# BELGIAN SOCIETY OF

# PHYSIOLOGY AND PHARMACOLOGY

## NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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**Spring Meeting**

**Friday, April 26th 2024**

**PROGRAMME**

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**Venue**

**Palace of the Academies**
**Royal Academy of Medicine of Belgium**
**"Rubens room"**
**Rue Ducale / Hertogstraat 1**
**1000 Brussels**

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**Local host**

**Prof. Dr. Philippe Gailly**
**Institute of Neuroscience (IoNS)**
**Laboratory of Cell Physiology (FYCL)**
**UCLouvain**
**Belgium**

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**with support of the**

**Royal Flemish Academy of Belgium for Science and the Arts**

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**Palace of the Academies**
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**1000 Brussels**

09.15-10.00 Welcome with coffee and tea

**Keynote lecture**

10.00-11.00 **TRP ionotropic cannabinoid receptors**
Prof. Dr. Peter Zygmunt (Lund University, Sweden)

**Oral communications (morning session)**

11.00-11.15 **Delineating the Binding Pocket of Meclofenamate in the TRPM4 ion channel**
Andy Pironet and Rudi Vennekens (KULeuven)

11.15-11.30 **Analysis of cyst hetero- and homogeneity in autosomal dominant polycystic kidney disease**
Jean-Paul Decuypere, Daniel Borras, Priyanka Koshy, Abhishek Garg, Djalila Mekahli, Rudi Vennekens (KULeuven & UZ Leuven)

11.30-11.45 **Investigating myocarditis following mRNA COVID-19 vaccination in exercising mice**
S. Eens, M. Van Hecke, S. Van den Bogaert, K. Favere, N. Cools, E. Fransen, T. Roskams, H. Heidbuchel, PJ. Guns (UAntwerp)

11.45-12.00 **β3-adrenoceptor as a new promising target against proliferative retinopathies**
Melecchi A., Amato R., Canovai A., Cammalleri M., Dal Monte M., Bagnoli P. (University of Pisa, Italy)

12.00-12.15 **Understanding the impact of heart failure treatment on cancer growth**
C. Civati, B. Goovaerts, B. van Berlo, V.F.M. Segers, G.W. De Keulenaer (UAntwerp)

**12.15 – 12.45 Lunch**

**12.45 – 13.30 Guided Poster Session**

**Posters:**

- **Exploring apelinergic system signaling in autosomal dominant polycystic kidney disease**
  Corinna Heinze, Jean-Paul Decuypere, Rudi Vennekens, Djalila Mekahli (KU Leuven)

- **Investigating the structure and pharmacology of SK channels through molecular docking and in vitro patch clamp**
  Mouchet A.1, Vitello R.2, Brans A.1, Seutin V.3, Liégeois J.F.2, Kerff F.1 (ULiège)

- **The endothelial glycocalyx: a functional and morphological investigation in a mouse model**
  Benjamin Hanotieau, Louise Mary, Sophie Dogné, Nathalie Kirschvink (UNamur)

- **Brain Death-Induced Renal Injury in pigs: Unveiling Glycocalyx alteration and the Protective Role of Tacrolimus**
  Kaoutar Idouz, Benoit Rondelet, Laurence Dewachter, Asmae Belhaj, Nathalie Kirschvink, Sophie Dogné (UNamur, CHU UCL Namur & ULB)

- **Conditional deletion of TRPC1 modulates synaptic plasticity, long term depression, and memory extinction in Fragile X Syndrome mice**
  Farah Issa, Xavier Yerna, Thibaud Parpaite, Caren Jabbour, Olivier Schakman, Nicolas Tajeddine, Roberta Gualdani, Philippe Gailly (UCLouvain)

- **PHARMACOLOGICAL CHARACTERIZATION OF THE ORPHAN G PROTEIN-COUPLED RECEPTOR GPR85**
  Manon Stoffels, Monika Wozniak, Thanigaimalai Pillaiyar, Julien Hanson (ULiège & U Tübingen)

- **Investigating cell type diversity in the maturing bed nucleus of the stria terminalis in mice**
  Yana van de Poll, Yasmin Cras, Tommas Ellender (UAntwerp)

## Oral communications (afternoon session)

13.30-13.45 **Induction of Autophagy normalizes Arterial Stiffness in Mice**
Van Praet M., Neutel C.H.G., Jacobs C., Roeyen E., Wesley C., Krüger D, Guns P.J., De Meyer G.R.Y., Martinet W. and Roth L. (UAntwerp)

13.45-14.00 **Bcl-2-based strategies to target dysregulated Ca2+ signaling as an early event in Alzheimer's disease**
Callens Manon, Vervliet Tim, Chernyuk Daria, Polozova Marina, Gordeev Andrey, Pchitskaya Ekaterina, Chigriai Margarita, Bezprozvanny Ilya, Bultynck Geert (KULeuven & Peter the Great St. Petersburg State Polytechnic University)

14.00-14.15 **The effect of hypercholesterolemia and aging on nociception in rodents**
Justyna B Startek, Alina Milici, Silvia Pinto, Evy Van Den Broek, Karel Talavera (VIB-KU Leuven)

