# BELGIAN SOCIETY OF

# PHYSIOLOGY AND PHARMACOLOGY

## NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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**Spring Meeting**

**Friday, April 30th 2021**

**PROGRAMME**

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**Venue**

**Online**

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**Local host**

**Dr. Genevieve Dupont and Dr. Sumeet Pal Singh**
**ULB**
**Belgium**

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

**BELGIAN SOCIETY OF PHYSIOLOGY AND PHARMACOLOGY**

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## Keynote lecture

10.00-11.00 **Mechanocheminal basis for mesendoderm internalization during gastrulation**
Prof. Dr. Carl-Philipp Heisenberg (Institute of Science and Technology, Austria)

## Oral communications (morning session)

11.00-11.15 **GPR101 drives growth hormone hypersecretion and gigantism in mice via constitutive activation of Gs and Gq/11**
Dayana Abboud, Adrian F. Daly, Nadine Dupuis, Mohamed Ali Bahri, Asuka Inoue, Andy Chevigné, Fabien Ectors, Alain Plenevaux, Bernard Pirotte, Albert Beckers & Julien Hanson (University of Liège)

11.15-11.30 **Identification and validation of novel autophagy-inducing small molecules via high-throughput screening**
Farnaz Sedigheh Takhsha, Winnok De Vos, Vera Goossens, Isabel Pintelon, Dominique Audenaert, Pieter Van Der Veken, Guido R.Y. De Meyer, Wim Martinet (University of Antwerp)

11.30-11.45 **Muscle-to-Brain communication in the context of obesity: impact of physical exercise ?**
A. Delpierre, C. Deroux, L. Ris, A-E. Declèves, A. Legrand, A. Villers and A. Tassin (UMONS)

11.45-12.00 **The intracellular calcium chelator BAPTA-AM induces cell death in lymphoma: is it all about calcium chelation?**
Sneyers F., Vervloessem T., La Rovere R.M., Welckenhuyzen K., Bootman M.D., Bultynck G. (KULeuven)

12.00-12.15 **Extracellular calcium regulates epidermal growth and mechanical strength in zebrafish**
Ines Garteizgogeascoa, Ariel Valiente Gabioud, Oliver Griesbeck, Sumeet Pal Singh (ULB)

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**12.15 – 13.00 Lunch**

**Oral communications (afternoon session)**

13.00-13.15 **Pre-symptomatic elevated serum corticosterone levels and systemic insulin resistance as potential biomarkers in a hAPP23 overexpressing mouse model of Alzheimer's Disease**
Jhana O. Hendrickx, Sofie De Moudt, Elke Calus, Debby Van Dam, Guido R.Y. De Meyer (University of Antwerp)

13.15-13.30 **Identification and Functional Characterization of a novel TRPM7 mutation associated with trigeminal neuralgia**
Roberta Gualdani, Philippe Gailly, Jun-Hui Yuan, Giulia DiStefano, Andrea Truini, Giorgio Cruccu, Sulayman D. Dib-Hajj, Stephen G. Waxman (UCLouvain)

13.30-13.45 **β-arrestin2 recruitment at the β2 adrenergic receptor: A luciferase complementation assay adapted for undergraduate training in pharmacology**
Mattia Ferraiolo, Pauline Beckers, Nicolas Marquet, Martin Roumain, Lucie Ruiz, Nadine Dupuis, Julien Hanson, Emmanuel Hermans (UCLouvain)

13.45-14.00 **Acetyl-CoA carboxylase inhibition alters tubulin acetylation and aggregation in thrombin-stimulated platelets**
Marie Octave, Laurence Pirotton, Audrey Ginion, Valentine Robaux, Sophie Lepropre, Shakeel Kautbally, Victor M. Darley-Usmar, Jérôme Ambroise, Bruno Guigas, Martin Giera, M. Foretz, Luc Bertrand, Christophe Beauloy, Sandrine Horman (UCLouvain)

14.00-14.15 **The F337C mutation in the CLCN1 gene yields an original functional phenotype**
Kevin Jehasse, Kathleen Jacqueri, Alice de Froidmont, Camille Lemoine, Thierry Grisar, Katrien Stouffs, Bernard Lakaye, Vincent Seutin (ULiège)

