BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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Spring Meeting

Friday, April 27th 2018

PROGRAMME AND ABSTRACT BOOK

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Venue

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

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Local host

Prof. Dr. Julien Hanson
Laboratory of Molecular Biology – GIGA Institute
ULiège
Belgium

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with support of the

Royal Flemish Academy of Belgium for Science and the Arts

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1000 Brussels

Main Lecture

10.00-11.00 G PROTEIN-COUPLED RECEPTORS: DO WE REALLY UNDERSTAND HOW THEY WORK.
Prof. Dr. Evi Kostenis – University of Bonn, Section Molecular-, Cellular-, and Pharmacobiology

Oral Communications

11.00-11.15 EFFECTS OF T-VNS ON BEHAVIORAL AND BRAIN RESPONSES TO SOMATOSENSORY STIMULI
M. Dumoulin, G. Liberati, A. Mouraux, R. El Tahry (UCL) (Invited by Pr. P. Gailly)

11.15-11.30 DEFECTIVE AUTOPHAGY IN VASCULAR SMOOTH MUSCLE CELLS ALTERS VASCULAR REACTIVITY IN YOUNG C57BL6 MICE
D.G. De Munck, P. Fransen, S. De Moudt, L. Roth, G.R.Y. De Meyer, W. Martinet (UAntwerp)

11.30-11.45 A1AMPK PROTECTS AGAINST ENDOTHELIAL BARRIER DYSFUNCTION THROUGH REGULATION OF CX43 EXPRESSION AND HSP27 PHOSPHORYLATION
M. Angé, D. Castanares-Zapatero, L. Bertrand, C. Beauloye, S. Horman (UCL)

11.45-12.00 THE ACC INHIBITOR TOFA DECREASES PLATELET MITOCHONDRIAL FUNCTION AND THROMBIN-INDUCED ACTIVATION.
M. Octave, S. Lepropre, S. Kautbally, V.M. Darley-Usmar, L. Bertrand, C. Beauloye, S. Horman (UCL, University of Alabama at Birmingham)

12.00-12.15 CHARACTERIZATION OF GPR27 SIGNALLING PATHWAY WITH SURROGATE LIGANDS AND CONSTITUTIVELY ACTIVE MUTANT
C. Laschet, N. Dupuis, S. Vandevoorde, D. Abboud, B. Pirotte, J. Hanson (ULG)

12.15-12.30 TARGETING GLYCOLYSIS TO PREVENT ATHEROSCLEROTIC PLAQUE FORMATION IN MICE
P. Perotta, B. Van der Veken, G.R.Y. De Meyer, W. Martinet (UAntwerp)

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

Posters
(height 120 cm – width 100 cm)

1. A NEW EX VIVO SLICE PREPARATION TO STUDY IONIC CONDUCTANCES DURING SPERMATOGENESIS
   D. Gall, P. Lybaert (ULB)
2. FUNCTIONAL CHARACTERISATION OF A DISEASE-CAUSING MUTATION IN TRPM4
   A. Pironet, S. Kerselaers, N. Syam, A. Janssens, M. Benoit, T. Voets, R. Vennekens (KULeuven)
3. AMPKA1 PARTICIPATES IN THE REGULATION OF MIR199A TO SUSTAIN CARDIAC HYPERTROPHY
   V. Joris, T. Metzinger, H. Esfahani, J.-L. Balligand, D. Catalucci, G. Condorelli , S. Horman, C. Dessy (UCL, UOS of Milan, IREC)
4. Poster withdrawn

5. PHOENIXIN-20-AMIDE DOES NOT ACTIVATE HETEROLOGOUSLY EXPRESSED GPR173
   S. Vandevoorde, C. Laschet, N. Dupuis, J. Hanson (ULG)

6. GENERATION OF GPR27 MUTANTS FOR THE STUDY OF ITS COUPLING WITH G PROTEINS
   M. Charles, C. Laschet, B. Pirotte, J. Hanson (ULG)

7. THE ROLE OF TRPV2 IN ENDOMETRIAL RECEPTIVITY
   C. Van den Eynde, J. Vriens (KULeuven)

8. GPR101 ORPHAN RECEPTOR: A NOVEL CAUSE OF GROWTH HORMONE DEREGULATION
   D. Abboud, A. Daly, P. Molina, N. Dupuis, C. Laschet, P. Geubelle, B. Pirotte, A. Beckers, J. Hanson (ULG)

9. IN VITRO INHIBITION OF α-AMYLASE ACTIVITY BY CITRULLUS COLOCYNTHIS FRUITS EXTRACTS
   N. Benariba, H. Laoufi, K.M. Berrekama, R. Djaziri, W.J. Malaisse (ULB, University Abou Bekr Belakaïd, Tlemcen, Algeria)

10. EFFECTS OF INSULIN AND *CITRULLUS COLOCYNTHIS* SEED AQUEOUS EXTRACTS UPON SODIUM TRANSPORT ACROSS A6 KIDNEY CELL MONOLAYERS
    N. Benariba, K. Louchami, R. Djaziri, A. Sener, W.J. Malaisse (ULB, University Abou Bekr Belakaïd, Tlemcen, Algeria)

Oral Communications

14.00-14.15 EPIGENETIC REGULATION OF OXIDATIVE STRESS AND ANGIOGENESIS BY MIR199A FAMILY IN LOCAL AND SYSTEMIC TISSUES OF GRAVES' PATIENTS.
J. Craps, V. Joris, M. Mourad, A. Buemi, C. Daumerie, B. Lengelé, C. Behets, L. Baldeschi, A. Boschi, C. Dessy, M.-C. Many (UCL)