14.15-14.30 **Kinins: Locally formed peptides during inflammation with potential use in tissue regeneration**
Leonardo MARTIN, Michael BADER, João PESQUERO (UAntwerp, Max-Delbrück Center for Molecular Medicine, Berlin, Germany & Federal University of São Paulo, Brazil)

14.30-14.45 **Implication of cholesterol in mechanosensitive ion channel deregulation in glioblastoma**
AVALOSSE Noémie; DUMITRU Andra-Cristina; VERSAEVEL Marie; GABRIELE Sylvain; GAILLY Philippe & TYTECA Donatienne (UCLouvain & UMons

## 14.45 – 15.15 Coffee – Tea and Networking

## Closing lecture

15.15-16.00 **Hypoxia and Hypoxia-Inducible Factor Signaling in Muscular Dystrophies**
Alexandra Tassin (UMons)

## 16.00 General Assembly for the Members

ORAL PRESENTATION 1

**Delineating the Binding Pocket of Meclofenamate in the TRPM4 ion channel**

Andy Pironet and Rudi Vennekens

KULeuven

INTRODUCTION | Cardiac arrhythmias pose a major health threat, yet often prove difficult to prevent and treat. Current anti-arrhythmic treatments lack efficacy and approved anti-arrhythmic drugs have considerable adverse effects, including drug-induced pro-arrhythmia. Inhibiting TRPM4, a Ca2+-activated non-selective cation channel expressed in all segments of the human heart, is a crucial potential treatment mechanism. We have recently identified meclofenamate as a potent TRPM4 inhibitor. We showed that inhibition of TRPM4 with meclofenamate suppresses cardiac arrhythmias in living mice in a TRPM4-dependent manner via suppression of a Ca2+-dependent background current, which contributes to cardiac cellular excitability. In order to further contribute to drug development targeting TRPM4, we investigated the binding pocket of meclofenamate in the TRPM4.

METHODS | We performed whole-cell and inside-out patch clamp on HEK-293T cells transiently transfected with either the wild-type or mutant TRPM4 channel.

RESULTS | Using whole-cell patch clamp, we observed that binding of meclofenamate was largely abolished in cells expressing either the p.L907A mutation or the p.S924A mutation. Inside-out patch clamp recordings showed a 4.8x increase in the IC50 value of meclofenamate by the p.L907A mutant and a 3.6x increase by the p.S924A mutant. Furthermore, despite location of the L907 residue in the vicinity of a Ca2+ binding site, we showed that meclofenamate does not interfere with Ca2+ sensitivity of the human TRPM4 channel.

CONCLUSION | The presented data establishes the involvement of L907 and S924 residues in the binding pocket of meclofenamate in TRPM4, informing further drug development targeting TRPM4 in suppressing cardiac arrhythmias.

ORAL PRESENTATION 2

**Analysis of cyst hetero- and homogeneity in autosomal dominant polycystic kidney disease**

Jean-Paul Decuypere1 , Daniel Borras2 , Priyanka Koshy3 , Abhishek Garg2 , Djalila Mekahli1,4, Rudi Vennekens5

1KU Leuven, PKD Research Group, Leuven, Belgium, 2KU Leuven, Lab of Cell Stress & Immunity, Leuven, Belgium, 3UZ Leuven, Dept of Pathology, Leuven, Belgium, 4UZ leuven, Dept of Pediatrics, Leuven, Belgium, 5KU Leuven, Lab of Ion Channel Research, Leuven, Belgium

INTRODUCTION | Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, most frequently caused by mutations in PKD1. Kidney cysts arise from tubular epithelial cells that aberrantly dedifferentiate and proliferate, regulated by various molecular changes. However, the exact sequence of events and their relative importance in cyst formation remains unclear.

METHODS | We performed RNA-Seq analysis on 40 individual cystic membranes of kidneys of 4 PKD1 patients, 4 microcystic tissues from 2 ADPKD kidneys and 4 samples from 4 healthy kidneys. Principal component, cluster and trajectory analysis was performed to explore cyst heterogeneity. Cystic membranes were stained with proximal (PT) or distal (DT) tubular markers.

RESULTS | The heterogeneity among individual cysts was independent of patient, gender, cyst size, location or fluid color. It was characterized by a gradual increase of interstitial (remodeling) and inflammation markers, and a decrease in differentiation. The decrease of PT markers already occurred in microcystic tissue and was stronger compared to DT segments. Several cysts showed expression of multiple tubular segments. Although none of the macrocystic membranes stained positive for PT, microcysts did. Small macrocysts (diameter in the mm range) were lined by both PT- and DT-positive regions.

CONCLUSION | We tracked down the molecular alterations in ADPKD progression and determined whether they occur similarly in all cysts or at different levels. Moreover, loss of PT markers is more prominent in macrocysts compared to the decrease in distal markers. Finally, inner cyst walls can consist of cyst-lining cells of multiple origins.