**14.15 – 14.30 Coffee – Tea**

14.30-14.45 **Doxorubicin induces arterial stiffness: a comprehensive in vivo and ex vivo evaluation of vascular toxicity in mice**
Matthias Bosman, Kasper Favere, Cédric H.G. Neutel, Griet Jacobs, Guido R.Y. De Meyer, Wim Martinet, Emeline M. Van Craenenbroeck, Pieter-Jan D.F. Guns (University of Antwerp)

14.45-15.00 **Targeting increased colonic trypsin-like activity and abdominal pain in a rat model of irritable bowel syndrome using a serine protease inhibitor**
Hanning N, De bruyn M, Ceuleers H, Boogaerts T, Berg M, Smet A, De Schepper HU, Joossens J, van Nuijs ALN, De Man JG, Augustyns K, De Meester I, De Winter BY (University of Antwerp)

15.00-15.15 **A feedback loop regulating capacitation in murine sperm**
Bertrand de Prelle, Pascale Lybaert, David Gall (ULB)

15.15-15.30 **Pharmacological properties of essential oil from Aeollanthus pubscens in Benin**
Philippe Sessou, Mahudro Yovo, Gwladys S. Komagbe, Maximin Senou, Guy Alain Alitonou, Félicien Avlessi, Souaïbou Farougou, Dominique Sohounhloue (University of Abomey-Calavi, Benin).

PRESENTATION 1

**GPR101 drives growth hormone hypersecretion and gigantism in mice via constitutive activation of Gs and Gq/11**

Dayana Abboud, Adrian F. Daly, Nadine Dupuis, Mohamed Ali Bahri, Asuka Inoue, Andy Chevigné, Fabien Ectors, Alain Plenevaux, Bernard Pirotte, Albert Beckers & Julien Hanson

University of Liège, Liège, Belgium

Growth hormone (GH) is a key modulator of growth and GH over-secretion can lead to gigantism. One form is X-linked acrogigantism (X-LAG), in which infants develop GH secreting pituitary tumors over-expressing the orphan G-protein coupled receptor, GPR101(Trivellin et al, 2014). The role of GPR101 in GH secretion remains obscure. We studied GPR101 signaling pathways and their effects in HEK293 and rat pituitary GH3 cell lines, human tumors and in transgenic mice with elevated somatotrope Gpr101 expression driven by the rat Ghrhr promoter (GhrhrGpr101). We reported that Gpr101 causes elevated GH/prolactin secretion in transgenic GhrhrGpr101 mice but without hyperplasia/tumorigenesis (Abboud et al, 2020). Furthermore, we showed that GPR101 constitutively activates not only Gs, but also Gq/11 and G12/13, which leads to GH secretion but not proliferation. These signatures of GPR101 signaling, notably PKC activation, are also present in human pituitary tumors with high GPR101 expression. These results underline a role for GPR101 in the regulation of somatotrope axis function.

PRESENTATION 2

**Identification and validation of novel autophagy-inducing small molecules via high-throughput screening**

Farnaz Sedigheh Takhsha, Winnok De Vos, Vera Goossens, Isabel Pintelon, Dominique Audenaert, Pieter Van Der Veken, Guido R.Y. De Meyer, Wim Martinet

University Antwerp

Introduction Autophagy is a reparative and life-sustaining process that sequestrates unnecessary or dysfunctional cellular components in double membrane structures called autophagosomes. Because defective autophagy is currently emerging as a hallmark of many diseases, there is a strong need for potent and selective autophagy-inducing compounds.

Methods We optimized and validated an image-based high-throughput assay to quantify small molecule-induced autophagosome formation in L929 fibroblasts that were stably transfected with a construct encoding the autophagosome marker LC3 (microtubule-associated protein light chain 3) fused to green fluorescent protein (GFP). To achieve this goal, bafilomycin A1 was used as a positive control and rapamycin-derivative everolimus as a reference compound. Hits from the initial screen were re-evaluated in HeLa cells, stably transfected with an mRFP-GFP-LC3 construct. The latter approach allowed us to distinguish compounds that can truly induce autophagic flux from those that induce autophagosome accumulation.

