BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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Autumn Meeting

Friday, November 22nd 2019

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. Geert Bultynck and Dr. Tim Vervliet
Laboratory of Molecular and Cellular Signaling
KULeuven
Belgium

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with support of the

Royal Flemish Academy of Belgium for Science and the Arts

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Venue

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1000 Brussels

9.30 – 10.00 Registration - coffee and tea

Keynote lecture

10.00-11.00 BURNING THE CANDLE FROM TWO SIDES - A NOVEL VIEW OF HUNTINGTON'S DISEASE
Prof Dr Axel Methner (Johannes Gutenberg-Universität Mainz, Germany)

Oral communications (morning session)

11.00-11.15 THE ROLE OF MACROPHAGE RIPK1 IN THE DEVELOPMENT OF ATHEROSCLEROTIC PLAQUES IN APOE KNOCKOUT MICE
I. Coornaert, G. Marcassoli, G.R.Y. De Meyer, W. Martinet (University of Antwerp)

11.15-11.30 HYALURONIDASE SPAM1 KNOCKOUT PREVENTS SUBCHONDRAL BONE LOSS AND CARTILAGE DEGRADATION IN MICE MODEL OF OSTEOARTHRITIS
S. Lafont, C. Behets (UCL)

11.30-11.45 ANTI-METALLOTHIONEIN ANTIBODIES ALTER MACROPHAGE PHENOTYPE AND REPRESENT A NOVEL THERAPY FOR ACETAMINOPHEN-INDUCED LIVER INJURY
L. Devisscher, S. Van Campenhout, S. Lefere, S. Raevens, L. Tilleman, F. Van Nieuwerburgh, H.P. Van Eeckhoutte, A. Hoorens, M.A. Lynes, A. Geerts, D. Laukens, H. Van Vlierberghe (University of Ghent, University of Connecticut, Storrs, USA)

11.45-12.00 A LIPID SITE SHAPES THE AGONIST RESPONSE OF A PENTAMERIC LIGAND-GATED ION CHANNEL
C.M. Hénault, C. Govaerts, R. Spurny, M. Brams, A. Estrada-Mondragon, J. Lynch, D. Bertrand, E. Pardon, G.L. Evans, K. Woods, B.W. Elberson, L.G. Cuello, G. Brannigan, H. Nury, J. Steyaert, J.E. Baenziger, C. Ulens (KU Leuven)

12.00-12.15 SPONTANEOUS ARTERIAL AGEING IN C57BL6 MICE REVEALS PASSIVE AND ACTIVE AORTIC STIFFENING IN THE ABSENCE OF PERIPHERAL BLOOD PRESSURE ALTERATIONS
S. De Moudt, J.O. Hendrickx, D.G. De Munck, A.J. Leloup, W. Martinet, G.R.Y. De Meyer, P. Fransen (University of Antwerp)

12.15-12.30 GLUCOSE CONTROLS GLUCAGON SECRETION BOTH DEPENDENTLY AND INDEPENDENTLY OF α-CELL $[Ca^{2+}]c$ WHEREAS SULFONYLUREAS CONTROL GLUCAGON SECRETION VIA A-CELL $[Ca^{2+}]c$ AND SOMATOSTATIN
B. Singh, F. Khattab, P. Gilon (UCL)

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12.30 – 13.45 Lunch – Guided Poster Session

13.15 – 13.45 General Assembly

Posters

(height 120 cm – width 100 cm)

1. INVESTIGATING BIDIRECTIONAL RELATIONSHIP BETWEEN COCAINE-INDUCED LOCOMOTOR BEHAVIOUR AND POSTTRAUMATIC STRESS DISORDER-LIKE IN DBA/2J MICE
   T. Matonda ma Nzuzi, V. Didone, T. van Ingelgom, V. Seutin, E. Tirelli, E. Quertemont (University of Liège)

2. MUSCLE-TO-BRAIN COMMUNICATION IN THE CONTEXT OF OBESITY: IMPACT OF PHYSICAL EXERCISE?
   A. Delpierre, A. Villers, C. Deroux, L. Ris, A-E. Declèves, A. Legrand, A. Tassin (University of Mons)

3. REGULATION OF δ-CELL $[Ca^{2+}]_i$ AND SOMATOSTATIN SECRETION BY GLUCOSE
   L. Ruiz, A. Delcourt, P. Gilon (UCL)

4. HETEROGENEOUS Ca^{2+} RELEASE AT CARDIOMYOCYTE DYADS DURING EXCITATION-CONTRACTION COUPLING AND ITS MODULATION BY Gαq AGONISTS
   K. Demydenko, K. Sipido, H. L. Roderick (KU Leuven)

