# BELGIAN SOCIETY OF

# PHYSIOLOGY AND PHARMACOLOGY

## NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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**Autumn Meeting**

**Friday, October 19th 2018**

**PROGRAMME**

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**Venue**

**Palace of the Academies**
**Royal Academy of Medicine of Belgium**
**"The Marble Room"**
**Rue Ducale / Hertogstraat 1**
**1000 Brussels**

—

**Local host**

**Prof. Dr. Geert Bultynck**
**Laboratory of Molecular and Cellular Signaling**
**KULeuven**
**Belgium**

———

**with support of the**

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

**BELGIAN SOCIETY OF PHYSIOLOGY AND PHARMACOLOGY**

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**1000 Brussels**

9.30 – 10.00 Registration - coffee and tea

**Keynote lecture**

10.00-11.00 CT CALCIUM SIGNALING AND CHEMOTHERAPY: NEW PLAYERS AND THE PREVENTION OF SIDE EFFECTS
Prof. Dr. Barbara E Ehrlich (Yale University, New Haven)

**Oral communications (morning session)**

11.00-11.15 THE FUNCTION OF TMBIM-CONTAINING PROTEINS IN $Ca^{2+}$ SIGNALING
B. Seitaj, Q. Liu, J.B. Parys, A. Methner, G. Bultynck (KULeuven)

11.15-11.30 3D IMAGING OF INTRAPLAQUE NEOVASCULARIZATION IN $APOE^{-/-}$ $FBN1^{C1039+/-}$ MICE USING iDISCO TECHNOLOGY
P. Perrotta, I. Pintelon, P. Quax, G.R.Y. De Meyer, W. Martinet (UAntwerpen, LUMC-Leiden)

11.30-11.45 THE GATING PORE BLOCKER 1-(2,4-XYLYL)GUANIDINE INHIBITS THE SPONTANEOUS FIRING OF MIDBRAIN DOPAMINE NEURONS
K. Jehasse, L. Massotte, S. Ringlet, J. Roeper ,B. Lakaye, V. Seutin (ULiège, Goethe University)

11.45-12.00 3PO INHIBITS GLYCOLYSIS BUT DOES NOT BIND TO 6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE-2,6-BISPHOSPHATASE-3 (PFKFB3)
B. Emini Veseli, P. Perrotta, H. Macut, A. Abdali, P. Van Wielendaele, G. Monaco, A.-M. Lambeir, M.-L. Gelmi, G. Bultynck, W. Martinet, G.R.Y De Meyer (UAntwerpen, KULeuven, University of Milan)

12.00-12.15 AT1 RECEPTOR MEDIATED INHIBITORY MODULATION OF THE BAROREFLEX BY ANG II INVOLVES NO DEPENDENT LOCAL GABA RELEASE IN THE NUCLEUS TRACTUS SOLITARII OF NORMOTENSIVE RATS
L. Légat, I. Smolders, A. Dupont (VUB)

12.15-12.30 ROLE OF AUXILIARY SUBUNITS OF CAV1 CHANNELS IN TH2 CELLS - THERAPEUTIC IMPLICATIONS IN ALLERGIC ASTHMA
N. Rosa, E. Triffaux, V. Robert, M. Mars, M. Klein, G. Bouchaud, A. Canivet, A. Magnan, J.-C. Guéry, L. Pelletier, M. Savignac (University of Toulouse)

12.30-12.45 THE ROLE OF $Ca^{2+}$ IN L-AZETIDIN-2-CARBOXYLIC ACID-INDUCED ER STRESS AND AUTOPHAGY
G. Roest, E. Hesemans, K. Welkenhuyzen, T. Luyten, N. Engedal, G. Bultynck, J.B. Parys (KU Leuven, University of Oslo)

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**12.45 - 14.00 Lunch – Guided Poster Session**

**13.30 – 14.00 General Assembly**

**Posters**
(height 120 cm – width 100 cm)

1. EFFECTS OF LACTISOLE ON PANCREATIC ISLET B-CELLS ELECTROPHYSIOLOGY
   J.V. Sanchez-Andres, W.J. Malaisse (ULB, Universitat Jaume I, Castellan)

2. PHARMACOLOGICAL PROFILE OF THE INa IN HUMAN IPSC DERIVED CARDIOMYOCYTES COMPARED TO HETEROLOGOUS EXPRESSION MODELS
   D. Van de Sande, I. Kopljar, A. Teisman, D.J. Gallacher, D.J. Snyders, H. Rong, A.J. Labro (UAntwerpen)

3. BDA-366, A NOVEL BCL-2 BH4 DOMAIN ANTAGONIST, INDUCES APOPTOSIS THROUGH A BCL-2 INDEPENDENT MECHANISM IN DLBCL AND CLL
   T. Vervloessem, B. Sasi, D.Efremov, G. Bultynck (KULeuven, International Center for Genetic Engineering & Biotechnology, Trieste)

4. ACTIVATION OF CX43 HEMICHANNELS BY TNFA IN HELA CELLS STABLY OVEREXPRESSING CX43 AND IN THE ENDOTHELIAL CELL LINE EA.HY926
   S. Verwaerde, M. De Smet, T. Delvaeye, A. Lissoni, P. Vandenabeele, L. Leybaert (UGent)

5. MODULATION OF ARIPIPRAZOLE'S PHARMACOLOGICAL PROPERTIES BY ADENOSINE A2A RECEPTOR
   M. Ferraiolo, N. Desmet, A. Pochet, E. Hermans (UCL)

6. SEARCH FOR THE SOURCE OF THE RETINAL RELAXING FACTOR
   L. Vanden Daele, C. Boydens, J. Devoldere, K. Remaut, J. Van De Voorde (UGent)

7. RECIPROCAL LONG-LASTING EFFECTS BETWEEN ETHANOL LOCOMOTOR SENSITIZATION AND PTSD-LIKE CONDITION IN DBA/2J MICE
   T. Matonda-ma-Nzuzi, V. Didone, V. Seutin, E. Tirelli, E. Quertemont (ULG, University of Kinshasa)

8. THE β AND α2δ AUXILIARY SUBUNITS OF CAV1 CHANNELS ARE REQUIRED FOR TH2 LYMPHOCYTE FUNCTION AND ACUTE ALLERGIC AIRWAY INFLAMMATION
   N. Rosa, E. Triffaux, V. Robert, M. Mars, M. Klein, G. Bouchaud, A. Canivet, A. Magnan, J.-C. Guéry, L. Pelletier, Savignac (University of Toulouse, KULeuven)

9. IMPACT OF AICAR TREATMENT IN A MURINE MODEL OF ARISTOLOCHIC ACID NEPHROPATHY
   P. Mosseray, I. Jadot, O. Botton, J. Nortier, A.-E. Declèves, N. Caron (University of Namur)

10. EXPLOITING THE $Ca^{2+}$-INHIBITORY PROPERTIES OF BCL-2/BCL-XL'S BH4 DOMAIN AGAINST ACUTE PANCREATITIS
    T. Vervliet, J.V. Gerasimenko, P.E. Ferdek, M.A. Jakubowska, O.H. Petersen, O.V. Gerasimenko, G. Bultynck (KULeuven, Cardiff University)

11. GLUCOSE POTENTLY INHIBITS GLUCAGON SECRETION IN MICE WITH A-CELL-SPECIFIC DELETION OF $K_{ATP}$ CHANNELS
    B.K. Lai, H. Chae, A. Gómez-Ruiz, N. Antoine, P.L. Herrera, S. Seino, P. Gilon (UCL, University of Geneva, Kobe University)