Results In non-autophagic conditions, GFP-LC3 transfected L929 cells revealed diffuse green fluorescence in the cytoplasm, which became punctate after treatment with autophagy-inducing small molecules. A pilot screen with 10,240 compounds allowed identification of 316 molecules that induce autophagosome formation in the cytoplasm. Thirty compounds, for which their effects on autophagosome accumulation were confirmed in triplicate, were further validated in mRFP-GFP-LC3 transfected HeLa cells. Among the compounds tested, one molecule clearly triggered formation of mRFP-positive/GFP-negative cytoplasmic dots, indicative of autophagic flux stimulation.

Conclusion Starting from a library of 10,240 small molecules, we have identified one compound that significantly induced autophagic flux in mammalian cells.

PRESENTATION 3

**Muscle-to-Brain communication in the context of obesity: impact of physical exercise ?**

A. Delpierre (1), C. Deroux (2), L. Ris (2), A-E. Declèves (3)(4), A. Legrand (1), A. Villers (2) and A. Tassin (1)

(1) Lab. of Respiratory Physiology and Rehabilitation, UMONS (2) Lab. of Neurosciences, UMONS (3) Lab. of Metabolic and Molecular Biochemistry, UMONS (4) Research Institute for Health Sciences and Health Technology, UMONS

Exercise training (ET) has a positive effect on brain health. During ET, skeletal muscle releases specific myokines including potential regulators of hippocampal function such as irisin, released by cleavage of FNDC5. Also expressed in the brain, FNDC5 contributes increasing the level of brain-derived neurotrophic factor (BDNF). However, the contribution of muscle-derived Irisin on cognitive function remains controversial, as well as the influence of obesity or ET modalities. The goal of our study is to determine (i) inter-relationships between FNDC5/Irisin pathway and cognition depending on ET modalities and (ii) whether muscle-to-brain crosstalk is altered in the context of obesity. Two ET modalities were compared in Low-Fat (LF) and High-Fat (HF) fed mice: voluntary (enriched environment) and forced ET (endurant). Irisin plasmatic level is increased by ET, whatever ET modality or diet. As concerns FNDC5, voluntary ET is associated to an increased protein level in LF but not in HF mouse muscles while forced ET does not modify FNDC5 protein level in muscular or brain tissues. Enrichment in mice improves spatial learning and memory. However, the BDNF protein level is not modified by voluntary ET in the cortex and hippocampus. Forced ET does not modify spatial learning and memory and BDNF protein level in the hippocampus. However, BDNF protein level is increased in the brain cortex by endurance- training and surprisingly, by HF diet. In conclusion, ET increases irisin plasmatic level and enrichment improves cognitive function in mice. FNDC5 protein level is dependent on training modalities, is tissue-specific and influenced by diet.

PRESENTATION 4

**The intracellular calcium chelator BAPTA-AM induces cell death in lymphoma: is it all about calcium chelation?**

Sneyers F., Vervloessem T., La Rovere R.M., Welckenhuyzen K., Bootman M.D., Bultynck G.