14.15-14.30 MICE LACKING FUNCTIONAL TRPV2 DISPLAY LATE-ONSET INTRA-UTERINE GROWTH RESTRICTION
K. De Clercq, E. Persoons, T. Voets, G. Kerckhofs, J. Vriens (KULeuven, UCL)

14.30-14.45 LOCAL INHIBITION OF ANGIOGENESIS COMBINED WITH REPEATED BLOOD CLOT EMBOLIZATION INDUCES CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION IN RABBITS
R. Quarck, A. Wagenaar, B. Tielemans, P. Mendes-Ferreira, F. Perros, P. Dorfmüller, C. Belge, M. Delcroix (KULeuven, INSERM and Université Paris-Sud)

14.45-15.00 INHIBITING O-GLCNACYLATION BY AMP-ACTIVATED PROTEIN KINASE, A NEW WAY TO REVERSE CARDIAC HYPERTROPHY DEVELOPMENT?

J. Dontaine, F. Mailleux, R. Gélinas, A. Ginion, L. Bultot, C. Beauloye, S. Horman, L.Bertrand (UCL, IREC)

Coffee – Tea

ORAL 1: 11:00 – 11:15

EFFECTS OF T-VNS ON BEHAVIORAL AND BRAIN RESPONSES TO SOMATOSENSORY STIMULI

M. Dumoulin (1), G. Liberati (2), A. Mouraux (2), R. El Tahry (3), invited by Pr. P. Gailly (4)

(1): 7th grade medical student, Université Catholique de Louvain (UCL), Brussels, Belgium. (2): Institute of Neuroscience, UCL, Brussels, Belgium. (3): Department of Neurology, Saint-Luc University Hospital, Brussels, Belgium. (4): Department of Physiology and Pharmacology, UCL, Brussels, Belgium

INTRODUCTION | With only 1/3 of refractory epileptic patients benefitting from vagus nerve stimulation (VNS) therapy, a better understanding of the mechanisms of action of VNS is of fundamental importance. Transcutaneous-VNS (T-VNS), a non-invasive device stimulating the auricular branch of the vagus nerve, was suggested to influence nociception. Hence, its effects on behavioral and brain responses to nociceptive somatosensory stimuli could account for potential biomarkers of VNS effectiveness in reducing epileptic symptoms.
METHODS | 3 epileptic patients were tested at bedside. Active T-VNS stimulation (cymba conchae) and control stimulation (earlobe), randomized during a 2-day protocol, were delivered on the left ear for 3 consecutive hours. Before and after T-VNS/control stimulation, thermonociceptive (CO_2 laser, recruiting either A-δ or C fibers) and non-nociceptive (vibrotactile and cool) stimuli were applied on the right hand dorsum. We acquired perception thresholds, intensity, and quality for all four modalities, and recorded electroencephalography (EEG) responses elicited by three sets of 30 stimuli (one set per modality: heat recruiting A-δ fibers, cool, and vibrotactile).
RESULTS | T-VNS showed little influence on both behavioral and EEG responses to all four types of stimuli (vibrotactile, cool, A-δ and C fibers). These responses appeared to be divergent among the 3 tested patients.
CONCLUSION | With limitations from our restricted cohort and recording material, these divergent preliminary results could reflect the interpatient variability commonly seen in VNS therapy. Further studies among healthy subjects might better characterize the effects of t-VNS on behavioral and brain responses to somatosensory stimuli.

ORAL 2: 11:15 – 11:30

DEFECTIVE AUTOPHAGY IN VASCULAR SMOOTH MUSCLE CELLS ALTERS VASCULAR REACTIVITY IN YOUNG C57BL6 MICE

Dorien G. De Munck, Paul Fransen, Sofie De Moudt, Lynn Roth, Guido R. Y. De Meyer, Wim Martinet

Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium.

INTRODUCTION | Autophagy is an intracellular pathway that degrades and recycles defective organelles and proteins to maintain cellular homeostasis. Hence, defective autophagy is linked to many age-associated diseases. Recently, we demonstrated that autophagy in vascular smooth muscle cells (VSMCs) of large elastic arteries such as the aorta is important for Ca2+ mobilization and vascular reactivity. Whether autophagy plays a role in the smaller muscular arteries such as the femoral artery and thereby contributes to blood pressure regulation is unknown.

METHODS | Vascular reactivity of aortic and femoral artery segments was examined in mice containing a SMC-specific deletion of the essential autophagy gene Atg7 (Atg7F/FSM22α-Cre+) and age-matched Atg+/+ SM22α-Cre+ control mice using organ bath set-ups (aorta) and wire myograph (femoral artery).

RESULTS | Both aortic and femoral artery segments of Atg7F/F sm22-Cre mice were more sensitive to depolarization (high K+)-induced contractions (EC50 24.2±0.3mM, resp26.8±0.7mM). Contractions elicited by release of intracellular Ca2+ from the SR in the absence of extracellular Ca2+ were only affected in the femoral artery segments of autophagy deficient mice (AUC 77±10 vs33±4). In the aortic segments, basal NO release was enhanced and ex vivo analysis showed increased arterial stiffness at physiological pressures. In the femoral artery, which lacks basal NO release ex-vivo, VSMC were more sensitive to exogenous NO (pIC50 7.35±0.08logM, resp6.9±0.2logM).