ORAL PRESENTATION 3

## Investigating myocarditis following mRNA COVID-19 vaccination in exercising mice

S. Eens, M. Van Hecke, S. Van den Bogaert, K. Favere, N. Cools, E. Fransen, T. Roskams, H. Heidbuchel, PJ. Guns

Cardiovascular Research Group & Laboratory of PhysioPharmacology (GENCOR), University of Antwerp

Introduction: The mechanism underlying acute myocarditis associated with mRNA COVID-19 vaccination, as well as the potential predisposition for young, physically active men, remains poorly understood. The current study utilized a recently described mouse model of mRNA COVID-19 vaccine-induced myocarditis to explore the potential role of physical exercise as a contributing risk factor.

Methods: Groups of seven-week-old male BALB/c mice underwent either continuous treadmill running (30cm/s, daily) or remained sedentary for five weeks. Subsequently, two high doses of the mRNA COVID-19 vaccine or vehicle were intravenously administered with a 14-day interval, while continuing the physical activity regimen. Animals were sacrificed two to three days after the administration of the second dose.

Results: Vaccination elicited a robust, spike-specific, Th1-skewed T cell response and induced mild body weight loss. Vaccination led to a twofold increase in the incidence of discrete hepatic inflammation. Small foci of fibrovascular inflammation and focal cell loss were predominantly observed subepicardially in the right ventricle, regardless of vaccination or exercise status. Vaccination did not result in an elevation of plasma cardiac troponin I levels. Cardiac tissue from vaccinated mice showed upregulated mRNA expression of the pro-inflammatory genes IFNγ and IL-1β, independently of exercise. Ex vivo vascular reactivity and endothelial adhesion molecule expression in aortic tissue remained unaffected by vaccination.

Conclusion: Our data support the cardiac safety of mRNA COVID-19 vaccination. Despite the disproportionately high dosing and intravenous administration route, no evidence of myocarditis was observed. The potential involvement of physical exercise in vaccine-induced myocardial injury could not be determined.

ORAL PRESENTATION 4

## β3-adrenoceptor as a new promising target against proliferative retinopathies

Melecchi A., Amato R., Canovai A., Cammalleri M., Dal Monte M., Bagnoli P.

University of Pisa, Italy

Although the role of β-adrenoceptors (β-ARs) in retinopathy of prematurity (ROP) has mainly focused on the study of β1\β2-AR, β3-AR could represent another promising drug target given its lower desensitization after hypoxia-induced overexpression. Moreover, β3-AR stimulation, when overexpressed, showed beneficial effects in other organs such as the heart and adipose tissue. Here, the effects of β3-AR stimulation with BRL 37344 (BRL) were investigated on the oxygen-induced retinopathy (OIR) experimental model, a well-established model of ROP. Alterations in retinal angiogenesis together with loss of astrocyte and retinal ganglion cells (RGC) density and related loss of visual function are considered typical OIR hallmarks. BRL was administered daily and subcutaneously during the hypoxic stage of the OIR experimental model. Retinal vasculature and astrocyte or RGC density were evaluated by immunofluorescence and western blot analyses. Retinal function was evaluated by electroretinogram (ERG) recordings. β3-AR stimulation with BRL displayed a strain-dependent efficacy, restoring the physiological vascularization with no avascular areas or peripheral tufts only in the 129s, but not in C57/BL6 mouse retinas. BRL administration also maintained astrocyte and RGC density in the retinal central areas and preserved the visual function only in the 129s mouse strain. Our findings suggest that 3-AR is significantly involved in the hypoxia-induced retinal angiogenesis, and its stimulation with BRL may counteract the OIR-induced altered vasculature, astrocyte or RGC density in the central area of the retina, also preserving the visual function. Thus, β3-AR might represent a promising pharmacological target against proliferative retinopathies such as ROP.

ORAL PRESENTATION 5

**Understanding the impact of heart failure treatment on cancer growth**

C. Civati, B. Goovaerts, B. van Berlo, V.F.M. Segers, G.W. De Keulenaer

Laboratory of Physiopharmacology, University of Antwerp

INTRODUCTION: Heart failure (HF) constitutes a multifaceted syndrome characterized by systemic alterations that extend beyond the cardiovascular domain, potentially influencing cancer progression.

PURPOSE: This investigation aims to elucidate the intricate interplay between HF, particularly post-myocardial infarction (MI) left ventricular (LV) dysfunction, and the development of breast cancer along with its metastatic dissemination to the lungs.

METHODS: In a mouse model, myocardial infarction was induced, followed by treatment with carvedilol, lcz696, enalapril, or empagliflozin. Subsequently, 4T1 breast cancer cells were injected, and tumor growth and cardiac function were monitored.

RESULTS: Empagliflozin notably demonstrated a significant reduction in LV volumes ($p=0.0043$; $p=0.0186$). Empagliflozin significantly decreased both tumor weight ($p=0.0306$) and volume ($p=0.0268$). Furthermore, carvedilol significantly attenuated lung metastases ($p=0.0347$).

CONCLUSION: Despite the presence of HF, there was no exacerbation of cancer growth post-MI. However, specific HF treatments, particularly carvedilol and empagliflozin, showed promise in mitigating cancer growth post-MI, with carvedilol displaying additional efficacy in reducing metastatic spread. These findings underscore the potential of HF therapies to exert effects beyond their traditional cardiovascular targets, suggesting novel avenues for therapeutic exploration in cancer management. Nevertheless, further elucidation of the underlying mechanisms and molecular impacts of HF treatments on cancer progression is warranted.