KULeuven

A hallmark of B-cell malignancies such as diffuse large B-cell lymphoma (DLBCL) is anti-apoptotic Bcl-2 overexpression, enabling cancer cells to escape apoptosis by neutralizing pro-apoptotic proteins. Combining venetoclax, a selective Bcl-2 inhibitor, with the intracellular Ca2+ chelator BAPTA-AM significantly enhanced apoptosis in DLBCL cell models. Here, we aim to elucidate the mechanisms underlying the synergism between venetoclax and BAPTA-AM. We show that BAPTA-AM treatment induced apoptosis and sensitivity towards BAPTA-AM correlated with sensitivity towards S63845, a selective antagonist of Mcl-1, another anti-apoptotic Bcl-2-family member. We therefore assessed BAPTA-AM's effect on the expression of different Bcl-2-family members. BAPTA-AM treatment resulted in a complete and rapid loss of Mcl-1 through inhibition of its translation, preceding apoptosis. Remarkably, overexpression of a non-degradable Mcl-1 variant resulted in the rescue of BAPTA-AM-induced cell death. Moreover, cells with genetically engineered addiction to Mcl-1 were more sensitive to BAPTA-AM than those addicted to Bcl-XL. Thus, the heterogeneous response towards BAPTA-AM in DLBCL cells likely relates to their Mcl-1 addiction. Lastly, we found that BAPTA-AM rapidly inhibited glycolytic activity, possibly lying at the heart of translation inhibition and resulting in Mcl-1 downregulation. Surprisingly, all aforementioned effects of BAPTA-AM could be phenocopied by a BAPTA-AM variant compromised in buffering intracellular Ca2+. Moreover, EGTA-AM, a structurally different Ca2+ chelator with similar affinity for Ca2+ as BAPTA-AM, was much less effective in lowering Mcl-1-protein levels and did not provoke cell death. Thus, our results reveal an unconventional, "Ca2+-independent" mechanism by which BAPTA-AM limits survival of DLBCL, exploiting their dependence towards Mcl-1.

PRESENTATION 5

**Extracellular calcium regulates epidermal growth and mechanical strength in zebrafish**

Ines Garteizgogeascoa[1], Ariel Valiente Gabioud[2], Oliver Griesbeck[2], Sumeet Pal Singh[1]

[1]IRIBHM, ULB, Brussels, Belgium. [2]Max Planck Institute of Neurobiology, Martinsried, Germany

INTRODUCTION | Calcium is an important ion for biological life. Changes in intracellular calcium levels is a widely utilised signalling cue. However, intracellular calcium is only one aspect of calcium regulation in our body, the other being extracellular calcium. Extracellular calcium is maintained at a stable level in our body, around 1.4 mM. Mis-regulation of extracellular calcium levels leads to endocrine diseases. In-spite of its biological and medical significance, our understanding of extracellular calcium is rather lacking.

METHODS | A major reason for the paucity in our understanding of extracellular calcium is the lack of a sensor for its visualisation. To overcome this limitation, here, we describe the first genetically encoded sensor for extracellular calcium and its utility for in vivo imaging at cellular resolution. These aspects combined with zebrafish transgenesis and optical transparency at early stages of life, provides an indicator that detects dynamics in extracellular calcium levels. Further, we couple manipulation of extracellular calcium with Atomic Force Microscopy to quantify the mechanical properties of the tissue.

RESULTS | We show that extracellular calcium levels fall upon a break in epithelial barrier, when interstitial space is exposed to the low-calcium environment. We demonstrate that acute reduction of extracellular calcium leads to disintegration of epithelial tissue and makes it more “fluid”. Moreover, chronic reduction of extracellular calcium reduces the growth rate of epithelium during development.

CONCLUSION | Extracellular calcium levels show dynamics at local level and its reduction leads to loss of epithelial integrity.

PRESENTATION 6

**Pre-symptomatic elevated serum corticosterone levels and systemic insulin resistance as potential biomarkers in a hAPP23 overexpressing mouse model of Alzheimer's Disease**

Jhana O. Hendrickx[1], Sofie De Moudt[1], Elke Calus[2], Debby Van Dam[2,3], Guido R.Y. De Meyer[1]

[1]Laboratory of Physiopharmacology, University of Antwerp, Belgium, [2]Laboratory of Neurochemistry and Behaviour, Institute Born-Bunge, University of Antwerp, Belgium, [3]Department of Neurology and Alzheimer Center, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands

INTRODUCTION | Increasing epidemiological evidence highlights the correlation between systemic insulin resistance and Alzheimer's disease (AD). Because insulin resistance is well-known to be caused by high-dose stress hormone levels we aimed to investigate the systemic insulin functionality and circulating stress hormone levels in a mutant humanized APP overexpressing (hAPP23+/-) AD mouse model.

METHODS | Male age-matched hAPP23+/- and C57BL/6J J mice at 4 (n=10-11) and 12 months (n=11-21) were tested for memory and spatial learning by a Morris Water Maze (MWM) test. In addition, animals were metabolically tested with an intraperitoneal glucose and insulin tolerance test (GTT, ITT). Circulating insulin and corticosterone levels were determined in blood serum. Data are represented as mean ± SEM.