Invited lecture

13.45-14.45 TARGETED THERAPY IN PATIENT WITH PIK3CA RELATED OVERGROWTH SYNDROME
Prof Guillaume Canaud (Necker Hospital, Paris, France)

Oral communications (afternoon session)

14.45-15.00 MYELOID-SPECIFIC IRE1 ALPHA DELETION ATTENUATES TUMOUR DEVELOPMENT IN A NON-ALCOHOLIC STEATOHEPATITIS-INDUCED HEPATOCELLULAR CARCINOMA MOUSE MODEL
S. Van Campenhout, L. Tilleman, S. Lefere, A. Vandierendonck, A. Geerts, F. Van Nieuwerburg, H. Van Vlierberghe, L. Devisscher (University of Ghent)

15.00-15.15 RECONSTRUCTED SHEEP EPIDERMIS - A WAY TO MIMIC AN ANAEROBIC SKIN INFECTION?
N. Burton, V. De Glas, Y. Poumay, N. Kirschvink (University of Namur)

15.15-15.30 NAKED MOLE-RAT AND HYALURONAN: MYTHS AND REALITIES
D. del Marmol, S. Dogné, S. Holtze, T. Hildebrandt, K. Szafranski, Y. Poumay, B. Flamion (University of Namur)

15.30-15.45 PARTIAL INHIBITION OF GLYCOLYSIS REDUCES ATHEROGENESIS INDEPENDENT OF INTRAPLAQUE NEOVASCULARIZATION IN MICE
P. Perrotta, B. Van der Veken, P. Van Der Veken, I. Pintelon, L. Roosens, E. Adriaenssens, V. Timmerman, P.-J. Guns, G.R.Y. De Meyer, W. Martinet (University of Antwerp, Antwerp University Hospital)

15.45 – 16.15 Coffee – Tea

ORAL 1

THE ROLE OF MACROPHAGE RIPK1 IN THE DEVELOPMENT OF ATHEROSCLEROTIC PLAQUES IN APOE KNOCKOUT MICE

I. Coornaert, G. Marcassoli, G.R.Y. De Meyer, W. Martinet

Laboratory of Physiopharmacology, Department of Pharmaceutical Sciences, University of Antwerp, B-2610 Antwerp, Belgium.

INTRODUCTION | During atherogenesis, macrophages undergo necrosis and release their cytoplasmic content in the centre of the plaque, thereby forming a necrotic core. Further expansion of the necrotic core promotes plaque instability and rupture. Hence, targeting macrophage death is a promising strategy to stabilize atherosclerotic plaques. Recently, necroptosis was discovered as a form of regulated necrosis. The formation of a receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3 pro-necrotic complex, called the necrosome, is a crucial step in the induction of necroptosis. This study aimed to investigate the impact of a macrophage-specific RIPK1 deletion on atherogenesis.

METHODS | $RIPK1^{F/F}LysMCre^{+}ApoE^{-/-}$ and $RIPK1^{+/+}LysMCre^{+}ApoE^{-/-}$ mice were fed a western-type diet (WD) for 16 or 24 weeks to induce plaque formation.

RESULTS | Although total plasma cholesterol levels were slightly elevated after 16 weeks WD, the plaque area and percentage necrosis in $RIPK1^{F/F}LysMCre^{+}ApoE^{-/-}$ mice were significantly decreased. Moreover, plaques of $RIPK1^{F/F}LysMCre^{+}ApoE^{-/-}$ mice showed increased TUNEL positivity and a decreased macrophage content. After 24 weeks WD, plaque size and necrosis were similar between $RIPK1^{+/+}LysM\text{-}Cre^{+}ApoE^{-/-}$ and $RIPK1^{F/F}LysM\text{-}Cre^{+}ApoE^{-/-}$ mice. Similar to plaques of $RIPK1^{F/F}LysM\text{-}Cre^{+}ApoE^{-/-}$ mice after 16 weeks WD, increased TUNEL positivity was associated with a significant decrease in macrophage content. In vitro, macrophage RIPK1 deficiency resulted in inhibition of NF-κB activation. Moreover, RIPK1 deletion increased the sensitivity of macrophages to apoptosis. However, it was not sufficient to inhibit necroptosis induction.

CONCLUSION | Macrophage RIPK1 deficiency resulted in increased apoptosis, thereby slowing down plaque progression after 16 weeks WD. However, after 24 weeks WD, macrophage RIPK1 deficiency does not affect plaque size.