ORAL PRESENTATION 6

**Induction of Autophagy normalizes Arterial Stiffness in Mice**

Van Praet M., Neutel C.H.G., Jacobs C., Roeyen E., Wesley C., Krüger D, Guns P.J., De Meyer G.R.Y., Martinet W. and Roth L.

Laboratory of Physiopharmacology, University of Antwerp, Belgium

Introduction | Arterial stiffness, a known hallmark of vascular ageing, is an important risk factor for atherosclerosis. In several cardiovascular diseases a decline in autophagy, a cellular homeostatic protective process, has been observed. Therefore, we wanted to investigate if higher basal autophagy can prevent arterial stiffening by crossbreeding a mouse model with increased autophagy levels (*Becn1*$^{F121A}$) with a model of accelerated vascular ageing (*Fbn1*$^{C1039G+/-}$).

Material and methods | We used three and six month old male wild-type (n=3/6), *Becn1*$^{F121A}$ (n=3/6), *Fbn1*$^{C1039G+/-}$ (n=5-6/7) and *Fbn1*$^{C1039G+/-}$*Becn1*$^{F121A}$ (n=4-5/6) mice in which we measured *in vivo* blood pressure (CODA tail-cuff system), cardiac function and abdominal pulse wave velocity (PWV) as a measure of vascular stiffness (Vevo LAZR-X ultrasound). Afterwards, the proximal ascending aorta was isolated for *ex vivo* assessment of vascular reactivity and stiffness using an in-house designed organ bath set-up (ROTSAC). Subsequently, collagen and elastin content was histologically determined as well as wall thickness. Data were analysed using three-way ANOVA with Bonferroni correction (mean±SEM).

Results | No differences were observed between *Becn1*$^{F121A}$ and wild-type mice indicating no effect of autophagy induction on a healthy vasculature. At three months of age, induction of autophagy in *Fbn1*$^{C1039G+/-}$ mice did not affect blood pressure, cardiac function, percentage collagen and elastin and wall thickness. However, we did observe a decrease in abdominal PWV (5.1±1.8m/s vs. 1.0±0.5m/s, p=0.012) and in left ventricular internal diameter (2.5±0.2mm vs. 1.8±0.1mm, p=0.018). *Ex vivo*, both compliance and Peterson modulus (a parameter of stiffness) were normalized in *Fbn1*$^{C1039G+/-}$*Becn1*$^{F121A}$ mice as compared to *Fbn1*$^{C1039G+/-}$ mice (3.0±0.2µm/mmHg vs. 3.9±0.2µm/mmHg, p=0.005 and 570±44mmHg vs. 402±64mmHg, p=0.002). At six months of age, these parameters were still normalised in *Fbn1*$^{C1039G+/-}$*Becn1*$^{F121A}$ mice (2.2±0.2µm/mmHg vs. 2.9±0.5µm/mmHg, p=0.05 and 1163±247mmHg vs. 717±163mmHg, p=0.014). Interestingly, these effects could not be observed in female mice in which the *Fbn1*$^{C1039G+/-}$ pathology (elastin breaks and arterial stiffness) is less pronounced.

Conclusion | Inducing autophagy in the aorta is beneficial to prevent arterial stiffening of the aorta in a mouse model of accelerated vascular ageing.

ORAL PRESENTATION 7

**Bcl-2-based strategies to target dysregulated Ca2+ signaling as an early event in Alzheimer's disease**

Callens Manon(1), Vervliet Tim(1), Chernyuk Daria(2), Polozova Marina(2) , Gordeev Andrey(2) , Pchitskaya Ekaterina(2), Chigriai Margarita(2), Bezprozvanny Ilya(2) , Bultynck Geert(1)

1) KU Leuven, Laboratory of Molecular & Cellular Signaling, Leuven, Belgium (2) Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg State Polytechnic University, Saint Petersburg, Russia

INTRODUCTION | Aberrant Ca2+ signaling plays an important role in the early pathogenesis of Alzheimer's disease (AD). The familial form of AD is mainly caused by mutations in presenilin 1 (PSEN1). It has been shown that PSENs affect Ca2+ signaling but the underlying mechanism is not completely elucidated yet. In this project, we aim to better understand the role of PSEN1 WT/mutant on intracellular Ca2+ homeostasis. Additionally, we implement the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) which can inhibit the two main Ca2+ release channels in the ER as a tool to counteract the observed Ca2+ dysregulations.

METHODS | Single-cell Ca2+ measurements were performed on cells (over)expressing PSEN1 WT /mutants. The cells were loaded with Fura2-AM or transfected with genetically encoded Ca2+ indicators. Experiments were performed on HEK293 cells overexpressing ryanodine receptor type 2 (RyR2) and INS-1 cells. Bcl-2 based tools were used to evaluate their impact on Ca2+ signaling.

RESULTS | Our analysis revealed that PSEN1 mutations increase mitochondrial Ca2+ influx after stimulating the RyR, while not affecting ER Ca2+ release. This suggest a role of PSEN1 at the mitochondria-associated membranes (MAMs). Additionally, in vivo results from our collaborators indicate the neuroprotective potential of Bcl-2 through inhibition of RyRs. Furthermore, we show that PSEN1 establishes an ER-mediated Ca2+ leak mediated via RyRs.