RESULTS | MWM data revealed cognitive decline in APP23+/- at 12 months of age, as total path lengths increased compared to C57BL/6J. Elevated circulating corticosterone levels were measured in the serum of hAPP23+/- compared to C57BL/6J animals. Peripheral glucose homeostasis remained unchanged at both ages. However, at 4 months of age, hAPP23+/- mice presented with peripheral insulin resistance compared to control littermates, which stabilized at 12 months of age. Serum insulin levels were similar between genotypes at 4 months but were significantly elevated in APP23+/- mice at 12 months of age.

CONCLUSION | These results suggest that circulating corticosterone levels and peripheral insulin resistance could be potential metabolic biomarker in the pre-symptomatic phase of AD.

PRESENTATION 7

**Identification and Functional Characterization of a novel TRPM7 mutation associated with trigeminal neuralgia**

Roberta Gualdani[1,2], Philippe Gailly[1], Jun-Hui Yuan[2], Giulia DiStefano[3], Andrea Truini3, Giorgio Cruccu[3], Sulayman D. Dib-Hajj[2], Stephen G. Waxman[2]

[1]Institute of Neuroscience, Université catholique de Louvain, B-1200 Brussels, Belgium, [2]Department of Neurology, Yale School of Medicine, New Haven, CT and Center for Neuroscience and Regeneration Research, Veterans Affairs Connecticut Healthcare, West Haven, CT, USA, [3]Department of Human Neuroscience, Sapienza University, Rome, Italy

Trigeminal neuralgia (TN) is a unique pain disorder in which affected individuals experience intense paroxysmal pain in the territory of the trigeminal nerve. Although most cases of TN are sporadic, occurrence of familial TN suggests a genetic contribution to this disorder. Here we used Ca2+ and Na+ imaging and whole-cell patch clamp to assess a variant in the TRPM7 channel kinase c.2791G>A (p.Ala931Thr) found in a man whose father suffered from TN.
We found that A931T produced dramatic changes of TRPM7 channel properties. WT TRPM7 produced pronounced outward currents at positive potentials and small inward currents at negative potentials. The current-voltage relationship of A931T was significantly different from WT TRPM7, with 20-fold higher inward currents than TRPM7 WT and unchanged outward currents. Moreover the conductance-voltage relationship showed the activation of an inward component at hyperpolarizing potentials. Finally, the current recorded in A931T had the following properties: i) it was carried by Na+ ions under physiological conditions; ii) it was completely inhibited by the TRPM7 antagonist NS8593; iii) it was observed when Na+ was replaced by Guanidinium, but it was completely suppressed by application of NMDG+.
Our results support the notion that A931T, located in S3 and in close proximity to R975 in S4, generates an omega current that carries Na+ influx in physiological conditions. A931T mutation may have consequences in the trigeminal axonal integrity since small persistent Na+ currents are known to trigger reverse Na+/ Ca2+ exchange that can lead to increased intracellular Ca2+ and predispose axons to time-dependent injury.

PRESENTATION 8

**β-arrestin2 recruitment at the β2 adrenergic receptor: A luciferase complementation assay adapted for undergraduate training in pharmacology**

Mattia Ferraiolo 1,2 | Pauline Beckers1,2 | Nicolas Marquet 1 | Martin Roumain 2 | Lucie Ruiz 2 | Nadine Dupuis 3 | Julien Hanson 3 | Emmanuel Hermans 1,2

1 Neuropharmacology Laboratory, Institute of Neuroscience, UCLouvain, Brussels, Belgium 2 Faculty of Pharmacy and Biomedical Sciences, UCLouvain, Brussels, Belgium 3 Laboratory of Molecular Pharmacology, GIGA-Molecular Biology of Disease, ULiège, Liège, Belgium

INTRODUCTION | In the context of pharmacology teaching, hands-on activities constitute an essential complement to theoretical lectures. Frequently, these activities consist of exposing fresh animal tissues or even living animals to selected drugs and qualitatively or quantitatively evaluating functional responses. However, technological advancements in pharmacological research and the growing concerns for animal experimentation support the need for innovative and flexible in vitro assays adapted for teaching purposes.