ORAL 2

HYALURONIDASE SPAM1 KNOCKOUT PREVENTS SUBCHONDRAL BONE LOSS AND CARTILAGE DEGRADATION IN MICE MODEL OF OSTEOARTHRITIS

Lafont S. Behets C.

Université Catholique de Louvain

INTRODUCTION | Osteoarthritis (OA) is caractarized by a lost in hyaluronan (HA) content. Enzymes such hyaluronidases can stimulate this degradation and accelerate the pathogenesis of OA. The aim of our study was to analyze the knockout of the hyaluronidase spam1 and its application on OA development.

METHODS | OA was induced by surgery in wild type (WT) and Spam1$^{-/-}$ mice. Knee joints, were analysed by pQCT, micro-CT, histology, immunohistochemistry and qpcr at different times post-surgery (days 0 to 70).

RESULTS | Before the surgery, Spam1$^{-/-}$ mice show a significantly lower bone mineral density (BMD) than WT mice. In the first seven days post-surgery, Spam1$^{-/-}$ subchondral bone react, in an opposite way to the WT mice, by an increase in BMD and bone volume fraction (BV/TV) followed by a decrease in those parameters. After this adaptation, both mice genotyping recovered their pre-surgery BMD and Spam1$^{-/-}$ mice showed a significantly lower BMD than WT mice up to 70days post-surgery. From 14 to 70days post-surgery, Spam1$^{-/-}$ mice showed significantly lower Modified Mankin score, osteophyte volume and number of ectopic calcification than WT mice. In normal condition, Spam1$^{-/-}$ chondrocytes express significantly more Hyal1 and significantly less Hyal 2 than WT mice. In post-surgery condition, WT chondrocytes show a significantly higher percentage of surface stained with Hyal1 and Mmp13 than Spam1$^{-/-}$ chondrocytes.

CONCLUSION | The absence of Spam1 seems to play a role in the subchondral bone response and in the expression of enzymes that degrade HA and the ECM.

ORAL 3

ANTI-METALLOTHIONEIN ANTIBODIES ALTER MACROPHAGE PHENOTYPE AND REPRESENT A NOVEL THERAPY FOR ACETAMINOPHEN-INDUCED LIVER INJURY

Lindsey Devisscher[1], Sanne Van Campenhout[2], Sander Lefere[2], Sarah Raevens[2], Laurentijn Tilleman[3], Filip Van Nieuwerburgh[3], Hannelore P. Van Eeckhoutte[2], Anne Hoorens[4], Michael A. Lynes[5], Anja Geerts[2], Debby Laukens[2], Hans Van Vlierberghe[2]

[1, 2, 3, 4] Ghent University, Belgium; [5] University of Connecticut, Storrs, USA

INTRODUCTION | Acetaminophen (APAP) intoxication is a leading cause of drug-induced liver failure and the only pharmacological treatment option, N-acetylcysteine (NAC), is not effective for patients who are admitted too late, emphasizing the need for alternative treatment options. APAP induces hepatocyte death followed by the release of danger signals which enhance hepatic monocyte/macrophage infiltration and activation. Metallothioneins (MTs) have danger signaling functions and might represent novel therapeutic targets in APAP overdose.

METHODS AND RESULTS | We evaluated MTs expression in human and mouse liver tissue after APAP overdose and the effect of anti-MT antibodies on the transcriptional profile of hepatic macrophage populations and liver injury following APAP overdose in mice. Hepatic MT expression was significantly induced in APAP-intoxicated mice and in resected livers from patients. APAP intoxication resulted in increased serum transaminase levels and pro-inflammatory markers and extended necrotic regions on liver histology which were significantly less pronounced in mice treated with anti-MT antibodies. Anti-MT therapy attenuated pro-inflammatory macrophage polarization, demonstrated by RNA sequencing analyses of isolated liver macrophages and in LPS-stimulated bone-marrow derived macrophages. Importantly, both NAC and anti-MT antibodies were equally effective and administration of anti-MT antibody in combination with NAC exceeded the efficiency of both monotherapies in APAP-induced liver injury.

CONCLUSION |The neutralization of secreted MTs using a monoclonal antibody is a novel therapeutic strategy for APAP-induced liver injury. In addition, we provide evidence suggesting that secreted MTs are involved in macrophage polarization.

ORAL 4

A LIPID SITE SHAPES THE AGONIST RESPONSE OF A PENTAMERIC LIGAND-GATED ION CHANNEL

Hénault CM, Govaerts C, Spurny R, Brams M, Estrada-Mondragon A, Lynch J, Bertrand D, Pardon E, Evans GL, Woods K, Elberson BW, Cuello LG, Brannigan G, Nury H, Steyaert J, Baenziger JE, Ulens C.