CONCLUSION | Mutations in PSEN1 impact RyR2-mediated mitochondrial Ca2+ flux and this may contribute to other defects observed in early-AD pathology. Bcl-2-based strategies show promise in restoring Ca2+ signaling and counteracting the development of early AD features.

ORAL PRESENTATION 8

**The effect of hypercholesterolemia and aging on nociception in rodents**

Justyna B Startek, Alina Milici, Silvia Pinto, Evy Van Den Broek, Karel Talavera

Laboratory of Ion Channel Research (VIB-KU Leuven)

Hypercholesterolemia is a condition characterized by high levels of cholesterol in the bloodstream and is often associated with atherosclerosis, diabetes, and hypertension. These health conditions are commonly attributed to genetic factors and unhealthy lifestyles, especially among the aging population. Hypercholesterolemia can have a significant impact on the cardiovascular system and other organs such as the pancreas, liver, kidney, and brain. However, its effect on the peripheral nervous system and pain sensation has been largely underinvestigated. Previous studies on life-span changes and cholesterol levels in nociception and pain behaviors in both humans and rodents have yielded equivocal results, causing debate in the scientific community.

This study aimed to evaluate the effects of hypercholesterolemia and aging on pain sensation using two rodent models and multiple behavioral assays, including von Frey, dynamic cold and heat plate, and tail immersion assays. Blood lipid panels and glucose levels were also monitored throughout the study. Additionally, calcium imaging of DRG neurons isolated from both mice strains at different ages was conducted. The study found that increased cholesterol levels and aging can cause mild sensory impairment. However, it remains unclear whether this reflects a direct decrease in the sensitivity of nociceptors in the peripheral nervous system, as other factors such as differences in weight and ability to perform motor responses cannot be ruled out. Nevertheless, the study suggests that high levels of cholesterol and advancing age can lead to abnormalities in DRG and sensory neurons function and may increase susceptibility to neuropathies.

ORAL PRESENTATION 9

**Kinins: Locally formed peptides during inflammation with potential use in tissue regeneration**

Leonardo MARTIN, Ph.D*; Michael BADER, Ph.D; João PESQUERO, Ph.D.

Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium (L.M.); Max-Delbrück Center for Molecular Medicine, Berlin, Germany (M.B.); Center for Research and Molecular Diagnosis of Genetic Diseases, Federal University of São Paulo, São Paulo, Brazil (J.P.)

INTRODUCTION: Kinins are a set of peptides present in the blood/tissues and involved in cardiovascular regulation, inflammation, and pain. Bradykinin reduces fibrosis in renal and cardiac damage models through the B2 receptor. The B1 receptor expression is induced by damage, and blocking of the kallikrein-kinin system seems to affect the progression of muscular dystrophy.

METHODS: We present a brief collection of data from the main findings on the use of kinins for tissue regeneration, especially focusing on the differential effects for B1 and B2 receptors in post-injury skeletal muscle repair.

RESULTS: Injured B1KO mice showed a faster healing progression of the injured area with a larger amount of central nucleated fiber post-injury when compared to control mice. In addition, they exhibited higher neovasculogenic capacity, maintaining optimal tissue perfusion for the post-injury phase; had higher amounts of myogenic markers with less inflammatory infiltrate and tissue destruction. This was followed by higher amounts of SMAD7 and lower amounts of p-SMAD2/3, which resulted in less fibrosis. In contrast, B2KO and B1B2KO mice showed more severe tissue destruction and excessive fibrosis. B1KO animals had better results in post-injury functional tests compared to control animals.

CONCLUSION: We demonstrate that injured skeletal muscle tissues have a better repair capacity with less fibrosis in the presence of B2 receptor and absence of B1 receptor, including better performances in functional tests. These data suggest that kinin receptors may represent new therapeutic targets for the development of new therapies for tissue regeneration.

ORAL PRESENTATION 10

**Implication of cholesterol in mechanosensitive ion channel deregulation in glioblastoma**

AVALOSSE Noémie1,2; DUMITRU Andra-Cristina3; VERSAEVEL Marie4; GABRIELE Sylvain4; GAILLY Philippe2 & TYTECA Donatienne1

1: de Duve Institute, UCLouvain, Brussels, Belgium; 2: Institute of Neurosciences, UCLouvain, Brussels, Belgium; 3: Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-La-Neuve, Belgium; 4: Research Institute for Biosciences, UMons, Mons, Belgium

INTRODUCTION | Glioblastoma multiforme (GBM) is the most aggressive and frequent brain tumour in adults. New therapeutic approaches are therefore needed and require to better understand the mechanisms behind GBM development and progression. We evaluate if transmembrane mechanosensitive channels are deregulated in GBM and the implication of cholesterol and extracellular matrix in the process.