12. THE CONTROL OF GLUCAGON SECRETION BY GLUCOSE AND SULFONYLUREAS
    B. Singh, L. Ruiz, H. Chae, B.K. Lai, N. Antoine, P. Gilon (UCL)

## Oral communications (afternoon session)

14.00-14.15 CONNEXIN 43 HEMICHANNELS AS A NOVEL TARGET TO MEDIATE RADIATION-INDUCED ENDOTHELIAL CELL DAMAGE
R. Ramadan, E. Vromans, D. Chuo Anang, E. Decrock, S. Baatout, L. Leybaert, A. Aerts (SCK•CEN Mol, UGent, UHasselt)

14.15-14.30 NEUREGULIN-1 TREATMENT COUNTERACTS ANGIOTENSIN II-INDUCED CARDIAC AND RENAL FIBROSIS IN ENDOTHELIAL NO SYNTHASE DEFICIENT MICE
H. Shakeri, S. De Moudt, A. Leloup, G. Jacobs, G.R.Y. De Meyer, G. De Keulenaer, P. Fransen, V. Segers, P.-J. Guns (UAntwerpen)

14.30-14.45 CALCIUM SIGNALS FROM IP3 RECEPTORS SUPPRESS BASAL AUTOPHAGY
K. Rietdorf, T. Chehab, G. Roest, G. Bultynck, J.B. Parys, F. McDonald, D. Bister, Martin Bootman (The Open University UK, KULeuven, Kings College London)

14.45-15.00 RYR2 REGULATES CX43 HEMICHANNEL $[Ca^{2+}]i$-DEPENDENT ACTIVATION IN CARDIOMYOCYTES
A. Lissoni, M. De Smet, T. Martins-Marques, N. Wang, H. Girão, Paco Hulpiau, L. Leybaert (UGent, Hogeschool West-Vlaanderen - Brugge, University of Coimbra)

15.00-15.15 THE CITRUS FLAVANONE HESPERETIN PREFERENTIALLY INHIBITS SLOW-INACTIVATING CURRENTS OF AN LQT3 SYNDROME $Na^+$ CHANNEL MUTATION
J. Alvarez-Collazo, A. López-Requena, L. Galán, J.L. Alvarez, K. Talavera (KULeuven, VIB Leuven, Institute of Cardiology and Cardiovascular Surgery, Havana)

**15.15 – 15.45 Coffee – Tea**

15.45-16.00 MUTUAL ANTAGONISM BETWEEN IP3 AND ANTI-APOPTOTIC BCL-2: NOVEL INSIGHTS IN THE MODULATION OF IP3R ACTIVITY AT THE LEVEL OF THE LIGAND-BINDING DOMAIN
H. Ivanova, L.E. Wagner II, A. Tanimura, E. Vandermarliere, T. Luyten, K. Welkenhuyzen, H. De Smedt, L. Martens, D.I. Yule, J.B. Parys, G. Bultynck (KULeuven, University of Rochester)

16.00-16.15 IDENTIFICATION AND EFFICIENCY ASSESSMENT OF POTENTIAL MIRNAS TARGETS FOR DEVELOPING NEW PHARMACOLOGICAL DRUGS AGAINST CHEMOTHERAPY-INDUCED OVARIAN DAMAGE USING MICE MODEL
C. Alexandri, B. Stamatopoulos, F. Rothe, I. Demeestere (ULB)

**Closing lecture**

16.15-17.00 INSP3-MEDIATED $Ca^{2+}$ SIGNALLING DURING CARDIAC HYPERTROPHY AND FAILURE
Prof. Dr. Llewelyn Roderick (KULeuven)

Oral presentation 1

## THE FUNCTION OF TMBIM-CONTAINING PROTEINS IN $Ca^{2+}$ SIGNALING

B. Seitaj, Q. Liu, J.B. Parys, A. Methner, G. Bultynck (KULeuven)

The family of Transmembrane Bax Inhibitor motif (TMBIM)-containing proteins was implicated in the regulation of Ca2+ signaling. The founding member, TMBIM6, lowered the steady-state Ca2+ levels in the endoplasmic reticulum (ER) thereby protecting cells against pro-apoptotic stimuli. Our team demonstrated that TMBIM6 functions as a Ca2+-leak channel and excitingly, the recently determined structure of a bacterial homolog of TMBIM6, BsYetJ, supported our functional analysis, identifying a C-terminal Ca2+-channel pore formed by critical Asp residues conserved among the family. Here, we focused on the bacterial BsYetJ and the mitochondrial TMBIM5 investigating whether other TMBIM-proteins could impact Ca2+ signaling. Using unidirectional 45Ca2+-flux assays in permeabilized HeLa cells, we found that addition of BsYet accelerates the Ca2+ efflux from the ER in a dose dependent way. Pre-application with supramaximal IP3 concentration completely abrogated the ability of BsYetJ to induce Ca2+ release, showing that the bacterial BsYetJ protein establishes Ca2+ fluxes across the ER. We then studied the effect of TMBIM5 overexpression on mitochondrial Ca2+ fluxes in HEK293 cells. Our TMBIM5-GFP was targeted to the mitochondria, while TMBIM5-GFP lacking the N-terminal mitochondrial-targeting sequence (TMBIM5/1-43) did not. Compared to control cells, TMBIM5-GFP overexpression enhanced agonist-induced Ca2+ rise in mitochondria. Importantly, overexpression of TMBIM5, but not of TMBIM5/1-43, inhibited staurosporin-induced cell death. Further work is focusing on the role of TMBIM5-mediated Ca2+ transport and cell survival processes. Overall, our work indicates that already early on during evolution, TMBIM-related proteins were capable to mediate Ca2+ fluxes across membranes and that likely TMBIM5 is enabling mitochondrial Ca2+ fluxes.

Oral presentation 2

## 3D IMAGING OF INTRAPLAQUE NEOVASCULARIZATION IN APOE$^{-/-}$FBN1$^{C1039+/-}$ MICE USING iDISCO TECHNOLOGY

P. Perrotta, I. Pintelon, P. Quax, G.R.Y. De Meyer, W. Martinet (UAntwerpen, LUMC-Leiden)

Multiple lines of evidence suggest that intraplaque (IP) neovascularization promotes destabilization and rupture of advanced atherosclerotic plaques. Although pharmacological inhibition of neo-vascularization is well established in cancer treatment, the potential benefits for atherosclerosis remain largely unexplored owing to the limited number of animal models that develop IP neovessels and the lack of reliable quantification methods for IP angiogenesis.

To address these limitations, we used ApoE$^{-/-}$ mice carrying an heterozygous mutation (C1039+/-) in the fibrillin-1 (Fbn1) gene. Similar to advanced atherosclerosis in human, ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice show very pronounced atherosclerosis and highly unstable plaques after feeding a western diet (WD), with evident IP neovascularization in the brachiocephalic artery and common carotid arteries. To obtain a longitudinal visualization of the IP vascular network, we applied 3D confocal microscopy. The success of this technique heavily relies on an optimized tissue-clearing process, termed iDISCO, that allows penetration of light in tissue while maintaining an intact plaque morphology.

ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice were fed WD for 18 weeks to stimulate plaque formation. Thereafter, carotid arteries were dissected and fixed with 4% paraformaldehyde. Tissue samples were stained with primary antibodies against the endothelial cell marker CD31 for 3 days, followed by incubation with fluorescently-labelled secondary for 2 days. After immuno-staining, tissues underwent iDISCO clearing and analysis using confocal microscopy. Using this protocol, IP neovessels of ApoE$^{-/-}$Fbn1$^{C1039G+/-}$ mice were successfully reconstructed in 3D. Plaques from ApoE$^{-/-}$ mice that underwent the same procedure did not reveal IP neovascularization.

Overall, we may conclude that 3D imaging represents a new approach to visualize plaque neo-vascularization and could allow better investigation of antiangiogenic therapy in atherosclerosis.

Oral presentation 3

# THE GATING PORE BLOCKER 1-(2,4-XYLYL)GUANIDINE INHIBITS THE SPONTANEOUS FIRING OF MIDBRAIN DOPAMINE NEURONS

K. Jehasse, L. Massotte, S. Ringlet, J. Roeper ,B. Lakaye, V. Seutin (ULiège, Goethe University)

INTRODUCTION | Midbrain dopamine (DA) neurons are slow intrinsic pacemaker cells displaying a slow depolarization during the interspike interval. The current underlying this depolarization has been calculated to be around 2 pA, which is equivalent to the current carried by the main pore of ±3 voltage-gated channels. We therefore hypothesized that the pacemaker current might be generated by gating pores, which have a much smaller conductance. We tested this hypothesis using guanidinium and 1-(2,4-xylyl)guanidine (XG) which activates and blocks, respectively, gating pore currents.

METHODS | We performed extracellular (EC) and patch-clamp (PC) recordings on acute rat and mouse brain slices in a conventional bicarbonate-based Ringer solution supplemented with blockers of synaptic inputs. In PC experiments, the intracellular solution was K-methylsulphate-based.

RESULTS | Guanidinium produced unspecific effects as judged from its effect on non-pacemaker neurons (CA1 pyramidal neurons). On the other hand, XG inhibited the firing of DA neurons (n=3, PC rat, n=6, PC mouse) in a concentration dependent manner (n=18, EC) with a total inhibition at 3 mM in all DA neurons. XG also completely inhibited the slow pacemaking of serotonergic neurons in the dorsal raphe (n=3, EC). It had negligible effects in most other neurons. Importantly, in voltage-clamped DA neurons, XG did not increase the cell conductance in the -50 to -60 mV range, showing it does not inhibit pacemaking by e.g. opening K+ channels.

CONCLUSION | We suggest that XG specifically inhibits slow neuronal pacemaking. Its exact mechanism of action remains to be determined.

Oral presentation 4

**3PO INHIBITS GLYCOLYSIS BUT DOES NOT BIND TO 6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE-2,6-BISPHOSPHATASE-3 (PFKFB3)**

B. Emini Veseli, P. Perrotta, H. Macut, A. Abdali, P. Van Wielendaele, G. Monaco, A.-M. Lambeir, M.-L. Gelmi, G. Bultynck, W. Martinet, G.R.Y De Meyer (UAntwerpen, KULeuven, University of Milan)

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, glycolysis inhibition may represent a novel strategy to prevent IP neovascularization and plaque formation. 6-phosphofrukto-2-kinase/fructose-2,6-bisphosphatse isoform 3 (PFKFB3) is a key enzyme of the glycolysis pathway and plays an essential role in angiogenesis. 3PO [3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one] is frequently used as a glycolysis inhibitor and is thought to inhibit PFKFB3. However, this latter effect of 3PO has never been studied in detail.

METHODS AND RESULTS | We showed that 3PO inhibits glycolysis in endothelial cells by using Seahorse technology and a cell based assay, inhibits capillary tube formation, EC migration, and formation of aortic sprouts. In order to demonstrate binding of 3PO to PFKFB3, we used two independent enzyme binding assays, i.e. isothermal titration calorimetry (ITC) and microscale thermophoresis (MST). To provide a complete thermodynamic profile of the molecular interaction, we used up to 6 mM (with ITC) and 1 mM (with MST) of 3PO. However, we found that 3PO did not bind to PFKFB3. In contrast, 25 µM of AZ PFKFB3 67, a potent and specific PFKFB3 inhibitor, was sufficient to determine the binding constant by using ITC and MST.

CONCLUSION | Our findings demonstrate that 3PO inhibits glycolysis and angiogenesis in a PFKFB3-independent manner.

Oral presentation 5

**AT1 RECEPTOR MEDIATED INHIBITORY MODULATION OF THE BAROREFLEX BY ANG II INVOLVES NO DEPENDENT LOCAL GABA RELEASE IN THE NUCLEUS TRACTUS SOLITARII OF NORMOTENSIVE RATS**

L. Légat, I. Smolders, A. Dupont (VUB)

INTRODUCTION | In the central cardiovascular control, the nucleus tractus solitarii (NTS) plays an important role and there is evidence indicating that actions of gamma-aminobutyric acid (GABA) and angiotensin II (Ang II) within the NTS are similar, suggesting that these neuromediators may interact to influence the sympathetic tone. Our main objective was therefore to pharmacologically characterize the responses evoked by Ang II infusion within the NTS in normotensive Wistar rats. METHODS | In vivo microdialysis was used for measurement of extracellular glutamate and GABA levels and for infusion of Ang II in the NTS of normotensive Wistar rats. The mean arterial pressure response and heart rate were monitored with a pressure transducer. Angiotensin II type 1 receptor (AT1R) selectivity was assessed using the AT1R antagonist candesartan; a nitric oxide synthase inhibitor was used to assess the involvement of nitric oxide in the evoked responses by infusion of Ang II.

RESULTS | Within the NTS, Ang II significantly increased extracellular GABA levels. This was associated with a significant increase in mean arterial pressure. GABA increase as well as mean arterial pressure increase were abolished by co-administration of the AT1R antagonist, candesartan. Local administration of the nitric oxide inhibitor alone decreased the GABA dialysates concentration in the NTS and abolished the Ang II induced GABA release.

CONCLUSION | We suggest that the Ang II mediated inhibitory modulation of the baroreflex is dependent on activation of nitric oxide synthase and subsequent enhanced release of GABA in the NTS of normotensive rats.

Oral presentation 6

**ROLE OF AUXILIARY SUBUNITS OF CAV1 CHANNELS IN TH2 CELLS - THERAPEUTIC IMPLICATIONS IN ALLERGIC ASTHMA**

N. Rosa, E. Triffaux, V. Robert, M. Mars, M. Klein, G. Bouchaud, A. Canivet, A. Magnan, J.-C. Guéry, L. Pelletier, M. Savignac (University of Toulouse)

Background: T lymphocytes express not only cell membrane ORAI Calcium release-activated calcium channel but also voltage-gated calcium channel (Cav) 1 channels. In excitable cells, these channels are composed of the ion-forming pore α1 and auxiliary subunits (β and α2δ) needed for proper trafficking and activation of the channel. Previously, we disclosed the role of Cav1.2 α1 in mouse and human TH2 but not TH1 cell functions and showed that knocking down Cav1 a1 prevents experimental asthma.

Methods: We used Cavβ antisense oligonucleotides to knock down Cavβ and gabapentin, a drug that binds to and inhibits α2δ1 and α2δ2, to test their effects on TH2 functions and their capacity to reduce allergic airway inflammation.