Chris Ulens is affiliated with Laboratory of Structural Neurobiology, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium.

INTRODUCTION | Phospholipids are key components of cellular membranes and are emerging as important functional regulators of different membrane proteins, including pentameric ligand-gated ion channels (pLGICs). Here, we take advantage of the prokaryote channel ELIC (Erwinia ligand-gated ion channel) as a model to understand the determinants of phospholipid interactions in this family of receptors.

METHODS AND RESULTS | A high-resolution structure of ELIC in a lipid-bound state reveals a phospholipid site at the lower half of pore-forming transmembrane helices M1 and M4 and at a nearby site for neurosteroids, cholesterol or general anesthetics. This site is shaped by an M4-helix kink and a Trp-Arg-Pro triad that is highly conserved in eukaryote GABAA/C and glycine receptors. A combined approach reveals that M4 is intrinsically flexible and that M4 deletions or disruptions of the lipid-binding site accelerate desensitization in ELIC, suggesting that lipid interactions shape the agonist response.

CONCLUSION | Our data offer a structural context for understanding lipid modulation in pLGICs.

ORAL 5

SPONTANEOUS ARTERIAL AGEING IN C57BL6 MICE REVEALS PASSIVE AND ACTIVE AORTIC STIFFENING IN THE ABSENCE OF PERIPHERAL BLOOD PRESSURE ALTERATIONS

S. De Moudt, J.O. Hendrickx, D.G. De Munck, A.J. Leloup, W. Martinet, G.R.Y. De Meyer, P. Fransen

Laboratory of Physiopharmacology, University of Antwerp

INTRODUCTION | Arterial stiffness (AS) has gained recognition as an independent predictor of cardiovascular (CV) events. Although AS was assumed to be an adaptive response to increased blood pressure (BP), AS has been shown to precede hypertension in two experimental mouse models, thereby revealing an incomplete understanding of AS pathophysiology.

METHODS | The current study presents CV characterization of spontaneously ageing C57Bl6 mice (2,4,6, 12-months of age) (male, n>10), including in vivo analysis of peripheral BP (Coda), aortic pulse wave velocity (aPWV, Vevo), and echocardiography (Vevo). Additionally, isometric reactivity (organ baths) and AS (ROTSAC) of the thoracic aorta was thoroughly studied ex vivo.

RESULTS | C57Bl6 mice displayed significant AS from 6-months of age, both in vivo (aPWV: 2-month 2.0 ± 0.1 up to 12-month 3.4 ± 0.2 m/s) and ex vivo (Peterson modulus, Ep: resp. 291 ± 6; 330 ± 4 mmHg). BP remained unaltered (SBP: resp. 102 ± 5; 103 ± 3 mmHg). Echocardiography revealed a declining left-ventricular (LV) relaxations, and LV hypertrophy. Ex vivo studies exposed intensification of adrenoreceptor-dependent contractions with age (60% increase in maximal isometric contraction), resulting in active AS. This was accompanied by a 37% increase in voltage-dependent calcium channels (VGCC) contribution. Interestingly, NO signalling was totally unaffected. Also a 10% increase in passive AS was observed.

CONCLUSION | Spontaneous AS of C57Bl6 mice occurred from 6-months of age, in the absence of increased peripheral BP. Both active and passive AS was observed, and active AS was restricted to vascular smooth muscle cell disease.

ORAL 6

GLUCOSE CONTROLS GLUCAGON SECRETION BOTH DEPENDENTLY AND INDEPENDENTLY OF α-CELL $[Ca^{2+}]c$ WHEREAS SULFONYLUREAS CONTROL GLUCAGON SECRETION VIA α-CELL $[Ca^{2+}]c$ AND SOMATOSTATIN

B. Singh, F. Khattab, P. Gilon

Université Catholique de Louvain (UCL)

INTRODUCTION | The mechanisms by which glucose and sulfonylureas control glucagon secretion are hotly debated. In this work, we focused on their effects on α-cell $[Ca^{2+}]c$, a key element controlling exocytosis, and investigated whether $[Ca^{2+}]c$ changes correlated or not to changes of glucagon secretion. α-cell $[Ca^{2+}]c$ was measured using a transgenic mouse model expressing a Ca^{2+}-sensitive fluorescent probe, GCaMP6f, specifically in α-cells.