CONCLUSION | Results indicate a differential response of large elastic arteries (aorta) and muscular arteries (femoral artery) to autophagy deficiency in their VSMC. This may be suggestive for a role of the autophagic process in blood pressure regulation and general blood vessel compliance.

ORAL 3: 11:30 – 11:45

Α1AMPK PROTECTS AGAINST ENDOTHELIAL BARRIER DYSFUNCTION THROUGH REGULATION OF CX43 EXPRESSION AND HSP27 PHOSPHORYLATION

Marine Angé[1,2], Diego Castanares-Zapatero1[,3], Luc Bertrand[1], Christophe Beauloye[1,4], Sandrine Horman[1]

[1] Université catholique de Louvain, Institut de Recherche Experimentale et Clinique, Brussels, Belgium; [2] Cliniques Universitaires Saint Luc, Division of Pediatrics, Brussels, Belgium; [3] Cliniques Universitaires Saint Luc, Division of Intensive Care, Brussels, Belgium; [4] Cliniques Universitaires

INTRODUCTION | Endothelial dysfunction plays a major role in sepsis pathogenesis, and is featured by hyperpermeability. AMP-activated protein kinase (AMPK) is known to regulate actin cytoskeleton organization and interendothelial junctions (IEJs), contributing to endothelial barrier integrity. We have already demonstrated its role in defence against sepsis induced hyperpermeability, but the underlying mechanisms remain unknown. This project aims to identify molecular targets involved in the beneficial action of AMPK against endothelial barrier dysfunction.
METHODS | Experiments have been performed in human microvascular dermal endothelial cells. Expression and/or activity of α1AMPK have been silenced by siRNA or stimulated by pharmacological AMPK activators (AICAr, 991). We have investigated the effect of this modulation on the expression/phosphorylation of Connexin 43 (Cx43) and Heat shock protein of 27 kDa (HSP27), two essential proteins for IEJs maintenance and actin dynamics respectively.
RESULTS | We show that α1AMPK is required to sustain Cx43 expression as it was drastically reduced in cells transfected with α1AMPK siRNA whereas HSP27 expression was not affected. However, both AMPK activators increased HSP27 phosphorylation on Ser82, in a α1AMPK-dependent manner, while Cx43 phosphorylation is unaffected. Our results also reveal that Hsp27 phosphorylation concurred with F-actin stress fibers appearance at cell periphery, suggesting a beneficial role for p-HSP27 as well as F-actin stress fibers in endothelial barrier function through reinforcing the endothelial tethering.
CONCLUSION | In conclusion, our work identifies the regulation of Cx43 expression and HSP27 phosphorylation as potential protective responses underlying the beneficial action of AMPK against endothelial barrier dysfunction.

ORAL 4: 11:45 – 12:00

THE ACC INHIBITOR TOFA DECREASES PLATELET MITOCHONDRIAL FUNCTION AND THROMBIN-INDUCED ACTIVATION.

M. Octave*[1], S. Lepropre[1], S. Kautbally[1], V. M. Darley-Usmar[2], L. Bertrand[1], C. Beauloye[1], S. Horman[1]

[1]Pôle de recherche cardiovasculaire, Institut de Recherche Expérimentale et Clinique, université catholique de Louvain, Bruxelles, Belgium [2]Department of Pathology, UAB Mitochondrial Medicine Laboratory, Center for Free Radical Biology University of Alabama at Birmingham, Birmingham, USA

INTRODUCTION | Acetyl-CoA carboxylase (ACC) is a key regulator of lipid synthesis and oxidation. Given the primary roles of lipids in platelet energy storage and signaling, we hypothesized that ACC inhibition might have consequences on platelet bioenergetics and functions.
METHODS | Platelets were treated with 30µM TOFA, an ACC inhibitor, for 2 hours before thrombin stimulation. Mitochondrial oxygen consumption rate (OCR) was measured using the Seahorse Flux Analyzer. Platelet functions were assessed by aggregometry and flow cytometric studies.
RESULTS | Indices of mitochondrial function assessed by OCR (pmoles/min/µg prot) before and after sequential injection of mitochondrial respiratory chain inhibitors (oligomycin, FCCP, rotenone/antimycin A) were decreased in TOFA-treated platelets relative to control (basal: control 9.01±0.53, TOFA 6.81±0.30; ATP-linked: control 7.79±0.65, TOFA 2.70±0.30; reserve capacity: control 8.19±1.37, TOFA 1.77±0.89; $p<0.05$). In addition, TOFA also suppresses mitochondrial function in response to thrombin, notably through a decrease in reserve capacity and ATP-linked respiration. These bioenergetics changes were accompanied by a significant defect in dense granules secretion and aggregation in response to low thrombin concentrations, whereas alpha-granules secretion was not affected, suggesting that the default in aggregation likely resulted from a lower autocrine effect of ADP. Underlying mechanisms involve reduced PKC activity, and in particular two PKC substrates activity, cytohesin-2 and PKD, known to control dense granule secretion and aggregation.
CONCLUSION | It is concluded that the inhibitory effect of TOFA on platelet secretion and on aggregation may be mediated by the reduction of mitochondrial energy production and PKC pathway activation.

