glutamate.

P10

TRANSCELLULAR TRANSPORT IN THE BBB IN INFLAMMATORY CONDITIONS

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Situated between the blood circulation and the brain, the blood-brain barrier (BBB) protects the brain from circulating toxins while securing a specialized environment for neuro-glial signaling. Unique features of BBB-endothelial cells (ECs) include highly restrictive tight junctions that prevent paracellular diffusion, and low prevalence of non-specific transcytosis. Previous work demonstrated that the EC cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is an important determinant of BBB function and that connexin hemichannels (CxHCs) contribute to $[Ca^{2+}]_i$ dynamics and BBB alterations. BBB compromise, as observed in inflammatory conditions, involves paracellular leakage and/or increased, non-selective transcytotic events, the latter being the subject of this study. CxHCs may contribute to transcytosis by providing a direct diffusion pathway for small MW molecules (<1kDa), or they may exert control over $[Ca^{2+}]_i$ that is critical in the transcellular pathway. We used lipopolysaccharide (LPS) to trigger a BBB permeability increase in mice. This increase was prevented by intravenous injection of the Ca^{2+} chelator BAPTA-AM and the CxHC blocking peptide (Gap27). Dye uptake studies were performed in immortalized bEnd3 cells and primary mouse brain capillary ECs, using low (376Da) and high (10kDa) MW probes that allow separating endocytosis from CxHC-mediated dye uptake. Identification of vesicles in BBB-ECs was done by immunostaining for early endosome antigen-1 and TSG101. All experiments were performed in combination with inhibitors of vesicular transport (BrefeldinA), CxHCs (Gap27), or $[Ca^{2+}]_i$ changes (BAPTA-AM). Our results suggest that transcytosis may play a role in increased BBB permeability in inflammatory conditions and that Ca^{2+} signaling and CxHCs are also involved in this transcellular transport.

P11

TRP CHANNEL FUNCTION IN IPSC-DERIVED SENSORY NEURONS

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Using somatic cells to generate induced pluripotent stem cells (iPSC) is an established method in research and has multiple applications and advantages. Result is an increasing amount of cell types that have been successfully differentiated from iPSC's including hematopoietic cells, cardiomyocytes, smooth muscle cells, pancreas, liver and renal tissue. Directing differentiation into neuronal cells has the great benefit of bypassing the problematic isolation of human neuronal cells. After cortical neurons and motor neurons, a protocol using dual SMAD inhibition can drive differentiation into sensory neuron like cells. Although expression of canonical markers of sensory neurons is already validated, we are interested in the in-depth characterization of TRP channels in these induced neurons to verify a nociceptor phenotype. In this study, we use qPCR, Fura-2-based microfluorimetry and patch clamp experiments to evaluate the expression and function of the typical TRP channels at different time points in the development of the sensory neurons. Not only is a large variety of TRP channels important for somatosensation present, they furthermore exhibit similar behavior as in sensory neurons isolated from mice. Interestingly, electrophysiological recordings show a temporarily increase of TRPM3 responses in an early time point in differentiation, which can indicate an important role for this channel in the development of sensory neurons. To conclude, iPSC derived sensory neurons functionally express all TRP channels known to be important in somatosensory neurons. The possibility of inducing human sensory neurons in less than five weeks creates enormous potentials. For example, this method can be used to investigate development of sensory neurons *in vitro* and explore the physiology of pain in a human context. Moreover, it opens doors to generate patient-derived neurons for disease modeling and target validation.

P12

MEASURING SUBCELLULAR ACTIVITY OF TRPM3 CHANNELS USING TETHERED CALCIUM SENSORS

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TRPM3 is a calcium-permeable plasma membrane channel, activated by pregnenolone sulfate (PS), heat and membrane depolarization. TRPM3 is expressed in nerve terminals of sensory nociceptive (trigeminal and dorsal root ganglia) neurons where it evokes heat and (inflammatory) pain responses. Subcellular TRPM3 activity is difficult to measure with commonly used synthetic Ca^{2+} indicators (i.e. Fura2-AM, Fluo4-AM). Therefore we fused a fast genetically encoded calcium indicator (GCaMP6) and a red fluorescent mCherry molecule to the C-terminus of a TRPM3 channel. Ratiometric GCaMP-mCherry has a high SNR in the presence of a Ca^{2+} buffered cellular environment. With the use of total internal reflection (TIRF) microscopy, TRPM3-mediated local calcium signals in transfected HEK293T cells can be measured in different areas of the plasma membrane. Results from TRPM3-GCaMP-mCherry-tagged HEK cells, will be further used to study the localization and subcellular activity of TRPM3 ion channels in sensory mouse neurons.