METHODS | Several mouse models were used including Lox-STOP-Lox-GCaMP6f, GluCre/Lox-STOP-Lox-GCaMP6f and GluCre/Lox-STOP-Lox-GCaMP6f/$Sst^{-/-}$. Secretion and $[Ca^{2+}]c$ experiments were performed on perifused islets or dispersed islet cells in the presence of alanine, arginine and glutamine (each at 2 mM). Immunodetections were performed on dispersed islet cells.

RESULTS | Validation experiments on GluCre/Lox-STOP-Lox-GCaMP6f mice showed that the Ca^{2+}-probe was reliable, was specifically expressed in α-cells, and did not interfere with glucagon secretion. Sulfonylureas increased α-cell $[Ca^{2+}]c$ but inhibited glucagon secretion. However, they failed to inhibit glucagon secretion in somatostatin KO mice. Furthermore, application of exogenous somatostatin strongly inhibited glucagon secretion and α-cell $[Ca^{2+}]c$. On the other hand, glucose modulated glucagon secretion both dependently and independently of α-cell $[Ca^{2+}]c$, depending on the glucose concentration. Moreover, it decreased the efficacy of Ca^{2+} on exocytosis, in mice expressing or not somatostatin.

CONCLUSION | Using a novel transgenic mouse model, we show that sulfonylureas control glucagon secretion via α-cell $[Ca^{2+}]c$ and somatostatin. Moreover, we show that glucose modulates glucagon secretion via two distinct mechanisms depending on the glucose concentration. Finally, we show that glucose decreases the efficacy of Ca^{2+} on exocytosis in α-cells.

ORAL 7

MYELOID-SPECIFIC IRE1 ALPHA DELETION ATTENUATES TUMOUR DEVELOPMENT IN A NON-ALCOHOLIC STEATOHEPATITIS-INDUCED HEPATOCELLULAR CARCINOMA MOUSE MODEL

Sanne Van Campenhout[1], Laurentijn Tilleman[2], Sander Lefere[1], Astrid Vandierendonck[1], Anja Geerts[1], Filip Van Nieuwerburgh[2], Hans Van Vlierberghe[1], Lindsey Devisscher[3]

[1]Department of Internal Medicine and Pediatrics, Ghent University, Belgium [2]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Ghent University, Belgium [3]Department of Basic and Applied Medical Sciences, Ghent University, Belgium

INTRODUCTION | Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, inflammation and macrophage infiltration and is rising causes of hepatocellular carcinoma (HCC). Inositol-requiring enzyme 1 alpha (IRE1a) is involved in macrophage cytokine production. We evaluated the effect of myeloid-specific IRE1a deletion on NASH and subsequent HCC development.

METHODS | NASH-HCC was induced by streptozotocin (STZ) injection and western diet (WD) feeding for 17 weeks. Myeloid IRE1a knockout (KO) and wild type (WT) mice were evaluated for obesity, diabetes, NASH and HCC development. Flow cytometric analyses and RNA sequencing was performed on FACS isolated liver macrophages.

RESULTS | Impaired glucose tolerance, advanced NASH and HCC was observed after STZ+WD feeding. KO STZ+WD fed mice showed lower fasting glucose levels and an improved glucose tolerance and attenuated HCC development after 17 weeks of WD feeding despite similar degree of NASH compared to WT mice. Liver resident macrophages, Kupffer cells (KCs), were depleted in NASH-HCC whereas monocytes had infiltrated the liver. Transcriptomic analyses revealed different phenotypes for liver KCs, macrophages and monocytes at steady state and, even more pronounced, at NASH-HCC. In healthy mice, pathways involved in immune system activation and metabolism were downregulation in KCs from KO mice whereas pathways involved in cell division and metabolism were upregulated in monocytes from KO mice compared to WT cells. NASH-HCC attenuated the differential gene expression profile of KO and WT liver isolated macrophages.

CONCLUSION | Our results show that myeloid-specific IRE1a deletion results in an altered transcriptional profile of hepatic macrophages and attenuates diabetes induction and NASH-related HCC development.

ORAL 8

RECONSTRUCTED SHEEP EPIDERMIS - A WAY TO MIMIC AN ANAEROBIC SKIN INFECTION?

BURTON Nicolas (1), DE GLAS Valérie (2), POUMAY Yves (2), KIRSCHVINK Nathalie (1)

University of Namur - Namur Research Institute of Life Sciences (NARILIS) - (1) Department Veterinary Medicine & (2) Cell and Tissue Laboratory (LabCeTi) - Belgium

INTRODUCTION | Footrot is a highly contagious disease affecting the foot of sheep, causing heavy economic losses due to lameness and reducing animal welfare. The causative bacterium is the gram negative anaerobe Dichelobacter nodosus. The aim of this study was to develop a reconstructed sheep epidermis (RSE) by 1) selecting the most appropriate skin digestion protocol; 2) comparing two conditions of cell culture; 3) performing functional and histological characterization of RSE.