ORAL 5: 12:00 – 12:15

CHARACTERIZATION OF GPR27 SIGNALLING PATHWAY WITH SURROGATE LIGANDS AND CONSTITUTIVELY ACTIVE MUTANT

C. Laschet[1,2], N. Dupuis[1,2], S. Vandevoorde[1,2], D. Abboud[1,2], B. Pirotte[2] and J. Hanson[1,2]

[1] ULG, Laboratory of Molecular Pharmacology, GIGA-Molecular Biology of Diseases, CHU, tour GIGA (+4), 11, av. de l'hôpital, 4000 Liège, Belgium [2] ULG, Laboratory of Medicinal Chemistry, CIRM, CHU, B36 (+5), 15, av. Hippocrate, 4000 Liège, Belgium.

GPR27 (SREB1) belongs to the super-conserved receptor expressed in the brain (SREB) subfamily, including GPR85 (SREB2) and GPR173 (SREB3). SREBs, which are only present in vertebrates, are the most evolutionarily conserved GPCRs and have the particularity to be mainly expressed in the central nervous system. For instance, GPR85 share 100% amino acid sequence homology between human, mouse and rat orthologues. This high level of conservation indicates that the functions of the SREB family are essential in vertebrates. No endogenous ligand has been proposed for GPR27 and it is still considered orphan. Dupuis et al. have recently identified two surrogate ligands promoting a specific recruitment of β-arrestin 2 to GPR27 upon stimulation. No activation of the closely related GPR85 or GPR173 was observed with these compounds, suggesting good selectivity. These two molecules were the first surrogate ligands described for GPR27 and may be considered as precious pharmacological tools to investigate GPR27 signalling pathway. Interestingly, no activation of G proteins was recorded for GPR27 stimulated with these two compounds. These observations were corroborated by similar results obtained with a mutant form of GPR27 (F6.44H) characterized by some level of constitutive activity. This mutant increased the interaction with β-arrestin 2 compared to wild type receptor but no G protein activation was detected despite being tested in various assays. Thus GPR27 may be considered as an atypical GPCR comparable to, for instance, CXCR7 or LGR5, which are GPCR members being devoid of G protein signalling.

ORAL 6: 12:15 – 12:30

TARGETING GLYCOLYSIS TO PREVENT ATHEROSCLEROTIC PLAQUE FORMATION IN MICE

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

Laboratory of Physiopharmacology, University of Antwerp

INTRODUCTION | Atherosclerosis is an inflammatory disorder characterized by accumulation of lipid- rich plaques in the arterial wall. Clinical evidence has recently shown that intraplaque (IP) angiogenesis promotes unstable vascular disease and thus contributes to increased risk of myocardial infarction, stroke and peripheral arterial disease. Because endothelial cells (ECs) rely on glycolysis for up to 85% of their energy demand, we investigated whether glycolysis inhibition may represent a novel strategy to prevent IP neovascularization and plaque formation.

METHODS AND RESULTS | We found that a small molecule, namely 3PO [3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one] (20-40 μM), markedly reduced glycolysis in ECs and inhibited their mobility/sprouting in vitro. We further found that 3PO (20 μM) induced autophagy in ECs (increased LC3-II and autophagic vacuoles detected by TEM). We then tested the effect of glycolysis inhibition on IP angiogenesis and atherogenesis using in vivo atherosclerosis models. In $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice, an established model of advanced atherosclerosis and IP angiogenesis, 3PO (50 μg/g body weight, i.p.) reduced neovascularization by almost 40% in a curative regimen (4 times/week for 4 weeks) and by 50% in a preventive regimen (2 times/week for 10 weeks). Interestingly, 3PO also reduced plaque formation in both treatments. The latter effect was confirmed in regular $ApoE^{-/-}$ mice, which develop plaques without IP angiogenesis.

CONCLUSION | In conclusion, inhibition of glycolysis represents a novel mechanism to prevent plaque formation in mice.

ORAL 7: 14:00 – 14:15

EPIGENETIC REGULATION OF OXIDATIVE STRESS AND ANGIOGENESIS BY MIR199A FAMILY IN LOCAL AND SYSTEMIC TISSUES OF GRAVES' PATIENTS.

Julie Craps*[1], Virginie Joris*[2], Michel Mourad[3], Antoine Buemi[3], Chantal Daumerie[3], Benoit Lengelé[1], Catherine Behets[1], Leilo Baldeschi[4], Antonella Boschi[4], Chantal Dessy**[2] and Marie-Christine Many**[1].

[1]IREC - MORF, [2]IREC - FATH, [3]Endocrinology Department, [4]Ophthalmology Department, Faculté de Médecine, Université catholique de Louvain, Bruxelles, Belgium. *Co-first authors,** Co-last authors

INTRODUCTION |Graves' disease (GD) is a Th2 autoimmune disease affecting thyroid and peripheral tissues such as orbital fat tissue. miR199a-3p (3p) and miR199a-5p (5p) are putative modulator of angiogenesis, endothelial dysfunction, oxidative stress (OS) and adipogenesis which are common features of GD pathophysiology. Accordingly, we previously showed that miRs 3p/5p and T4 are redundant regulators of the NOS/NO pathway in endothelial cells. We now aim to investigate the expression of both miR sequences in thyroid and fat tissues as well as in the plasma of GD patients.

METHODS |Thyroid and plasma samples were obtained from patients operated for multinodular goiters or for GD, orbital fat samples from blepharoplasty (controls) or thyroid associated orbitopathy (TAO). miRs expression was evaluated following Maxwell extraction, quantitative real-time PCR or in situ hybridization.