Results: Mouse and human TH2 cells express mainly Cavβ1, β3, and α2δ2 subunits. Cavβ antisense reduces T-cell receptor– driven calcium responses and cytokine production by mouse and human TH2 cells with no effect on TH1 cells. Cavβ is mainly involved in restraining Cav1.2 α1 degradation through the proteasome because a proteasome inhibitor partially restores the α1 protein level. Gabapentin impairs the T-cell receptor– driven calcium response and cytokine production associated with the loss of α2δ2 protein in TH2 cells.

Conclusion: These results stress the role of Cavβ and α2δ2 auxiliary subunits in the stability and activation of Cav1.2 channels in TH2 lymphocytes both in vitro and in vivo, as demonstrated by the beneficial effect of Cavβ antisense and gabapentin in allergic airway inflammation.

Oral presentation 7

## THE ROLE OF $Ca^{2+}$ IN L-AZETIDIN-2-CARBOXYLIC ACID-INDUCED ER STRESS AND AUTOPHAGY

G. Roest, E. Hesemans, K. Welkenhuyzen, T. Luyten, N. Engedal, G. Bultynck, J.B. Parys (KU Leuven, University of Oslo)

Accumulation of proteins in the ER causes ER stress and activates the unfolded protein response (UPR). This can result in autophagy and/or apoptosis. 6h treatment with 5mM L-azetidin-2-carboxylic acid (AZC) did not lead to cell death, but did induce phosphorylation of Eukaryotic translation initiation factor 2α (eIF2α), cleavage of Activating transcription factor (ATF) 6 and increased expression of Binding immunoglobulin protein (BiP). This indicates induction of the Protein kinase RNA-like ER kinase (PERK) and ATF6 arm of the UPR. Furthermore, lipidated microtubule-associated protein light chain (LC3-II) increased by 6h 5mM AZC treatment and further increased by co-treating with bafilomycin A1, indicating activation of autophagy. AZC-induced LC3-II was inhibited by the PERK inhibitor AMG PERK 44, indicating its dependence on the PERK arm of the UPR. Addition of 10µM or 20µM of the Ca2+-chelator BAPTA-AM for 6h reduced the levels of LC3-II induced by 5mM AZC. Furthermore, p-eIF2α/eIF2α ratios decreased. The Na+/K+ ATPase inhibitor ouabain affected LC3-II and p-eIF2α/eIF2α ratios independently from AZC, indicating that AZC-induced LC3-II and p-eIF2α are Ca2+ dependent. 6h pre-treatment with 5mM or 10mM AZC decreased the cytosolic Ca2+ detected with Fura2-AM upon stimulation with thapsigargin. Moreover, cytosolic [Ca2+] decreased quicker in AZC pre-treated cells. However, pre-treatment with AZC did decrease the ER Ca2+ store or increase mitochondrial Ca2+ uptake as measured with CEPIA probes targeted to the ER or mitochondria. These results strongly suggest that AZC-induced autophagy is PERK and Ca2+ dependent, and that a remodeling of the Ca2+ handling occurs at prolonged AZC treatment.

Oral presentation 8

**CONNEXIN 43 HEMICHANNELS AS A NOVEL TARGET TO MEDIATE RADIATION-INDUCED ENDOTHELIAL CELL DAMAGE**

R. Ramadan, E. Vromans, D. Chuo Anang, E. Decrock, S. Baatout, L. Leybaert, A. Aerts (SCK•CEN Mol, UGent, UHasselt)

Introduction: Medical applications of ionizing radiation (IR) have become widely used for diagnostic and therapeutic purposes. Emerging evidence indicates an excess risk for atherosclerosis after IR exposure. IR induces cellular effects which may induce endothelial cell dysfunction, an early marker for atherosclerosis. In addition, intercellular communication through gap junctions (GJs) and hemichannels, composed of connexin proteins (Cx), can modulate IR-induced responses and therefore the atherosclerotic process. However, the role of Cx channels in IR-induced atherosclerosis has never been described.

Methods: Telomerase immortalized human Coronary Artery/Microvascular endothelial cells were X-irradiated. Cx gene and protein expression, GJ and hemichannel function were investigated. In addition, ROS production, senescence, cell death and inflammatory responses were assessed with or without applying a hemichannel blocker (TAT-Gap19).

Results: IR exposure induced acute and persistent upregulation of Cx43 and downregulation of Cx40 gene and protein expression. Moreover, IR increased GJ communication and induced hemichannel opening. Furthermore, IR induced cell death, senescence, inflammation and ROS production. These effects were significantly reduced in the presence of the Cx43 hemichannel-targeting peptide TAT-Gap19.

Conclusions: An increase in intercellular communication after IR exposure may alter the transfer of IR damaging signals (ROS, senescence, cell death, inflammation) between the cells, resulting in an increase in endothelial cell damage, which could be protected by blocking the hemichannels. Moreover, similar alterations in Cx expressions have been reported in the literature in endothelial cells covering atherosclerotic plaques. Therefore, these results suggest that IR may contribute to atherosclerosis progression, and blocking hemichannels may have a potential radioprotective effect.

Oral presentation 9

**NEUREGULIN-1 TREATMENT COUNTERACTS ANGIOTENSIN II-INDUCED CARDIAC AND RENAL FIBROSIS IN ENDOTHELIAL NO SYNTHASE DEFICIENT MICE**

H. Shakeri, S. De Moudt, A. Leloup, G. Jacobs, G.R.Y. De Meyer, G. De Keulenaer, P. Fransen, V. Segers, P.-J. Guns (UAntwerpen)

INTRODUCTION | Endothelial cells are crucial for maintaining cardiovascular health. Functionally, the endothelium regulates vascular tone and cardiac function by production of nitric oxide (NO) by endothelial nitric oxide synthase (eNOS). Decreased eNOS activity is associated with different cardiovascular disorders, including high blood pressure, atherosclerosis and cardiac hypertrophy. Besides NO, endothelial cells produce other proteins with important functions. Neuregulin-1 (NRG-1) is an endothelium-derived growth factor, which plays crucial roles in cardiovascular development. NRG-1 induces growth and differentiation of different cell type, but has cardioprotective properties. Although the cardioprotective effects of endothelium-derived NO and NRG-1 are well established, the interaction between both endothelial signalling pathways is unstudied in vivo.

METHODS | 32eNOS knock out (eNOS-/-) mice and 32 wild type littermates were treated either with Angiotensin-II (Ang-II, 400 ng.kg -1 .min-1 for 28 days via osmotic minipumps)or received a sham surgery. Mice were randomized to receive either daily recombinant human NRG-1 injections (20 µg/kg, intraperitoneal) or vehicle for 4 weeks. Cardiac function was assessed in vivo by echocardiography.

RESULTS |AngII treatment did not cause higher blood pressure in this experimental setting, both heart and kidney weight was significantly higher in eNOS-/- animals treated with AngII, which was normalized after NRG-1 treatment. Moreover, Masson's trichrome staining for fibrosis showed an increase in cardiac and renal fibrosis in eNOS-/- animals treated with AngII. NRG-1 treatment inhibited this effect significantly.

CONCLUSION | This study shows for the first that the protective effects of NRG-1 on fibrosis and hypertrophy are independent from the NO/eNOS pathway.