METHODS AND RESULTS | Among 6 digestion protocols tested on 5 skin samples, the protocol based on 1 hour of incubation in 0.17% of trypsin at 37°C provided the most efficient result in terms of keratinocyte count (851.217±405.797 cells/mL) and viability 24h after digestion (85,5±3,7%). Keratinocyte cultures were performed with and without Rho kinase (ROCK) inhibitor Y-27632. In presence of Y-27632, isolated keratinocytes showed an increase in proliferation and delayed differentiation while cultures without inhibitor stopped growing. RSE were prepared with third passage keratinocytes seeded onto polycarbonate fiber and exposed to an air-liquid interface with a medium without Y-27632 allowing differentiation. Differentiated RSE were obtained 11 days after seeding. Histological staining using hematoxylin-erythrosine and immunohistological staining allowed morphological analysis of RSE confirming differentiation of basal, spinous, granular and cornified layer. Transepithelial electrical resistance was measured and confirmed functional integrity of RSE (12.589±310 Ω cm2, n=6).

CONCLUSION | This study describes the development of a RSE and provides a promising tool for the characterization of mechanisms involved in Dichelobacter nodosus infection, such as adhesion, invasion and proliferation of bacterium in epidermis.

ORAL 9

NAKED MOLE-RAT AND HYALURONAN: MYTHS AND REALITIES

Delphine del Marmol, Sophie Dogné, Susanne Holtze, Thomas Hildebrandt, Karol Szafranski, Yves Poumay, Bruno Flamion

Namur University (Belgium), Molecular Physiology Research Unit, NARILIS

INTRODUCTION |The naked mole-rat (Heterocephalus glaber) (NMR) is an eusocial rodent living in subterranean tunnels. Besides an exceptionally long lifespan, NMRs also exhibit pronounced resistance to cancer. This characteristic has been ascribed to an unusually high content of very high molecular weight (>6. 106 Da) hyaluronan (HA). HA functions are known to be size-dependent, with high molecular weight HA displaying anti-inflammatory and anti-cancerous properties.

METHODS AND RESULTS | HA metabolism in NMRs, with mouse and guinea pigs as controls, was studied in the skin, muscles, lymph nodes, kidneys, plasma and in skin fibroblasts supernatant using HA peroxidase detection and Alcian blue, ELISA-like assay, as well as size exclusion chromatography and electrophoresis on agarose gels to determine its molecular weight profile. Obvious differences in HA quantity and molecular weight were found between NMR and guinea pig (especially in lymph nodes) but contrary to previous results no high molecular weight (>4000 kDa) HA was found in any NMR samples. RNASeq analyses showed high expression of some HA-related genes in NMR vs mouse tissues.

CONCLUSION |These results suggest that HA turnover and partners differ in NMR vs other rodents but no evidence of extremely high HA accumulation was found in NMR tissues or blood.

ORAL 10

PARTIAL INHIBITION OF GLYCOLYSIS REDUCES ATHEROGENESIS INDEPENDENT OF INTRAPLAQUE NEOVASCULARIZATION IN MICE

Paola Perrotta[1], Bieke Van der Veken[1], Pieter Van Der Veken[2], Isabel Pintelon[3], Laurence Roosens[4], Elias Adriaenssens[5], Vincent Timmerman[5], Pieter-Jan Guns[1], Guido R.Y. De Meyer[1], Wim Martinet[1]

[1]Physiopharmacology, University of Antwerp, [2] Medicinal Chemistry, University of Antwerp, [3]Cell Biology and Histology, University of Antwerp, [4]Antwerp University Hospital, [5]Peripheral Neuropathy Research Group, University of Antwerp

INTRODUCTION | Intraplaque (IP) neovascularization is an important feature of unstable human atherosclerotic plaques. However, its impact on plaque formation and stability is poorly studied. Because proliferating endothelial cells (ECs) generate up to 85% of their ATP from glycolysis, we investigated whether pharmacological inhibition of glycolytic flux by the small molecule 3PO [3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one] could have beneficial effects on plaque formation and composition.