RESULTS |Thyroids from GD patients presented a significant reduction of 3p/5p expression. Interestingly, 3p/5p plasmatic circulation is also decreased in GD patients. GD orbital adipocytes also demonstrated a significant downregulation of these miRs. In addition, 5p in situ hybridization revealed a decrease in GD thyrocytes and GD adipocytes compare to controls whereas endothelial cells seem to express these miRs in the same way.

CONCLUSION |We have identified a new cluster of miRs differently regulated in the context of GD. A dramatic reduction in miR199a-3p/5p expression in GD-thyroid extracts, GD-plasma and GD-orbital fat are observed in GD tissues. Taken together, our results are in agreement with a potential implication of these miRs as regulators of OS, angiogenesis and the systemic manifestations of GD.

ORAL 8: 14:15 – 14:30

MICE LACKING FUNCTIONAL TRPV2 DISPLAY LATE-ONSET INTRA-UTERINE GROWTH RESTRICTION

Katrien De Clercq, Eleonora Persoons, Thomas Voets, Greet Kerckhofs, & Joris Vriens

Department of development and regeneration, KULeuven, Leuven | Department of molecular and cellular medicine, KULeuven, Leuven | Biomechanics Lab - Institute of Mechanics, UCL, Louvain-la-Neuve

INTRODUCTION |Members of the Transient Receptor Potential (TRP) superfamily are excellent candidates to regulate cation influx, as they are non-selective cation channels. TRPV2 is known to be involved in a myriad of physiological functions. Interestingly, TRPV2-/- mice were reported with reduced birth weight and increased susceptibility to perinatal death, suggesting intra-uterine growth restriction (IUGR). However, the function of TRPV2 in reproduction remains to be elucidated.
METHODS | TRPV2 expression was assessed in whole placentas and individual placental layers using quantitative RT-PCR (QRT-PCR). Fetal and placental weight of TRPV2 -/- and TRPV2+/+ littermates was assessed from E14.5 to term. Contrast-enhanced microfocus computed tomography (CE-CT) was used to evaluate placental morphology.
RESULTS | QRT-PCR on whole placentas through different time points of gestation revealed that the expression of TRPV2 was significantly increased at E14.5 and E15.5, with major expression in junctional zone and labyrinth. Interestingly, total placental weight of TRPV2-/- and TRPV2+/+ animals was similar at E14.5. However, from E15.5 until term, placental weight of TRPV2-/- was reduced. Remarkably, TRPV2-/- fetal weight decreased significantly from E16.5 onward, suggesting that a placental defect is responsible for the IUGR phenotype. CE-CT analysis indicated that, in line with the placental weight, the placental volume was also reduced from E15.5 onward, without affecting placental morphology. Finally, in situ hybridisation assays revealed a robust expression of TRPV2 in trophoblast giant cells.
CONCLUSION |The IUGR phenotype in mice lacking TRPV2 starts at E15.5 in the placenta and might be due to a role in trophoblast giant cells.

ORAL 9: 14:30 – 14:45

LOCAL INHIBITION OF ANGIOGENESIS COMBINED WITH REPEATED BLOOD CLOT EMBOLIZATION INDUCES CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION IN RABBITS

Rozenn Quarck, PhD; Allard Wagenaar, Birger Tielemans, MSc; Pedro Mendes-Ferreira, PhD Frédéric Perros, PhD; Peter Dorfmüller, MD, PhD; Catharina Belge, MD, PhD; Marion Delcroix, MD, PhD

RQ, AW, BT, CB, MD: Respiratory Division, CHROMETA, KU Leuven, Leuven, Belgium; FP, PD: UMR-S 999, INSERM and Université Paris–Sud, Le Plessis-Robinson (France)

INTRODUCTION | Chronic thromboembolic pulmonary hypertension (CTEPH) is a severe disease characterized by unresolved and organized thrombi obstructing the pulmonary arterial bed, resulting in PH and right ventricular (RV) failure. The disease is notoriously underdiagnosed and its pathogenesis remains poorly understood. The need of reliable animal models of CTEPH remains a major challenge. We hypothesized that inhibition of fibrinolysis and/or angiogenesis may prevent clot resolution and promote CTEPH progression. We aimed to investigate whether repeated embolization with blood clots containing an inhibitor of fibrinolysis or angiogenesis may induce CTEPH in rabbits.

METHODS | Adult male New Zealand rabbits were weekly embolized (x 7) with autologous blood clots alone, containing tranexamic acid (TXA), a fibrinolysis inhibitor, Sugen, an inhibitor of VEGF receptor or together with intravenous injection of TXA. A sham-operated group received saline. Right heart catheterization was performed 8 weeks after the last embolization. Right ventricular systolic pressure (RVSP) was monitored by telemetry in a subset of animals. RV hypertrophy and lung vessel morphometry were analyzed.

RESULTS | Repeated embolization with clots containing Sugen resulted in a 2.3-fold increase increase in mean pulmonary arterial pressure ($p<0.05$), a 4-fold increase in pulmonary vascular resistance index ($p<0.00001$) without any change in cardiac output; it also induced fibro-thrombotic lesions in large proximal pulmonary arteries and remodeling of distal pulmonary arteries in occluded and non-occluded areas.