METHODS | We herein report the implementation of a screening-compatible luciferase complementation assay in the framework of pharmacological training at the faculty of Pharmacy and Biomedical Sciences. The assay was based on a cellular model of HEK cells stably expressing the β2 adrenergic receptor and the β-arrestin2, respectively coupled to the C-terminal and the N-terminal fragments of the firefly luciferase enzyme.

RESULTS | The assay allowed students to quantitatively characterize the competitive antagonism of propranolol, and to calculate pEC50, pKB, and pA2 values after a guided data analysis session. Moreover, the newly implemented workshop delivered highly reproducible results and was generally appreciated by students.

CONCLUSION | We report that the luciferase complementation-based assay proved to be a straightforward, robust, and cost-effective alternative to experiments performed on animal tissues, constituting a useful and flexible tool to enhance and update current hands-on training in the context of pharmacological teaching.

PRESENTATION 9

**Acetyl-CoA carboxylase inhibition alters tubulin acetylation and aggregation in thrombin-stimulated platelets**

Marie Octave, Laurence Pirotton, Audrey Ginion, Valentine Robaux, Sophie Lepropre, Shakeel Kautbally, Victor M. Darley-Usmar, Jérôme Ambroise, Bruno Guigas, Martin Giera, M. Foretz, Luc Bertrand, Christophe Beauloy, Sandrine Horman

Université catholique de Louvain

INTRODUCTION Acetyl-CoA carboxylase (ACC), the first enzyme regulating lipid synthesis, promotes thrombus formation by increasing platelet phospholipid content. Inhibition of its activity decreases lipogenesis and increases acetyl-CoA content which can serve as substrate for protein acetylation. This posttranslational modification plays a key role in the regulation of platelet aggregation, via tubulin acetylation. Our aim is to demonstrate that ACC inhibition may affect platelet functions via an alteration of lipid content and/or tubulin acetylation.

METHODS Platelets were treated 2 hours with CP640.186, a pharmacological ACC inhibitor, prior to thrombin stimulation. Platelet functions were assessed by aggregometry and flow cytometry. Lipogenesis was measured via 14C-acetate incorporation into lipids. Lipidomics analysis was carried out on the commercial Lipidyzer platform. Protein phosphorylation/acetylation were evaluated by western blot.

RESULTS Treatment with CP640.186 decreased platelet lipogenesis. However, the quantitative lipidomics analyses showed that preincubation with the compound didn't affect global platelet lipid content. Interestingly, this short-term ACC inhibition was sufficient to increase tubulin acetylation level, at basal state and after thrombin stimulation. It was associated with an impaired platelet aggregation, in response to low thrombin concentration, while granules secretion wasn't affected. Mechanistically, we highlighted a decrease in Rac1 activity, associated with a reduced phosphorylation of its downstream effector PAK2. Surprisingly, actin cytoskeleton wasn't impacted but we evidenced a significant decrease in ROS production which could result from a decreased NOX2 activity.

CONCLUSION Pharmacological ACC inhibition decreases platelet aggregation upon thrombin stimulation. The mechanism depends on increased tubulin acetylation, with subsequent alteration of Rac1/PAK2/NOX2 signaling pathway.

PRESENTATION 10

**The F337C mutation in the CLCN1 gene yields an original functional phenotype**

Kevin Jehasse[1], Kathleen Jacqueri [2], Alice de Froidmont[1], Camille Lemoine[1], Thierry Grisar[3], Katrien Stouffs[4], Bernard Lakaye[3], Vincent Seutin[1]

[1]Laboratory of Neurophysiology, GIGA Institute, ULiège; [2]Department of Electrical Engineering and Computer Science, ULiège; [3]Laboratory of Molecular Regulation of Neurogenesis, GIGA Institute, ULiège; [4]Neurogenetics Research Group, VUB

INTRODUCTION | Mutations in the CLCN1 gene, which codes for the muscle ClC-1 channel, underlie the phenotype of myotonia congenita. We analyzed the phenotype of the Phe337Cys mutation.