METHODS AND RESULTS | $ApoE^{-/-}$ mice treated with 3PO (50 µg/g, i.p.; 4x/week, 4 weeks) showed a metabolic switch toward ketone body formation. Treatment of $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice with 3PO (50 µg/g, i.p.) either after 4 weeks of western diet (WD) (preventive, twice/week, 10 weeks) or 16 weeks of WD (curative, 4x/week, 4 weeks) inhibited IP neovascularization by 50% and 38%, respectively. Plaque formation was significantly reduced in all 3PO-treated animals. This effect was independent of IP neovascularisation. In vitro experiments showed that 3PO favors an anti-inflammatory M2 macrophage subtype and suppresses an M1 pro-inflammatory phenotype. Moreover, 3PO induced autophagy, which in turn impaired NF-κB signalling and inhibited TNF-α-mediated VCAM-1 and ICAM-1 up-regulation. Consistently, a preventive 3PO regimen reduced endothelial VCAM-1 expression in vivo. Furthermore, 3PO improved cardiac function in $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice after 10 weeks of treatment.

CONCLUSION | Partial inhibition of glycolysis restrained IP angiogenesis without affecting plaque composition. However, less plaques were formed, which was accompanied by downregulation of endothelial adhesion molecules, an event that depends on autophagy induction. Inhibition of coronary plaque formation by 3PO resulted in an overall improved cardiac function.

POSTER 1

INVESTIGATING BIDIRECTIONAL RELATIONSHIP BETWEEN COCAINE-INDUCED LOCOMOTOR BEHAVIOUR AND POSTTRAUMATIC STRESS DISORDER-LIKE IN DBA/2J MICE

Thierry Matonda ma Nzuzi, Vincent Didone, Théo van Ingelgom, Vincent Seutin, Ezio Tirelli, Etienne Quertemont

Laboratory of Neurophysiology, GIGA Neurosciences, ULiège, Belgium; Psychology & Neuroscience of Cognition – PsyNCogn, ULiège, Belgium

INTRODUCTION | Cocaine use disorder and posttraumatic stress disorder (PTSD) are bound in a complex reciprocal relationship. To date, this relationship remains poorly understood. Some scarce animal studies have shown that PTSD-like (PTSDL) increases cocaine-induced locomotor behaviour (CILB) whereas others studies did not. On the other side, for the reverse relationship, little is known on the influence of CILB on expression of PTSDL due to the scarcity of studies. This study aimed, on the one hand, to assess whether PTSDL modulates CILB. In the other hand, this study aimed to assess whether CILB modulates the expression of PTSDL, especially in a dose-dependent manner.

METHODS | Experiments were conducted on female inbred DBA/2J mice. PTSDL was induced by an electric footshock and assessed by the expression of conditioned fear, sensitized fear and anxiety-like behaviours. CILB was induced by chronic repeated intraperitoneal injections of cocaine. In experiment 1, mice underwent PTSDL procedure and then underwent CILB with 15 mg/kg cocaine. In experiment 2, mice underwent CILB with 7.5 mg/kg and 15 mg/kg cocaine before PTSDL.

RESULTS | PTSDL did not modulate both acquisition and expression of CILB. In the reverse relationship, CILB increased, independently of the PTSDL effect, the expression of both sensitized fear ($p = 0.025$) and conditioned fear ($p = 0.03$).

CONCLUSION | PTSDL does not increase CILB. For the reverse relationship, CILB modulates the expression of the sensitized fear and the conditioned fear of PTSDL indifferently of stress.

POSTER 2

MUSCLE-TO-BRAIN COMMUNICATION IN THE CONTEXT OF OBESITY: IMPACT OF PHYSICAL EXERCISE?

A. Delpierre, A. Villers, C. Deroux, L. Ris, A-E. Declèves, A. Legrand and A. Tassin

UMons

INTRODUCTION | Exercise training (ET) has a positive effect on brain health. Although molecular mechanisms underlying ET benefits are still poorly understood, a cross-talk between skeletal muscle and brain has been described. During ET, muscle releases specific myokines among which potential regulators of hippocampal function, like Irisin. This exerkine is a PGC1α-dependant myokine released by cleavage of FNDC5. Also expressed in the brain, FNDC5 contributes to increase the level of brain-derived neurotrophic factor. However, the contribution of muscle-derived Irisin on cognitive function remains controversial, 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 in function of ET modalities and (ii) whether muscle-to-brain crosstalk is altered in the context of obesity.

METHODS AND RESULTS |To this aim, two ET modalities were compared in mice: spontaneous ET (environmental enrichment) and endurance ET (training sessions on a treadmill). Mice were fed either with a Low-Fat (LF) or an High-Fat (HF) diet. ET reduces weight gain and fasting glycaemia in obese mice. Environmental enrichment improves spatial learning and memory (Morris Water Maze test), particularly in obese animals. Irisin plasmatic level is enhanced by a HF diet and endurance ET. In muscles, FNDC5 protein level is also modified by ET and diet. In brain, ET improves BDNF protein level.