Oral presentation 10

**CALCIUM SIGNALS FROM IP3 RECEPTORS SUPPRESS BASAL AUTOPHAGY**

K. Rietdorf, T. Chehab, G. Roest, G. Bultynck, J.B. Parys, F. McDonald, D. Bister, Martin Bootman (The Open University UK, KULeuven, Kings College London)

Cytosolic calcium signals have been proposed to regulate autophagy, but both inhibitory and stimulatory effects of calcium have been shown in published work [1]. Here, we examined the influence of IP3 receptor activity on basal autophagy in cells maintained in nutrient-rich medium. We measured the autophagic flux, cytosolic and mitochondrial calcium signals, cellular ATP levels, ER stress after transfection of HEK or HeLa cells with a 5'-phosphatase enzyme that converts IP3 into IP2. Cells expressing the IP3-phosphatase showed increased autophagic flux, increased number of GFP-LC3-labelled autophagosomes and decreased cellular ATP levels. ER stress, proportions of cells in different phases of the cell cycle and morphology of autophagosomes were not affected. Cells expressing the 5'-phosphatase enzyme showed reduced cytosolic and mitochondrial calcium signals evoked by application of IP3-generating agonists, and a lower number of spontaneous cytosolic calcium oscillations in cells maintained in nutrient-rich medium. A catalytically inactive 5'-phosphatase mutant did not have the same effects. The increased number of autophagosomes and decrease in ATP levels after expression of the 5'-phosphatase enzyme could be prevented by supplementing the nutrient-rich medium with methyl pyruvate, an activator of mitochondrial metabolism [2]. These data are consistent with the notion that transfer of calcium from IP3 receptors to mitochondria is necessary to enhance mitochondrial metabolism, maintain cellular ATP levels and thereby inhibit the activation of autophagy. Calcium signals arising from IP3 receptors suppress basal autophagy even when cells are in nutrient-replete conditions.

[1] Bootman et al., 2018 (Cell Calcium) [2] Cardenas et al., 2013 (Cell Reports)

Oral presentation 11

## RYR2 REGULATES CX43 HEMICHANNEL [Ca2+]i-DEPENDENT ACTIVATION IN CARDIOMYOCYTES

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INTRODUCTION| Connexin hemichannels are closed under resting condition, but open in response to increased intracellular Ca2+ concentration ([Ca2+]i). Connexin 43 (Cx43) is the most prevalent connexin in the heart. We here aimed to investigate the mechanisms that lead to [Ca2+]i-dependent Cx43 hemichannel opening in cardiomyocytes.

METHODS| Mouse ventricular cardiomyocytes were studied under whole-cell voltage-clamp at the resting potential of -70mV with simultaneous [Ca2+]i recording.

RESULTS| We recorded spiking unitary currents with the typical Cx43 unitary conductance of ~200pS upon activation of ryanodine receptor (RyR) with two agonists, caffeine and 4CmC, known to trigger the release of Ca2+ from the sarcoplasmic reticulum. In line with this, Cx43 hemichannel activity was suppressed in conditional Cx43 knock down animals. Surprisingly, elevating [Ca2+]i alone did not elicit any hemichannel response. However, simultaneous application of intracellular solutions with increased [Ca2+] and RyR agonist significantly promoted Cx43 hemichannel activity. After investigation, we found a direct interaction between Cx43 and RyR at the level of the intercalated discs in cardiomyocyte. A new mimetic peptide was designed to specifically target the interaction sequence and thus inhibit Cx43 hemichannel opening.

CONCLUSION| We conclude that the simultaneous activation of RyRs and [Ca2+]i elevation are essential to trigger hemichannel openings. The study provides a direct link between Cx43 hemichannel opening and the intracellular Ca2+ cascade that leads to excitation-contraction coupling. We hypothesize that the openings of Cx43 hemichannel may, as a large conductance and non-selective Ca2+ permeable channel, have a significant impact on electrical and cytoplasmic Ca2+ signaling in cardiomyocyte.

Oral presentation 12

**THE CITRUS FLAVANONE HESPERETIN PREFERENTIALLY INHIBITS SLOW-INACTIVATING CURRENTS OF AN LQT3 SYNDROME Na+ CHANNEL MUTATION**

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INTRODUCTION | The citrus flavanone hesperetin (HSP) has been proposed for the treatment of several human pathologies, but its cardiovascular actions remain largely unexplored. Here we studied the effects of HSP on the cardiac electrical and contractile activities and on aortic contraction. We further evaluated the action of HSP on the voltage-gated Na+ channel NaV1.5 and compared it to its effects on a recombinant NaV1.5 channel baring a mutation (R1623Q) associated with lethal ventricular arrhythmias in the Long QT syndrome subtype 3 (LQT3).

METHODS | We used cardiac surface electrocardiogram and contraction force recordings to evaluate HSP effects in isolated rat hearts and aortic rings. Whole-cell patch-clamp was used to record voltage-dependent Na+ currents (INa) in rat ventricular cardiomyocytes and in HEK293T cells expressing hNaV1.5 wild type (WT) or mutant channels.

RESULTS | HSP blocked the rat and human NaV1.5 channels in voltage-dependent manner with an effective inhibitory concentration (IC50) of ≈ 100 µmol·L-1. Its inhibition was markedly decreased by disruption of the F1760 residue. HSP preferentially accelerated the inactivation phase of INa and decreased the Na+ net influx into the cell. The effects on the inactivation phase were more marked in the R1623Q mutant, even at low concentrations, at which HSP had minor effects on cardiac electrical and contractile activities and poor vasodilatory action.

CONCLUSION | HSP preferentially accelerates the inactivation phase of INa, more markedly in the mutant R1623Q. HSP could be used as a template to develop drugs against lethal cardiac arrhythmias in LQT3.

Oral presentation 13

**MUTUAL ANTAGONISM BETWEEN IP3 AND ANTI-APOPTOTIC BCL-2: NOVEL INSIGHTS IN THE MODULATION OF IP3R ACTIVITY AT THE LEVEL OF THE LIGAND-BINDING DOMAIN**

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Bcl-2 protein has emerged as critical regulator of intracellular Ca2+ dynamics by directly targeting and inhibiting the IP3 receptor (IP3R), intracellular Ca2+-release channel. Here, we show that such inhibition could be counteracted by high concentrations of IP3 or agonist in permeabilized cells and in single-channel recordings or in intact cells, respectively. Interestingly, deletion of the known binding site for Bcl-2 in the central, modulatory domain of IP3R did not prevent the inhibitory effect of Bcl-2. This could be attributed to the binding of Bcl-2's BH4 domain to the ligand-binding domain (LBD) of the IP3R, shown experimentally and fitting in silico models. In silico analysis revealed steric hindrance by IP3 of the Bcl-2 binding to LBD. Consistent with this, the binding of Bcl-2 and its BH4 domain to the LBD was antagonized by IP3 or adenophostin A (AdA). Vice versa, the BH4 domain interfered with IP3 binding to the LBD. Thus, by competing for the LBD, IP3 and Bcl-2 mutually affect their impact on IP3R function: high concentrations of IP3 prevents IP3R inhibition by Bcl-2 and Bcl-2 prevents IP3 binding to the IP3R.

Oral presentation 14

**IDENTIFICATION AND EFFICIENCY ASSESSMENT OF POTENTIAL MIRNAS TARGETS FOR DEVELOPING NEW PHARMACOLOGICAL DRUGS AGAINST CHEMOTHERAPY-INDUCED OVARIAN DAMAGE USING MICE MODEL**

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INTRODUCTION | It is well- known that gonadotoxicity of chemotherapy agents may lead to follicle depletion, premature ovarian failure and infertility. This study aims to develop a new ovarian protective drug by modulating genes, using miRNAs involved in apoptosis, DNA-damage and in activation of primordial follicles during chemotherapy.