METHODS | We used confocal microscopy imaging and whole cell patch clamp recordings in transfected HEK293 cells.

RESULTS | We found that the F337C mutant inserts normally in the plasma membrane, contrary to the Q412P mutant. In patch clamp recordings, we observed a reduced (50%) macroscopic conductance in the F337 mutant, combined with a reduced voltage dependence. To our knowledge, this phenotype has not been observed so far.

CONCLUSION | This mutation should prevent the channels to provide an adequate repolarization force during muscle action potential firing.

PRESENTATION 11

**Doxorubicin induces arterial stiffness: a comprehensive in vivo and ex vivo evaluation of vascular toxicity in mice**

Matthias Bosman, Kasper Favere, Cédric H.G. Neutel, Griet Jacobs, Guido R.Y. De Meyer, Wim Martinet, Emeline M. Van Craenenbroeck, Pieter-Jan D.F. Guns

Laboratory of Physiopharmacology - University of Antwerp

Arterial stiffness is an important predictor of cardiovascular risk. Clinical studies have demonstrated that arterial stiffness increases in cancer patients treated with the chemotherapeutic doxorubicin (DOX). However, the mechanisms of DOX-induced arterial stiffness remain largely unknown. This study aimed to evaluate artery stiffening in DOX-treated mice using in vivo and ex vivo techniques.
Male C57BL/6J mice were treated for 2 weeks with 2 mg/kg (low dose) or 4 mg/kg (high dose) of DOX weekly. Arterial stiffness was assessed in vivo with ultrasound imaging (abdominal aorta pulse wave velocity (aaPWV)) and applanation tonometry (carotid-femoral PWV) combined with ex vivo vascular stiffness and reactivity evaluation. The high dose increased aaPWV, while cfPWV did not reach statistical significance. Phenylephrine (PE)-contracted aortic segments showed a higher Peterson's modulus (Ep) in the high dose group, while Ep did not differ when vascular smooth muscle cells (VSMCs) were relaxed by a NO donor (DEANO). In addition, aortic rings of DOX-treated mice showed increased PE contraction, decreased basal nitric oxide (NO) index and impaired acetylcholine-induced endothelium-dependent relaxation. DOX treatment contributed to endothelial cell loss and reduced endothelial nitric oxide synthase (eNOS) expression in the aorta. In conclusion, we have replicated DOX-induced arterial stiffness in a murine model and this aortic stiffness is driven by impaired endothelial function, contributing to increased vascular tone.

PRESENTATION 12

**TARGETING INCREASED COLONIC TRYPSIN-LIKE ACTIVITY AND ABDOMINAL PAIN IN A RAT MODEL OF IRRITABLE BOWEL SYNDROME USING A SERINE PROTEASE INHIBITOR**

Hanning N, De bruyn M, Ceuleers H, Boogaerts T, Berg M, Smet A, De Schepper HU, Joossens J, van Nuijs ALN, De Man JG, Augustyns K, De Meester I, De Winter BY

University of Antwerp

INTRODUCTION: Proteases have been suggested to contribute to the pathogenesis of irritable bowel syndrome (IBS). We explored the therapeutic effect of an intracolonically administered serine protease inhibitor on the proteolytic colonic profile and abdominal pain in a post-inflammatory rat model for IBS.
METHODS: An enema containing 2,4,6-trinitrobenzene sulfonic acid (TNBS) was used to induce colitis in male Sprague-Dawley rats, whereas controls received saline solution. Colonoscopies were performed to confirm colitis and follow up mucosal healing. After endoscopic healing was observed, the serine protease inhibitor UAMC-00050 (0.1 – 5 mg/kg) or its vehicle (5% DMSO) was administered intracolonically. Specific proteolytic activities in fecal and colonic samples were measured using fluorogenic substrates. Pharmacokinetic parameters were evaluated using bioanalytical measurements with LC-MS. The visceral mechanosensitivity to colorectal distensions was quantified by visceromotor responses (VMRs).
RESULTS: Post-inflammatory rats had a threefold increased trypsin-like activity in colonic tissue and elevated neutrophil elastase activity in fecal samples, compared to controls. Treatment with UAMC-00050 normalized trypsin-like activity in colonic tissue of post-colitis animals. Pharmacokinetic experiments revealed that UAMC-00050 acted locally, with limited systemic uptake after intracolonic administration. Post-colitis animals had higher VMRs than control animals, indicating presence of visceral hypersensitivity. Intracolonic administration of UAMC-00050 decreased the VMRs in a dose-dependent manner. In control animals, a high dose of UAMC-00050 (5 mg/kg) resulted in VMRs lower than in vehicle-treated controls.
CONCLUSION: These results support the potential and efficacy of locally administered serine protease inhibitors as clinically relevant therapeutics for the treatment of IBS patients with abdominal pain.