CONCLUSION | ET modalities and obesity influence FNDC5/Irisin pathway and cognitive functions in mice. Further studies are necessary to understand the contribution of muscle-derived Irisin to ET effects.

POSTER 3

REGULATION OF δ-CELL $[Ca^{2+}]_i$ AND SOMATOSTATIN SECRETION BY GLUCOSE

Lucie Ruiz, Arthur Delcourt, Patrick Gilon

UCL

INTRODUCTION | The islets of Langerhans play a key role in glucose homeostasis. They contain three major cell types: β-, α- and δ-cells that secrete insulin, glucagon and somatostatin (SST), respectively. Insulin is the main hypoglycemic hormone of the body, whereas glucagon is hyperglycemic. SST strongly inhibits insulin and glucagon secretion but the mechanisms by which glucose controls SST secretion are largely unexplored. These three cell types communicate between each other. Therefore, it is crucial to understand the mechanisms by which islet hormone secretion is regulated. The aim of this project is to study the mechanisms by which glucose controls δ-cell $[Ca^{2+}]$i and SST secretion.

METHODS | $[Ca^{2+}]$i was measured using a Ca^{2+}-sensitive fluorescent sensor, GCaMP6f, expressed specifically in δ-cells. SST secretion was measured by radioimmunoassay.

RESULTS | Glucose increases $[Ca^{2+}]$i in δ-cells and stimulates SST secretion. Interestingly, applying diazoxide to open ATP-sensitive potassium channels (KATP channels), abolishes $[Ca^{2+}]$i oscillations induced by glucose, suggesting an important role of KATP channels in the stimulation-secretion coupling in δ-cells. However, we also highlighted other mechanisms by which glucose stimulates SST secretion. We observed that Ca^{2+} influx via voltage-gated Ca^{2+} channels triggers a Ca^{2+}-induced Ca^{2+}-release, and also that raising the glucose concentration in the presence of diazoxide and high potassium (in order to clamp the membrane potential) increases SST secretion, independently from $[Ca^{2+}]$i changes.

CONCLUSION | This study shows that glucose stimulates SST secretion not only by closing KATP channels and increasing $[Ca^{2+}]$i but also through amplifying mechanisms of still unknown nature.

POSTER 4

HETEROGENEOUS Ca^{2+} RELEASE AT CARDIOMYOCYTE DYADS DURING EXCITATION-CONTRACTION COUPLING AND ITS MODULATION BY Gαq AGONISTS

Kateryna Demydenko, Karin Sipido, H Llewelyn Roderick

Lab of Experimental Cardiology, KU Leuven

INTRODUCTION |Cardiac contraction is activated by Ca^{2+} release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR2) following their activation by a transsarcolemmal Ca^{2+} influx through voltage-gated L-type Ca^{2+} channels (LCC). RyR2 on the SR together with juxtaposed LCCs on the sarcolemma form specialized microdomains, termed dyads. Multiple chemical signaling mechanisms, among them GPCRs liganded by ET-1 and Ang-II, elicit a number of effects upon the cardiomyocytes of the heart including inotropy. Here, we tested the hypothesis that GPCR agonists elicit their effects via alterations in the dynamics of Ca^{2+} release at individual dyads.

METHODS AND RESULTS| To address this question, we examined local Ca^{2+} release following stimulation with ET-1 and Ang-II in adult rat ventricular myocytes, using a dyad targeted GFP-based Ca^{2+} indicator (GCaMP6f-triadin) generated in the lab of Prof. Cheng. By using confocal linescan imaging of GCaMP fluorescence, we detected increases in fluorescence of the indicator at individual dyads during electrical pacing and in response to caffeine application. ET-1 application induced on average 30% increase in rate of electrically-evoked Ca^{2+} release at the dyads across the cell (max(ΔF0/ms)): 0.24±0.01711 vs 0.2055±0.01243 prior to treatment, p=0.003), whereas Ang-II was without effect. Neither ET-1 nor Ang-II treatment had an effect on spatial synchronicity of Ca^{2+} release and on caffeine induced dyadic Ca^{2+} release.

CONCLUSION |The results obtained above suggest that despite activating similar signaling cascades, Ang-II and ET-1 elicit different effects on the local Ca^{2+} release. These data provide evidence that enhanced rate of dyadic Ca^{2+} release rate contributes to the inotropic effects of ET-1.