METHODS | Postnatal mouse ovaries were collected at day 3 after birth (PND3) and cultured under control and treated conditions (4-HC, 20μM/ 1-24h). The miRNA expression profiling was performed by TaqMan Low Density Arrays and confirmed by custom-cards and individual qPCR-assays. Functional annotation clustering was performed on genes targeted by the selected miRNA, let-7a. The Lipofectamine-RNAiMAX was used for the in vitro delivery of let-7a into ovaries. After transfection's efficiency validation, apoptosis, proliferation and morphology of the follicles were assessed in 4 groups: control, let-7a, chemo (1h/4HC), chemo (1h/4HC)+ let-7a.

RESULTS | The expression profiling revealed that miRNAs are differently expressed in ovaries after chemotherapy exposure. Let-7a was the most downregulated miRNA among others and the mimic-let-7a was effectively delivered in ovaries in vitro. The overexpressed let-7a was confirmed after transfection and was able to prevent the upregulation of the selected mRNA-targets in response to chemotherapy and it had a beneficial effect on follicle survival.

CONCLUSION | MiRNAs expression is modulated by chemotherapy, giving the opportunity of developing potential drugs which aim to prevent ovarian damages. Mimic-let-7a prevents chemotherapy-induced apoptosis in the ovary while beneficial effect on follicular activation was not confirmed but we investigate it using in vivo model.

Poster presentation 1

**EFFECTS OF LACTISOLE ON PANCREATIC ISLET B-CELLS ELECTROPHYSIOLOGY**

J.V. Sanchez-Andres, W.J. Malaisse (ULB, Universitat Jaume I, Castellan)

Sucralose mimics the effects of glucose upon several variables of pancreatic islet B-cell metabolism and function, including bioelectrical activity. The present study aimed at investigating whether lactisole, which was recently found to act as an antagonist of the sweet taste receptor TIR3 in pancreatic islet B-cells, also opposes the effects of glucose and/or sucralose upon B-cell electrophysiology. A dual effect of lactisole, both inhibitory and stimulatory, was observed upon the electrical activity of mouse pancreatic islet B-cells. The present findings thus document that agents supposed to act specifically on the TIR3 sweet receptor may either mimic or oppose the effect of glucose upon islet B-cell electrical activity.

Poster presentation 2

**PHARMACOLOGICAL PROFILE OF THE INa IN HUMAN IPSC DERIVED CARDIOMYOCYTES COMPARED TO HETEROLOGOUS EXPRESSION MODELS**

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Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) are a promising in vitro screening model to evaluate cardiac research & safety in the pharmaceutical industry. Compared to the heterologous expression systems, classically used in electrophysiological research, hiPSC-CM represent a more relevant human cardiac model. It is still unknown if cardiac drugs might interact differently on hiPSC-CM. To test this, we evaluated the effect of well characterized sodium channel inhibitors on the cardiac voltage gated sodium channel Nav1.5 (which is in native cardiomyocytes known to be influenced by the β1 subunit) and compared the effects to those of the heterologous system Nav1.5 with or without β1 in COS-7 cells. Accordingly, two class Ia drugs (quinidine, ajmaline), two class Ib drugs (lidocaine, phenytoin) and one class Ic drug (flecainide) were tested. For all compounds, the effect on hiPSC-CM was comparable to that of the heterologous model expressing both Nav1.5 and β1. Especially for lidocaine and phenytoin, the response was substantially modified by the presence of β1. Consequently, the results from the heterologous model expressing only Nav1.5 diverged from those obtained on hiPSC-CM. In conclusion, the pharmacological response of the sodium current in hiPSC-CM shows a better correlation with the Nav1.5+β1 heterologous model than the Nav1.5 heterologous model. Our data firstly indicate that the functional sodium current in hiPSC-CM is mostly likely generated by the Nav1.5 + β1 channel complex.

Poster presentation 3

# BDA-366, A NOVEL BCL-2 BH4 DOMAIN ANTAGONIST, INDUCES APOPTOSIS THROUGH A BCL-2 INDEPENDENT MECHANISM IN DLBCL AND CLL

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General background: Anti-apoptotic Bcl-2 is an essential inhibitor of apoptosis. Via its hydrophobic cleft, Bcl-2 is able to inhibit the activation of pro-apoptotic family members thereby inhibiting Bax-dependent apoptosis. On the other side, the BH 4 domain of Bcl-2 operates at the level of the IP3R thereby inhibiting aberrant Ca2+ fluxes. Hence, many cancer cells upregulated anti-apoptotic Bcl-2. Therefore, Bcl-2 has become a prime target for anti-cancer therapy. Hence, recently a small molecule BDA-366 has been developed to target the BH4 domain of Bcl-2, thereby converting Bcl-2 into a pro-apoptotic protein and induces Bax-dependent apoptosis in lung cancer and multiple myeloma. BDA-366 toxicity in these cancer cells correlated with the Bcl-2-expression level, whereby high Bcl-2-expressing cells display the highest sensitivity to BDA-366.

Main findings: BDA-366 induced Bax-mediated, but Ca2+ independent apoptosis in both Bcl-2 dependent as independent DLBCL cell lines. The sensitivity of DLBCL cells to BDA-366 did not correlate with Bcl-2-expression levels. This indicates that the mechanism of action of BDA-366-induced cell death is different in DLBCL compared to SCLC cells. In fact; in DLBCL, we noticed that BDA-366 significantly shortened the half-life of Mcl-1, without affecting other anti-apoptotic proteins like Bcl-2 and Bcl-XL. Interestingly, combining BDA-366 with a selective Bcl-2 antagonist (venetoclax) significantly enhanced sensitivity and cell death towards venetoclax.

Conclusions: BDA-366 induced apoptosis in a collection of DLBCL cell lines. Moreover, the mechanism of BDA-366 induced cell death doesn’t correlate with the Bcl-2-expression levels. BDA-366 decreased the Mcl-1 levels, potentially enhancing the sensitivity towards venetoclax.

Poster presentation 4

**ACTIVATION OF CX43 HEMICHANNELS BY TNFA IN HELA CELLS STABLY OVEREXPRESSING CX43 AND IN THE ENDOTHELIAL CELL LINE EA.HY926**

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INTRODUCTION | Cx43 hemichannels (HCs) are normally closed but open in response to electrical or chemical triggers such as pro-inflammatory cytokines or elevation of cytoplasmic Ca2+ ([Ca2+]i). Here, we investigated the activation of Cx43 HCs by acute exposure to TNFα at the unitary current level.

METHODS | HeLa cells stably transfected with Cx43 were subjected to whole-cell voltage clamp experiments at +70 mV. EA.hy926 cells were clamped at several potentials between -60 mV and +60 mV and [Ca2+]I ranged from normal 50 nM to 1 µM to assess the promoting effect of higher [Ca2+]i on Cx43 HC activity. Different peptides such as the selective Cx43 HC blocker Gap19 and enhancer CT9 were used to confirm Cx43 HC involvement.

RESULTS | In stably transfected HeLa cells, unitary current events with a biophysical profile consistent with Cx43 HCs could be routinely observed. EA.hy926 cells showed a dose-dependent increase of Cx43 HC activity upon stimulation with [Ca2+]i up to 500 nM. Exposure to higher [Ca2+]i decreased Cx43 HC activity but could be rescued by applying CT9. Gap19 abolished the increase in unitary current events in EA.hy926 cells clamped at 500 nM [Ca2+]I at both negative and positive membrane potentials. TNFα application in EA.hy926 cells voltage clamped at -50 mV under conditions of low Ca2+ buffering (through the patch pipette) that allows physiological Ca2+ signaling, showed an increase in Cx43 HC activity.