CONCLUSION | Embolization with autologous blood clots containing Sugen promoted CTEPH development in rabbits. Obstruction of large proximal pulmonary arteries by fibro-thrombotic lesions and distal vascular remodeling highlights the similarity with the human pathology of CTEPH.

ORAL 10: 14:45 – 15:00

INHIBITING O-GLCNACYLATION BY AMP-ACTIVATED PROTEIN KINASE, A NEW WAY TO REVERSE CARDIAC HYPERTROPHY DEVELOPMENT?

Dontaine*, F. Mailleux, R. Gélinas, A. Ginion, L. Bultot, C. Beauloye, S. Horman, L. Bertrand

Pole of cardiovascular research (CARD), Université Catholique de Louvain, Institute of Experimental and Clinical Research (IREC), Bruxelles, Belgique

INTRODUCTION | We previously showed that AMPK activation prevents cardiac hypertrophy by decreasing protein O-GlcNAcylation (O-GlcNAc) levels. In order to better fit with physio-pathological conditions encountered in patients and reach therapeutic aim, we were interested to check if AMPK activation can also reverse a cardiac hypertrophic phenotype when already developed.
METHODS | Neonatal rat cardiomyocytes (NRVMs) were treated with the prohypertrophic agent phenylephrine (PE, 20 µM) for 24h, a time necessary to develop significant hypertrophy. NRVMs were then treated with or without the specific AMPK activator A769662 (12,5 µM and 25 µM) or more classical indirect AMPK activators (phenformin 0,03mM or AICAr 0,25 mM) for an additional 24h period. O-GlcNAcylation was concomitantly stimulated by the pharmacolocial O-GlcNAc inducer PUGNAc (50 µM). NRVM Hypertrophy was evaluated by measurement of cell surface area. O-GlcNAc levels were evaluated by western-blotting.
RESULTS | As previously shown, PE-dependent cardiomyocyte hypertrophy was accompanied by an increase in protein O-GlcNAc levels. Interestingly, A769662-mediated AMPK activation was able to reverse this PE-mediated O-GlcNAc increase, reaching levels similar to those found in control cells. This was nicely accompanied by a reduction in cell size. The same applied using the two other AMPK activators, phenformin and AICAr, demonstrating the universality of our phenomenon. Next, we evaluated the key role of O-GlcNAc by co-treating NRVMs with the O-GlcNAc inducer PUGNAc and showed that PUGNAc fully prevented the anti-hypertrophic action of the three AMPK activators.
CONCLUSION | Our results revealed that AMPK activation can reverse cardiomyocyte hypertrophy development by regulating O-GlcNAcylation levels.

12:30 – 14:00 Guided Poster Session

POSTER 1

A NEW EX VIVO SLICE PREPARATION TO STUDY IONIC CONDUCTANCES DURING SPERMATOGENESIS

David Gall and Pascale Lybaert

Laboratoire de Physiologie et Pharmacologie, Université Libre de Bruxelles, Belgium

Spermatogenesis is a complex developmental processes involving differentiation of spermatogonial stem cells into mature spermatozoa throughout the adult life. The molecular mechanisms that control germ cell differentiation and maturation in mammals are notoriously difficult to unravel, given the intricate anatomy and complex endo- and paracrinology of the testis. Ion channels and transporters are key elements in sperm–egg signaling, environmental sensing and are essential for fertilization, but little is known about their role in spermatogenesis. Here we present a new procedure to obtain acute testis slices that will allow us to study the evolution of the electrophysiological profile of germ cells during their maturation. Investigating these biological properties may provide critical insights for understanding stem cell biology and tissue development.

POSTER 2

FUNCTIONAL CHARACTERISATION OF A DISEASE-CAUSING MUTATION IN TRPM4

Andy Pironet, Sara Kerselaers, Ninda Syam, Annelies Janssens, Melissa Benoit, Thomas Voets, Rudi Vennekens

Laboratory of Ion Channel Research, KULeuven

Cardiac conduction disorders are a common cause of lethal cardiac arrhythmia. The origin of these cardiac conduction disorders are congenital in either structure or ion channel function. Progressive Familial Heart Block type 1 (PFHB1) is an inherited cardiac conduction disease which is caused by gain-of-function mutations in an ion channel, called TRPM4. Several point mutations related to this disease have already been characterised, but mechanistic basis on how these gain-of-function mutations in TRPM4 can contribute to this disease is still lacking. Recently, a novel point mutation (i.e. p.I376T) has been found in patients, but no characterisation of this mutant was done. Moreover, TRPM4 is also marked as a novel drug target since preliminary data show that Trpm4-/- are less vulnerable to cardiac arrhythmia, however, there are no safe and reliable drug therapies yet. To encounter these challenges, we characterised the p.I376T-mutation in human TRPM4 as well its murine orthogolue (i.e. p.I377T-mTRPM4) via Patch Clamp experiments in overexpressing HEK293T-cells and characterized a novel antagonist of TRPM4 which might serve as a novel anti-arrhythmic drug.