METHODS | 4 GBM cell lines (U87, U251, SNB19, LN229) were cultured on laminin, matrigel or polyacrylamide hydrogels with brain-mimicking stiffness range (AFM) and evaluated for (1) aggressiveness through EMT (RT-qPCR), proliferation (culture observation), survival (Akt phosphorylation) and invasion (Transwells); (2) mechanosensitive channel expression (RT-qPCR) and activation (Fura-2 imaging); (3) cholesterol level and extractability by methyl-β-cyclodextrin (Amplex Red); and (4) MMP expression (RT-qPCR) and invadopodia (cortactin immunofluorescence).

RESULTS | U87 and U251 were the most aggressive and SNB19 (a U251 subclone) the less aggressive. The four cell lines similarly expressed Piezo1, TRPC1 and TRPM7 but differently TRPV4. Despite similar aggressiveness, U87 showed strong and specific Piezo1 activation while U251 exhibited activation of all channels except TRPV4. U87 also showed a 1.5-fold lower invasion, 2-fold lower cholesterol content and extractability and few invadopodia vs U251. Furthermore, despite similar genetic background, SNB19 exhibited a 3-fold lower invasion, a 1.5-fold lower cholesterol content, less invadopodia and reduced MT1-MMP and MMP9 expression vs U251.

CONCLUSION | Overall, a lower invasion in GBM is associated with lower cholesterol content and matrix degradation but higher specific Piezo1 activation. The role of cholesterol in mechanosensing is currently tested on hydrogels with increasing stiffness.

CLOSING LECTURE

**Hypoxia and Hypoxia-Inducible Factor Signaling in Muscular Dystrophies**

T-H. Nguyen (1), M. Limpens (1), S. Bouhmidi (1) , L. Paprzycki (1), A. Legrand (1), A-E Declèves (2), P. Heher (3), A. Belayew (1), C R S. Banerji (3, 4), P S. Zammit (3) , A Tassin (1)

Laboratory of Respiratory Physiology, Pathophysiology and Rehabilitation, Research Institute for Health Sciences and Technology, University of Mons, 7000 Mons, Belgium.

Muscular dystrophies (MDs) are inherited muscle disorders characterized by progressive skeletal muscle wasting. Respiratory impairments and subsequent hypoxemia are encountered in a significant subgroup of patients in almost all MD forms. In response to hypoxic stress, compensatory mechanisms are activated especially through Hypoxia-Inducible Factor 1 α (HIF1α). In healthy muscle, hypoxia and HIF1α activation modify oxidative stress balance and metabolism. Moreover, we and others highlighted HIF1α as a key regulator of the myogenic program, a process taking place during muscle regeneration. In MDs, multifactorial pathological mechanisms could lead to HIF-1α activation in patient skeletal muscles. Indeed, in addition to the genetic defect per se, respiratory failure or blood vessel alterations could modify hypoxia response pathways. An altered hypoxia response characterizes the muscles of patients with FacioScapuloHumeral Dystrophy (FSHD) independently of hypoxia. FSHD is one of the most prevalent MD and is linked to the inappropriate expression of the DUX4 transcription factor in skeletal muscles. By further exploring the relationship between DUX4 and HIF1α, we showed that a DUX4-HIF1α axis exists but differs according to the stage of myogenic differentiation. Moreover, DUX4 interferes with HIF1α function in the early steps of the myogenic program. Given its role in muscle regeneration and metabolism, HIF1α remains challenging to target from a therapeutic perspective in the context of MDs, as we demonstrated in an FSHD-like murine model.

POSTER PRESENTATION 1

**Exploring apelinergic system signaling in autosomal dominant polycystic kidney disease**

Corinna Heinze, Jean-Paul Decuypere, Rudi Vennekens, Djalila Mekahli
KU Leuven

The apelinergic system (AS), i.e. the apelin receptor (APLNR) and its ligands apelin and apela, is a promising therapeutic target for conditions such as hypertension, heart failure and cancer due to its anti-inflammatory, anti-apoptotic, and pro-autophagic properties. Recent studies have shown that the AS may play a role in the treatment of kidney diseases. Nevertheless, the knowledge of the role of the AS, especially in the human kidney, is limited. We investigate the role of the AS in the human kidney, focusing on autosomal dominant polycystic kidney disease (ADPKD). ADPKD is an inherited and progressive kidney disease, in which cysts destroy kidney tissue eventually leading to kidney failure. Currently no treatment is available, and mechanistic insight in the process of cyst formation is limited. Our preliminary data show that Apelin and APLNR expression is elevated in ADPKD kidney cells. We also show that ADPKD patients with normal renal function have increased circulating apelin levels. Moreover, various intracellular signal transduction cascades that follow upon activation of the APLNR may be directly involved in the process of cyst formation and cyst growth in ADPKD. Thus, we hypothesize that the AS might be a novel drug target in the treatment for ADPKD. Therefore, we want to gain fundamental knowledge about the physiology of the AS in the human kidney, its role in the pathophysiology of ADPKD and its therapeutic potential in ADPKD.

POSTER PRESENTATION 2

**Investigating the structure and pharmacology of SK channels through molecular docking and in vitro patch clamp**

Mouchet A.1, Vitello R.2, Brans A.1, Seutin V.3, Liégeois J.F.2, Kerff F.1

1 Centre for Protein Engineering (CIP), InBios, University of Liège. 2 Laboratory of Medicinal Chemistry, C.I.R.M., University of Liège. 3 Laboratory of Neurophysiology, GIGA-Neurosciences, University of Liège.