PRESENTATION 13

**A feedback loop regulating capacitation in murine sperm**

Bertrand de Prelle, Pascale Lybaert, David Gall

Research Laboratory on Human reproduction, Université libre de Bruxelles

INTRODUCTION When mammalian spermatozoa are released in the female reproductive tract, they are incapable of fertilizing the ovocyte. They need a prolonged exposure to the alkaline medium of the female genital tract before their flagellum gets hyperactivated and the acrosome reaction can take place, allowing the sperm to enter the ovocyte. This process, called capacitation, is associated with a calcium influx through Catsper, a sperm-specific Calcium channel activated by an increase of intracellular pH (pHi). Here we propose a theoretical model for the activation of Catsper, including a feedback loop between the increase of pHi and the hyperpolarization (i.e. the transmembrane voltage shifting to more negative values).

METHODS In order to model the evolution of the pHi and the transmembrane voltage (Vm), we use first order differential equations including the effect of sNHE and SLO3, which are two other sperm-specific membrane transport proteins of protons and potassium ions respectively.

RESULTS Our model results in a feedback loop between pHi and Vm. The bistability resulting from this feedback loop implies that the capacitation's aspect of calcium influx can be reversed back.

CONCLUSION Our study shows that a feedback loop between pHi increase and hyperpolarization could switch the spermatozoa from an acidic and depolarized state to an alkaline hyperpolarized state. Actually, the possible reversibility of capacitation process could have major implications, relevant for the optimization of sperm preparation in assisted reproductive techniques and cryopreservation procedures.

PRESENTATION 14

**Pharmacological properties of essential oil from Aeollanthus pubscens in Benin**

Philippe Sessou, Mahudro Yovo, Gwladys S. Komagbe, Maximin Senou, Guy Alain Alitonou, Félicien Avlessi, Souaïbou Farougou, Dominique Sohounhloue

Research Unit on Communicable Diseases, Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O.Box 2009, Cotonou, Benin.

INTRODUCTION | Aeollanthus pubescens is an annual medicinal plant used traditionally as condiments, spices, flavourings as well as a medicine for the treatment of diarrhea, upper respiratory leaflet infections.

METHODS | The present study has evaluated the pharmacological properties of essential oil from this plant in Benin comprising its antiradical, antimicrobial, antiinflammatory and toxicological effects.

RESULTS | The results revealed that the oil containing thymol, carvacrol, thymyl acetate, carvacryl acetate, p-cymene and γ-terpinene as major compounds possessed high antimicrobial activities against multidrug resistant Salmonella spp and E coli isolated in poultry farms as well as on clinical strains namely E coli, S. aureus and Klebsiella pneumoniae isolated from urinary, diarrhea, septicemic infections patients samples. Furthermore, the study showed that the oil possessed high antiradical and low antiinflammatory effects. The oil had also no adverse effect on transaminases ALT and AST, urea, creatinine, cholesterol and hematological parameters in Wistar rats and did not exhibit any acute toxicity at 2000 mg/kg body weight. Microscopic liver and kidney examination of Wistar rats treated with 2000mg/kg of A. pubescens essential oil resulted in no significant changes and showed normal liver and kidney parenchyma between normal, gavaged and having received intramuscular injection extract groups

CONCLUSION | This study suggests that A. pubescens essential oils may be considered as generally recognized as safe and can be used in the fight against multidrug resistant bacteria.