POSTER 3

AMPKA1 PARTICIPATES IN THE REGULATION OF MIR199A TO SUSTAIN CARDIAC HYPERTROPHY

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INTRODUCTION | miR-199a has been shown to participate in process of cardiac hypertrophy by modulating autophagy and the maintenance of cell size. An activation of AMPK is also known in the hypertrophic heart. Aims of our study were to delineate the signaling cascades leading to regulation of both mature forms of miR199a during hypertrophy.
METHODS |In order to provoke hypertrophy, Wild type and Knock-out AMPKa1 mice were submitted to trans-aortic constriction (TAC). After 8 weeks, heart and plasma were processed for miR profiling. Rat cultured primary cardiomyocytes were treated with metformin (activating AMPK), phenylephrine (mimicking hypertrophy) and/or siRNA AMPKa1. MiR199a3p/5p expression and AMPK activity were measured by qPCR and Western Blotting.
RESULTS |Both miRs 199a3p/ 5p expression were increased in hypertrophic hearts compared to controls. Plasmas of these mice present an increase of miR199a5p only. Interestingly, in TAC-AMPKa1 KO mice, no increase of miR199a5p was measured in the heart or plasma during hypertrophy, indicating that AMPKa1 participates in the control of miR199a5p expression. Moreover, rat cardiomyocytes treated with phenylephrin or metformin were associated with an increase of miR199a5p expression and correlated with activation of AMPK pathway. Increased levels of miR199a5p were also measured in culture medium. In cardiomyocytes transfected with siRNA targeting AMPKa1, phenylephrin-induced miR199a5p upregulation was inhibited.
CONCLUSION| We demonstrate that expression of miR199a3p/5p is upregulated in the context of cardiac hypertrophy. In hypertrophic heart and phenylephrine-treated cardiomyocytes, the expression of miR199a5p is modulated by AMPKa1. In addition, cardiomyocyte miR199a5p is secreted in plasma and might regulate other cell types

POSTER 4
Withdrawn

POSTER 5

PHOENIXIN-20-AMIDE DOES NOT ACTIVATE HETEROLOGOUSLY EXPRESSED GPR173

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GPR173 (SREB3) is an orphan GPCR of the 'Superconserved Receptors Expressed in the Brain' family with high mRNA levels expressed in the hypothalamus, cortex and cerebellum. Phoenixin-20-amide (P20A), an endogenous hypothalamic ligand regulating ovarian cyclicity (Ullah et al., 2017; Stein et al., 2016), has been suggested to activate GPR173. In vitro studies on gonadotrophs showed that P20A stimulates GPR173, thereby increasing cAMP production, resulting in an elevated hypothalamic 'Gonadotrophin Releasing Hormone Receptor' (GnRHR) and kisspeptine expression, eventually increasing luteinizing hormone (LH) secretion (Treen et al., 2016; Stein et al., 2016). However, using the Promega GloSensor™ Technology, we failed to observe GPR173 mediated changes of cyclic AMP concentrations in HEK293 cellular models upon stimulation with P20A. Furthermore, it has been suggested that GPR173 could be stimulated by GnRH1_5 to trigger the recruitment of arrestin-3 in neurons co-expressing GnRHR, thereby inhibiting developmental neuronal migration (Larco et al., 2013). In our hands, with an arrestin-3-based firefly luciferase complementation assay in heterologously tranfected HEK293 cells, GPR173 was not able to recruit arrestin-3 upon stimulation with neither P20A nor GnRH1_5. Finally, GPR173 did neither bind any promiscuous g-proteins upon stimulation with P20A nor show any constitutive activity. Whether P20A influences the secretion of LH has not been studied, but P20A did not directly activate GPR173 in none of our assays. Nevertheless, transfected cellular models should be distinguished from native systems. Further studies are therefore necessary to determine if an endogenous partner is missing in our models and might be responsible for the previously reported results.

POSTER 6

GENERATION OF GPR27 MUTANTS FOR THE STUDY OF ITS COUPLING WITH G PROTEINS

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GPR27 is an orphan G protein-coupled receptor (GPCR) and a member of the super-conserved receptor expressed in the brain (SREB) subfamily. Recently, two surrogate agonists for GPR27 were identified[1]. Interestingly, it was found out that GPR27 activation induces the recruitment of beta-arrestin 2 but does not activate the G protein pathways[1]. The unexpected lack of G protein signalling could be due to biased signalling of the compounds or the consequence of an atypical nature of GPR27. Consistent with the idea that GPR27 is unable to couple to G proteins, we observed that two GPCR conserved motifs known to be critical for G protein-receptor interaction[2], the TM3 DRY and the TM7 NPXXY, were mutated to TRY and NPXXC in GPR27[1]. In order to understand the absence of G protein activation after stimulation of GPR27 with the surrogate ligands, we generated a modified GPR27 to rescue G protein signalling. First, we constructed a chimeric receptor where the three intracellular loops (ICL) and C terminus of GPR27 were replaced with those of the beta2 adrenoceptor that is known to couple to Gs. Moreover, the TRY and NPXXC motifs were replaced by DRY and NPXXY, respectively. The aim of this work is to validate our construct and to determine whether this modified GPR27 is able to activate Gs. This could confirm that WT GPR27 is an atypical GPCR with regard to G protein signalling. [1] Dupuis, et al. (2017). Mol Pharmacol, 91, 595. [2] Carpenter, et al. (2017). Curr Opin Struct Biol, 45, 124.