Small conductance calcium-activated potassium (SK) channels are gated by intracellular Ca2+ via calmodulin molecules. Three isoforms (SK1-3) have been identified, showing different but overlapping tissue expression in the central nervous system. SK1 and SK2 proteins display considerable overlap in cortex and hippocampus while SK3 expression is higher in the monoaminergic cell regions. SK channels play an important role in neuronal excitability by modulating the firing rate and firing pattern of neurons, and represent potential targets for the treatment of various CNS disorders. Therefore, the development of new non-peptidic blockers combining high affinity and selectivity towards SK2 or SK3 channels (and possibly versus one another) is crucial and requires a better knowledge of the structural features essential to the affinity of these ligands. In this project, we aimed to better understand the interaction between SK channels and the archetypical blocker apamin by studying the 3D structures of SK proteins and their activity. From models obtained using AlphaFold, we observed a particular conformation of the S3-S4 loop in SK1-3, which does not seem to be present in IK. Furthermore, in the first three subtypes, we observed the presence of a phenylalanine residue in this loop that appears to be located just outside the channel pore and could play a key role in the interaction with apamin. To validate this hypothesis, we generated different mutants of this phenylalanine in SK2 and SK3 subunits and studied their sensitivity to apamin and UCL1684 by coupling molecular docking to in vitro patch-clamp experiments.

POSTER PRESENTATION 3

**The endothelial glycocalyx: a functional and morphological investigation in a mouse model**

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INTRODUCTION | The endothelial glycocalyx (EG) is composed of glycosaminoglycans, proteoglycans and glycoproteins bound to the plasma membrane of endothelial cells and located to the luminal side of blood vessels (Reitsma and al., 2007). EG plays key roles in vascular physiology including regulation of blood vessels permeability, leukocytes adhesion, coagulation, and vascular tone (Reitsma and al., 2007). The latter induces a vasodilation when blood flow increase. All these functions are altered in cardiovascular diseases and lead to major clinical complications (Abassi and al., 2020 ; Dogné and al., 2020).

METHODS | To investigate the mechanosensor role of the EG in pathological conditions, ex vivo enzymatic degradation of the EG was developed on isolated mice mesenteric arteries. For that, heparinase III from F. heparinum, hyaluronidases from bovine testes or S. hyalurolyticus were perfused. Immunofluorescence staining was used to confirm the degradation (shedding) of the EG. Functional analysis was performed on treated vessels, under flow conditions thanks to pressure myography. Endothelium-dependent nitric oxide (NO) and endothelium-derived hyperpolarizing (EDH) pathways were investigated.

RESULTS | Flow-induced vasodilation after enzymatic treatments, was strongly reduced or completely absent in resistance arteries. Interestingly, both endothelium-dependent signaling pathways are impacted and more specifically, the EDH pathway. These evidences suggest that the degradation of the EG observed in cardiovascular diseases is implicated in vasomotion impairment.

CONCLUSION | The EDH pathway seems particularly affected after the enzymatic degradation of the EG. These findings highlight the importance of the EG preservation and/or restoration in patients suffering from cardiovascular diseases.

POSTER PRESENTATION 4

**Brain Death-Induced Renal Injury in pigs: Unveiling Glycocalyx alteration and the Protective Role of Tacrolimus**

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Brain death (BD) is a complex medical state that triggers systemic disturbances and a cascade of pathophysiological processes. This condition significantly impairs both kidney function and structural integrity, thereby presenting considerable challenges to graft viability and the long-term success of transplantation endeavors. This study aimed to investigate changes associated with brain death-induced renal injury in a 4-month-old female porcine model. The experimental groups included brain death placebo-pretreated (BD; n=9), brain death tacrolimus-pretreated (BD + TC; n=8), and control (ctrl, n=7) piglets, which did not undergo brain death induction. Our primary focus was to investigate endothelial dysfunction and changes in the glycocalyx. Additionally, we examined the distribution of apoptosis and inflammation. Furthermore, we aimed to assess the effect of tacrolimus (TC) on these renal alterations through graft preconditioning. Our findings revealed a cascade of events following BD, culminating in both functional and structural renal damage. Previous studies conducted in this porcine model of brain death have highlighted heightened immune responses, oxidative stress, and hormonal imbalances as consequences of BD. These factors collectively, may have contributed to endothelial activation and subsequent glycocalyx degradation, as shown in the present poster. Moreover, tacrolimus demonstrated significant efficacy in helping to attenuate apoptosis, maintaining glycocalyx integrity, regulation of neutrophils infiltration, and mitigating renal injury following brain death (BD). This study offers new insights into the pathophysiology of brain death (BD)-induced renal injury, emphasizing the potential of TC pretreatment as a promising therapeutic intervention for organ preservation, with the additional benefit of limiting the risk of rejection.