CONCLUSION | TNFα activates and modulates Cx43 HCs in stably transfected HeLa cells and EA.hy926 cells through a mechanism involving [Ca2+]i.

Poster presentation 5

**MODULATION OF ARIPIPRAZOLE'S PHARMACOLOGICAL PROPERTIES BY ADENOSINE A2A RECEPTOR**

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INTRODUCTION : Aripiprazole is an antipsychotic drug putatively acting as a partial agonist at the dopamine D2L receptor. The intrinsic activity of this drug at the dopamine receptor was shown to depend on the cellular environment. Therefore, diverse mechanisms may influence its biochemical activity. The response of dopaminergic ligands acting at the D2L receptor was proven to be modulated by its hetero-dimerization with the adenosine A2A receptor. Our hypothesis is that the interaction between these two receptors may influence the responses to aripiprazole.

METHODS : Cell membrane homogenates were prepared from rat striatum or from transfected HeLa cells expressing the D2L receptor alone or in combination with the A2A receptor. The functional response induced by dopamine and aripiprazole was monitored by measuring G-protein activation ([35S]GTPγS binding assay).

RESULTS : In transfected HeLa cells expressing the D2L receptor alone, aripiprazole did not increase [35S]GTPγS binding, suggesting neutral antagonism. In cells combining expression of the D2L and A2A receptors, aripiprazole decreased [35S]GTPγS binding, indicating an inverse agonist profile. At variance, in striatum samples where both D2L and A2A receptors are constitutively expressed, aripiprazole appeared as a neutral antagonist whereas inverse agonism was observed after A2A blockade using CGS15943.

CONCLUSION : We observed that the presence of A2A receptor could influence the pharmacological profile of aripiprazole acting at the D2L receptor, both in transfected cells and in brain tissues. Further studies are needed to better understand the molecular basis of such modulation and to clarify its implications on the clinical benefit of aripiprazole.

Poster presentation 6

**SEARCH FOR THE SOURCE OF THE RETINAL RELAXING FACTOR**

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INTRODUCTION | The retinal relaxing factor (RRF) is an unidentified paracrine factor, continuously released from retina and causing vasorelaxation. This study tried to identify the cellular source of the RRF. Furthermore, RRF release by voltage-dependent sodium channel activation and calcium-dependency of RRF release was investigated.

METHODS | Mice femoral arteries were mounted for in vitro isometric tension measurements. The vasorelaxing effect of avascular chicken retinas and of solutions incubated with MIO-M1 or primary Müller cell cultures were evaluated. The RRF release of other retinal cells was investigated using cell type inhibitors. Concentration-response curves of veratridine, a voltage-dependent sodium channel activator, were constructed in presence or absence of mouse retina to evaluate the RRF release. The calcium-dependency of RRF release was investigated by evaluating relaxations of RRF-containing solutions made out of chicken retinas in absence or presence of calcium.

RESULTS | Chicken retinas induced vasorelaxation, whereas solutions incubated with Müller cell cultures did not. Moreover, the gliotoxin DL-α-aminoadipic acid, the microglia inhibitor minocycline and the tetrodotoxin-resistant voltage-dependent sodium channel 1.8 inhibitor A-803467 could not reduce the RRF-induced relaxation. Concentration-response curves of veratridine were not enlarged in the presence of retina, and RRF-containing solutions made in absence of calcium induced a substantial, but reduced vasorelaxation.

CONCLUSION | The RRF is not released from vascular cells and probably neither from glial cells. The retinal cell type which does release the RRF remains unclear. Veratridine does not stimulate the RRF release in mice and RRF release in chickens is calcium-dependent as well as calcium-independent.

Poster presentation 7

**RECIPROCAL LONG-LASTING EFFECTS BETWEEN ETHANOL LOCOMOTOR SENSITIZATION AND PTSD-LIKE CONDITION IN DBA/2J MICE**

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INTRODUCTION | Mechanistic explanations of comorbiditie between alcohol use disorder (AUD) and Post-traumatic stress disorder (PTSD) remain unclear. This study investigated whether PTSD reduces the threshold for the acquisition of ethanol locomotor sensitization (ELS) in a mouse model. The second aim was to investigate whether prior ELS modulates the subsequent expression of PTSD.

METHODS | Female DBA/2J mice were submitted to a PTSD-like condition (PTSDLC), induced by an inescapable electric footshock, and to a paradigm of ELS. Firstly, mice were submitted to PTSDLC and then underwent ELS procedure. Mice were repeatedly injected with either saline, 1 g/kg ethanol (a non-sensitizing dose) or 2 g/kg ethanol (a sensitizing dose). Their locomotor activity was recorded in an open field. In second experiment, mice first underwent ELS procedure with the same doses and were then tested for their behavioral response to PTSDLC in fear conditioning chambers.

RESULTS |In the first experiment, PTSDLC did not allow ELS at the subthreshold dose of 1g/kg. However, with 2 g/kg ethanol, PTSDLC increased ELS compared to the control group. In the second experiment, ELS increased only the sensitized fear cluster of PTSDLC.

CONCLUSION | PTSDLC failed to reduce the dose threshold for the acquisition of ELS. But the PTSDLC strengthened the development of ELS with a sensitizing dose. This suggests that PTSD might interact with one of the mechanisms underlying the development of AUD. In the reverse direction, this study shows an effect of prior exposition to chronic administration of ethanol on “sensitized fear” PTSD cluster.

Poster presentation 8

# THE β AND α2δ AUXILIARY SUBUNITS OF CAV1 CHANNELS ARE REQUIRED FOR TH2 LYMPHOCYTE FUNCTION AND ACUTE ALLERGIC AIRWAY INFLAMMATION

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Background: T lymphocytes express not only cell membrane ORAI Calcium release-activated calcium channel but also voltage-gated calcium channel (Cav) 1 channels. In excitable cells, these channels are composed of the ion-forming pore α1 and auxiliary subunits (β and α2δ) needed for proper trafficking and activation of the channel. Previously, we disclosed the role of Cav1.2 α1 in mouse and human TH2 but not TH1 cell functions and showed that knocking down Cav1 a1 prevents experimental asthma.

Methods: We used Cavβ antisense oligonucleotides to knock down Cavβ and gabapentin, a drug that binds to and inhibits α2δ1 and α2δ2, to test their effects on TH2 functions and their capacity to reduce allergic airway inflammation.

Results: Mouse and human TH2 cells express mainly Cavβ1, β3, and α2δ2 subunits. Cavβ antisense reduces T-cell receptor– driven calcium responses and cytokine production by mouse and human TH2 cells with no effect on TH1 cells. Cavβ is mainly involved in restraining Cav1.2 α1 degradation through the proteasome because a proteasome inhibitor partially restores the α1 protein level. Gabapentin impairs the T-cell receptor– driven calcium response and cytokine production associated with the loss of α2δ2 protein in TH2 cells.

Conclusion: These results stress the role of Cavβ and α2δ2 auxiliary subunits in the stability and activation of Cav1.2 channels in TH2 lymphocytes both in vitro and in vivo, as demonstrated by the beneficial effect of Cavβ antisense and gabapentin in allergic airway inflammation.