POSTER 7

THE ROLE OF TRPV2 IN ENDOMETRIAL RECEPTIVITY

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INTRODUCTION| We have identified the transient receptor potential receptor vanilloid 2 (TRPV2) ion channel as a novel ion channel functionally expressed in the endometrial stroma of mice and human. Interestingly, TRPV2 mRNA levels were significantly upregulated towards the late luteal phase of the menstrual phase, suggesting a possible regulation of TRPV2 by reproductive hormones. Here, we aim to investigate the role of TRPV2 in the development of a receptive endometrium.
METHODS|The role of TRPV2 in endometrial proliferation was assessed in vitro by using the MTT assay. To investigate the role of TRPV2 in decidualization, we performed in vitro decidualization by applying cAMP and MPA for 5 days. Prolactin expression (RT-qPCR) and secretion (ELISA) was assessed to validate the protocol. TRPV2 expression (RT-qPCR) and functionality (Ca2+ imaging) was investigated after decidualization, and after application of growth factors important for endometrial receptivity.
RESULTS | Activation of TRPV2 by its agonist 9-tetrahydrocannabinol (THC) resulted in higher basal calcium levels and increased metabolic activity in primary cultures of ESC of mouse in MTT assays. In contrast, the TRPV2 mRNA expression levels and functionality were significantly reduced after induction of the decidualization process. This reduction of TRPV2 expression was mediated by cAMP, as we could observe a dose-dependent relationship between cAMP concentrations and TRPV2 expression. Furthermore, TRPV2 expression and functionality are increased upon incubation of ESC with the growth factors EGF, HB-EGF and TGF-α.
CONCLUSION | Altogether, these data suggest a major role of TRPV2 as regulator in the development of a receptive endometrium.

POSTER 8

GPR101 ORPHAN RECEPTOR: A NOVEL CAUSE OF GROWTH HORMONE DEREGULATION

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GPR101 is an orphan G-protein coupled receptor. Recently, a clinical study showed that GPR101 is strongly associated X-linked acrogigantism syndrome, a rare disorder characterized by excessive growth hormone secretion. Carriers of the GPR101 duplication on the X chromosome develop pituitary adenomas that do not respond to current therapies. The GPR101 function in growth regulation is elusive and this lack of information precludes its validation as a drug target. Therefore, we sought to characterize GPR101 signaling pathways. We cloned in expression vector the human GPR101 fused to an N-terminus FLAG and we generated cells stably expressing FLAG-GPR101. Subsequently, we determined the receptor precise cellular localization by using flow cytometry and confocal microscopy. Then, we used several pharmacological transduction assays and we developed a bioluminescent beta-arrestin complementation test to decipher GPR101 signaling pathways. We also screened for compounds and we generated several mutants that can increase or alter GPR101activity. GPR101 is characterized by a very high constitutive activity. We detected constitutive cAMP production that was linked to Gs activity, and we completed our study with an examination of receptor coupling to other G proteins. We also demonstrated that the basal recruitment of beta-arrestins leads to constitutive internalization of the receptor and relocates the receptor into endosomes. Furthermore, we investigated the origin of constitutive activity and identified putatively important residues (such as the E308) for constitutive signaling. These informations constitute an absolute prerequisite to establish a strong mechanistic link between GPR101 activation in the pituitary, and to treat people suffering from pituitary dysfunction.

POSTER 9

IN VITRO INHIBITION OF α-AMYLASE ACTIVITY BY CITRULLUS COLOCYNTHIS FRUITS EXTRACTS

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An aqueous extract, an H_20-methanol extract, a n-butanol extract and an ethyl acetate extract from Citrullus colocynthis fruits were tested at increasing concentrations (0.50, 0.83, 1.66 and 2.50 mg/ml) for their possible inhibitory effects upon the activity of Aspergillus oryzae α-amylase, the monosaccharide generated over 10 minutes incubation at 25°C being measured by the Bernfeld method. The polyphenol and flavonoid content of the extracts were also measured. Inhibition of α-amylase activity was indeed observed, with an aqueous extract > H_2O-methanol extract > n-butanol extract > ethyl acetate extract hierarchy in terms of the relative extent of enzymatic activity inhibition as assessed in experiments designed to document the concentration-response relationship. The relative potency of the four tested extracts paralleled their polyphenol content. Such a hierarchy was similar to that previously observed for either the decrease in glycaemia or regeneration of insulin-producing cell mentioned above. Consideration is eventually given to the possible link between the present enzymatic data and previous metabolic and cellular findings collected in diabetic rats.

POSTER 10

EFFECTS OF INSULIN AND CITRULLUS COLOCYNTHIS SEED AQUEOUS EXTRACTS UPON SODIUM TRANSPORT ACROSS A6 KIDNEY CELL MONOLAYERS

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Considering the effectiveness of insulin upon target cells as another process susceptible to be influenced by plant extracts, the present study compares the effects of either crude or defatted aqueous extracts from *Citrullus colocynthis* seeds upon Na^+ transport in A6 kidney cells. The intensity of electrical currents, taken as the potential difference divided by the epithelial resistance was measured in monolayers of A6 kidney cells exposed at the basolateral side to either crude untreated aqueous or defatted aqueous extracts, as well as fractions of these extracts containing material with low (< 5 kDa) or high (> 5 kDa) molecular weight, the effect of insulin being first documented in order to assess the functional integrity of the A6 monolayer. The defatted aqueous extract was more efficient than the untreated aqueous extract in terms of increasing the intensity of current in the A6 monolayer. The compounds of the defatted aqueous extract responsible for such a greater efficiency were located in the fraction containing high molecular weight material and acted in a time-related progressive fashion upon Na^+ transport. In the light of the present findings, further investigations may be justified in the perspective of a possible use of *Citrullus colocynthis* extracts in a therapeutic perspective.