POSTER PRESENTATION 5

**Conditional deletion of TRPC1 modulates synaptic plasticity, long term depression, and memory extinction in Fragile X Syndrome mice**

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INTRODUCTION | Group I metabotropic glutamate receptors (mGluR) are involved in various forms of synaptic plasticity that are believed to underlie declarative memory by inducing long-term depression (mGluR-LTD). We have previously shown that mGluR5 specifically activates channels containing TRPC1, an isoform of the canonical family of Transient Receptor Potential channels, highly expressed in the hippocampus.

METHODS | To explore the involvement of mGluR-TRPC1 in synaptic plasticity and unravel its subsequent signalling pathway, we used the Cre-tamoxifen conditional system to induce an acute deletion of the Trpc1 gene in a murine model exhibiting Fragile X Syndrome (FX). FX is a genetic disorder, coupled with the manifestation of autistic traits, and characterized by an enhanced activation of mGluR5, an accelerated memory extinction, and an exaggerated mGluR-LTD.

RESULTS | Acute deletion of Trpc1 reduced the social behavior deficits seen in FX mice, making them more sociable and less anxious. On the other hand, deletion of Trpc1 abolished the mGluR-LTD observed in FX mice and reduced their enhanced memory extinction rate as demonstrated in both Morris Water Maze and Passive Avoidance tests. Moreover, activation of mGluR5 by its agonist, DHPG, failed to increase Arc, an activity regulated cytoskeleton associated protein essential for mGluR-LTD, in hippocampal brain slices that lack TRPC1.

CONCLUSION | Here we show that TRPC1 is involved in maintaining a normal mGluR-LTD, and is required for memory extinction, an important process in synaptic plasticity. mGLuR-TRPC1 acts on Arc through eEF2K and ERK pathways, a calmodulin dependent kinase and extracellular signal-regulated kinase respectively.

POSTER PRESENTATION 6

# PHARMACOLOGICAL CHARACTERIZATION OF THE ORPHAN G PROTEIN-COUPLED RECEPTOR GPR85

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G protein-coupled receptors (GPCRs) represent the largest membrane receptors family and are the main target for about 30% of the drugs on the market. However, many GPCRs haven't been studied, with an unknown function and above all called "orphan", meaning that the endogenous ligand hasn't been found yet. The SREB (Super Conserved Receptors Expressed in the Brain) family are a particular group of GPCRs that are, as their name suggest, highly conserved in their amino acid sequence and in high concentration in the brain. GPR85 is part of the SREB family and is the highest conserved GPCR with 99.2% amino acid identity among 92 orthologs. The amino acid sequence is identical between humans and rodents. GPR85 expression in the brain and conserved sequence direct our focus on its central nervous system role, in addition to different studies which have suggested that this GPCR might be involved in mental diseases such as schizophrenia. Our research project aims on three main questions: - What is the binding site of GPR85? - How does GPR85 couple with G proteins? Or is it an atypical receptor that doesn't couple with G proteins? - What are the consequences of GPR85 activation in an endogenous system such as hippocampal neurons? Our main goal is to find the endogenous ligand and "de-orphan" this enigmatic receptor.

POSTER PRESENTATION 7

**Investigating cell type diversity in the maturing bed nucleus of the stria terminalis in mice**

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The bed nucleus of the stria terminalis (BNST), as part of the extended amygdala, has become a region of increasing interest with regard to its role in numerous human stress-related psychiatric disorders including post-traumatic stress disorders (PTSD), generalized anxiety disorders (GAD), obsessive compulsive disorder (OCD) and addiction. The BNST is a dimorphic and highly complex structure, which is already evident by its anatomy. For example, in rodents, the BNST can be divided into 12 to 18 sub-nuclei. Located in the ventral forebrain, the BNST is anatomically and functionally connected to many other limbic structures, including the amygdala, hypothalamic nuclei, basal ganglia, and hippocampus. Given its extensive connectivity, the BNST is thought to play a central and critical role in the integration of information on hedonic-valence, mood, and arousal states to calibrate wakefulness, process emotional information, and in general, shape motivated and stress/anxiety-related behaviours.

The BNST contains a wide variety of cell types that differ in their electrophysiological properties, morphology, spatial organization, neurochemical content, input and output organization, and function. Previous studies performed in adult mice (and rat) brains identified three distinct neuronal cell types (type I, II, & III) based on their electrophysiological properties and ion channel expression. Despite abundant data on the neurochemistry, connectivity, and functions of the BNST of adult rodents, information on its development is scarce. To date, it is unknown whether this heterogeneous group of BNST neuronal cell types mature differently and if differences in maturation impact functional connectivity. In this project, we aim to characterize the electrophysiological and morphological properties of BNST neuronal cell types at both early (P3-P9 and P9-P16) and late (P21-P35) stages of post-natal development by using whole-cell patch clamp and immunohistochemistry.

Rationale: Understanding normal brain development on a cellular level has proven to be critical to understand brain malfunction in human psychiatric disorders. Malfunction of the BNST, a brain region important in the regulation of mood and stress-/anxiety-related responses, has been associated with psychiatric PTSD, OCD, GAD, and depression. Even though great progress has been made in elucidating this complex brain region on multiple levels, normal development, and maturation of the BNST seems to be understudied. Here, we set out to explore how the electrophysiological and morphological properties of BNST neurons change throughout different stages of post-natal development.