Poster presentation 9

**IMPACT OF AICAR TREATMENT IN A MURINE MODEL OF ARISTOLOCHIC ACID NEPHROPATHY**

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INTRODUCTION | Aristolochic acid nephropathy (AAN) is a rapidly progressive tubulointerstitial nephritis induced by ingestion of aristolochic acids (AA). AAN is determined by two phases: (i) acute (AKI) and (ii) chronic kidney injury (CKD). AMP kinase regulates cellular metabolism and is activated during hypoxia. We investigated whether AMP kinase activator AICAR (5-amino-4-imidazolecarboxamide riboside-1-b-D-ribofuranoside) ameliorates AAN in mice.

METHODS | Eight-week-old C57Bl/6 male mice were divided into three groups: (i) control group; (ii) AA group (four intraperitoneal (i.p.) injections of 2.5 mg/kg of AA from D1 to D4); (iii) AA + AICAR group (seven i.p. injections of AICAR 0.5 mg/g from D-2 to D4). Each group was subdivided in two groups, the first one was sacrificed at D5 and the second one at D20. Serum creatinine, blood urea and urinary NAG levels were measured. Tubular impairments and fibrosis were scored. Expression of inflammation- and fibrosis-related genes were assessed.

RESULTS | AICAR treatment decreased serum creatinine, urea and NAG levels, attenuated macrophages infiltration into renal tissue and decreased expression of inflammation-related genes. Moreover AICAR treatment also decreased expression of fibrosis-related genes and limited the deposition of type I and III collagen fibers into tissue.

CONCLUSION | AICAR ameliorates kidney function after AA intoxication and protects against tubular impairments. Moreover, AICAR decreases inflammation induced by AAN and limits fibrosis development during the CKD phase. Activators of AMP kinase may represent a novel therapeutic approach for kidney diseases.

Poster presentation 10

## EXPLOITING THE Ca2+-INHIBITORY PROPERTIES OF BCL-2/BCL-XL'S BH4 DOMAIN AGAINST ACUTE PANCREATITIS

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Biliary acute pancreatitis (AP) is a serious condition which currently has no specific treatment. Taurolithocholic acid 3-sulfate (TLC-S) is one of the most potent bile acids causing cytosolic Ca2+ overload in pancreatic acinar cells (PACs), which results in premature activation of digestive enzymes and necrosis, hallmarks of AP. The inositol-1,4,5 trisphosphate receptor (IP3R) and the ryanodine receptor (RyR) play major roles in intracellular Ca2+ signaling. Inhibition of these endoplasmic reticulum-located channels suppresses TLC-S-induced Ca2+ release and necrosis, decreasing the severity of AP. Anti-apoptotic B cell lymphoma (Bcl)-2-family members, such as Bcl-2 and Bcl-XL, have emerged as important modulators of IP3Rs and RyRs. These proteins contain four Bcl-2 homology (BH) domains of which the N-terminal BH4 domain exerts critical roles in regulating intracellular Ca2+ release channels. The BH4 domain of Bcl-2, but not of Bcl-XL, binds to and inhibits IP3Rs, whereas both BH4 domains inhibit RyRs. Although clear cytoprotective effects have been reported for these BH4 domains, it remains unclear whether they are capable of inhibiting pathological Ca2+-overload, like that underlying AP. Here, we demonstrate in PACs that the BH4 domains of Bcl-2 and Bcl-XL inhibit RyR activity in response to the physiological agonist cholecystokinin. In addition, these BH4 domains inhibit pathophysiological TLC-S-induced Ca2+ overload in PACs via RyR inhibition which in turn protects these cells from TLC-S-induced necrosis. This study shows for the first time the therapeutic potential of BH4 domain function by inhibiting pathological RyR-mediated Ca2+ release and necrosis, events that trigger AP.

Poster presentation 11

**GLUCOSE POTENTLY INHIBITS GLUCAGON SECRETION IN MICE WITH A-CELL-SPECIFIC DELETION OF KATP CHANNELS**

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Introduction: In this study, we created mouse models lacking KATP channels specifically in α-cells to evaluate their role in the control of glucagon release.

Materials and methods: Transgenic mouse models were used: mice without KATP channels (Kir6.2-/-), mice with functional KATP channels and harboring two or one floxed Kir6.2 alleles (Kir6.2loxP/loxP, Kir6.2loxP/-). By crossing the above strains with Glu-cre mice, two α-cell-specific KATP channel KO models were generated: GluCre/Kir6.2loxP/loxP and GluCre/Kir6.2loxP/- mice. The percentage of cells without KATP channels was evaluated by measuring [Ca2+]c and testing their sensitivity to KATP channel modulators. The effects of G and KATP channel modulators were tested on glucagon and insulin secretion of perifused isolated islets.

Results: By [Ca2+]c measurements, the percentage of α-cells without KATP channels was 56% in GluCre/Kir6.2loxP/loxP and 88% in GluCre/Kir6.2loxP/- α-cells. Passing from 1 to 7 mM of G similarly inhibited glucagon secretion of GluCre/Kir6.2loxP/- and Kir6.2loxP/- islets. Interestingly, addition of diazoxide (250 µM) at 1 mM G strongly inhibited glucagon release of Kir6.2loxP/- islets but increased that of GluCre/Kir6.2loxP/- islets. The increase was abolished by pretreatement with pertussis toxin, which is compatible with an alleviation of the glucagonostatic effect of somatostatin released upon δ-cell KATP channel closure. Moreover, since glucagon secretion was more sustained in GluCre/Kir6.2loxP/- islets than in Kir6.2loxP/- islets, it appears that a closure of α-cell KATP channels exerts a glucagonotropic rather than a glucagonostatic effect.

Conclusion: Glucose inhibits glucagon secretion independently of α-cell KATP channels. Closure of δ-cell KATP channels exerts a strong tonic glucagonostatic effect

Poster presentation 12

**THE CONTROL OF GLUCAGON SECRETION BY GLUCOSE AND SULFONYLUREAS**

B. Singh, L. Ruiz, H. Chae, B.K. Lai, N. Antoine, P. Gilon (UCL)

INTRODUCTION | Glucose and sulfonylureas stimulate insulin secretion by a well-known mechanism. Closing ATP-sensitive K+ channels (KATP channels) by these agents induce an elevation of free cytosolic Ca2+ concentration ([Ca2+]c), thus triggering exocytosis. However, the mechanisms of control of glucagon secretion by these agents are still largely unknown. It is unclear whether their effects are mediated by a direct action on α cells or by an indirect paracrine action involving β or δ cells.

METHODS | We used a mouse model expressing a fluorescent Ca2+-sensitive protein (GCAMP6) specifically in α cells. This allowed us to know if there is any correlation between changes in [Ca2+]c and changes in glucagon secretion in response to glucose and sulfonylureas.

RESULTS | Results from validation of the probe showed that GCAMP6 was reliable, α cell specific and showed no interference with normal glucagon secretion in our mouse model. Moreover, a decrease of glucose concentration strongly stimulated glucagon secretion while it had no clear effect on [Ca2+]c. Interestingly, sulfonylureas increased both [Ca2+]c and glucagon secretion at high glucose concentration whereas they increased [Ca2+]c but decreased glucagon secretion at low glucose concentration.

CONCLUSION | Our data indicate that changes in [Ca2+]c are not correlated with changes in glucagon secretion when sulfonylureas are applied at low glucose concentration, showing that these agents might inhibit glucagon secretion via an indirect mechanism. On the other hand, the effect of glucose on [Ca2+]c and glucagon secretion show that glucose might act via a calcium independent pathway.