BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

—

Spring Meeting

Friday, April 21st 2017

PROGRAMME

—

Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
"Zaal Rubens"
Rue Ducale / Hertogsstraat 1
1000 Brussels

—

Local host

Prof. Dr. Philippe GAILLY
Institute of Neurosciences (IONS)
Laboratory of Cell Physiology
Université Catholique de Louvain
Avenue Mounier 53/B1.53.17
1200 Woluwé St. Lambert

—

with support of the

Royal Flemish Academy of Belgium for Science and the Arts

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Palace of the Academies
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Main Lecture

10.00-10.45 Piezo1 channel in the endothelium: a special mechanical force sensor.

Prof. Dr. David BEECH – Leeds Cardiovasculair Research Center, University of Leeds (United Kingdom)

Oral Communications

10.45-11.00 F. SEGHERS, X. YERNA, O. SCHAKMAN, P. GAILLY (UCLouvain)
Progress in the role of TRPV4 as an osmo- and mechanosensor in systemic osmoregulation and renal functions.

11.00-11.15 A. HENNES, K. HELD, K. DE CLERCQ, T. VOETS, J. VRIENS (KU Leuven)
The calcium permeable mechanosensitive Piezo1 as cellular sensor in endometrial epithelial cells.

11.15-11.30 S. LEPANNETIER, S. TEMPESTA, F. SEGHERS, O. SCHAKMAN, P. GAILLY (UCLouvain)
Role of TRPC1 ion channel in hippocampal neurons.

11.30-11.45 K. HELD, A. JANSSENS, T. VOETS, J. VRIENS (KU Leuven)
Novel regulation mechanism of the nociceptor TRPM3 by redox reagents.

11.45-12.00 K. PHILIPPAERT, P. MACDONALD, P. LIGHT, R. VENNEKENS (KU Leuven, Univ. Alberta Canada)
Enhanced function of human and murine pancreatic beta-cells by stimulating TRPM5.

12.00-12.15 C. BOYDENS, B. PAUWELS, L. VANDEN DAELE, J. VAN DE VOORDE (UGent)
Inhibition of cyclic GMP export by multidrug resistance protein 4: a new strategy to treat erectile dysfunction?

12.15-12.30 A. HANTHAZI, P. JESPERS, G. VEGH, C. DUBOIS, JY. SPRINGAEL, L. DEWACHTER, K. MC ENTEE (ULBruxelles)
Effects of chemerin on rat pulmonary artery smooth muscle cell proliferation, resistance to apoptosis and migration.

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

Posters

(height 120 cm – width 100 cm)

1. C. VAN DEN EYNDE, J. VRIENS (KU Leuven)
   Development and optimization of a Fura-2 based high-throughput screening procedure for the simultaneous identification of agonists and antagonists of the Ca^{2+} permeable cation channel TRPV2.

2. N. EMERIAU, M. DE CLIPPELE, S. PARYS, N. TAJEDDINE (supported by P. GAILLY, UCLouvain)
   Involvement of store-operated calcium entry in cisplatin toxicity.

3. R. GUALDANI, M. M. CAVALLUZZI, F. TADINI-BUONINSEGNI, G. LENTINI (UCLouvain, Univ. Firenze, Univ. Bari, Italy) (supported by P. GAILLY, UCLouvain)
   Molecular insights into hERG potassium channel blockade by Lubeluzole.

4. L. ALONSO-CARBAJO, Y.A. ALPIZAR, J.R. LÓPEZ-LÓPEZ, M.T. PÉREZ-GARCÍA, K. TALAVERA (KU Leuven, Univ. Valladolid, Spain)
   Cellular localization and function of TRPM3 channels in mouse resistance arteries.

5. D. ABBOUD, A. DALY, N. DUPUIS, C. LASCHET, P. GEUBELLE, B. PIROTTE, A. BECKERS, J. HANSON (ULiège)
   GPR101 orphan receptor: a novel cause of growth hormone deregulation.

6. C. LASCHET, N. DUPUIS, P. GEUBELLE, D. ABBOUD, A. SONI, B. PIROTTE, J. HANSON (ULiège)
   Targeted mutagenesis of orphan GPCRs of the SREB family.

7. A. LISSONI, M. DE SMET, N. WANG, L. LEYBAERT (UGent)
   Cx43 hemichannel opening in ventricular cardiomyocytes neccesitates simultaneous ryanodine receptor activation and elevation of intracellular Ca^{2+}, and is modulated by CaMKII and p38MAPK.

8. A. TUTUNARU, J. DUPONT, K. IDA, V. MAROLF, D. SERTAIN, C. SANDERSEN (ULiège)
   Comparison of detomidine-ketamine and midazolam-ketamine for sedation in Yucatan pigs.

9. K. IDA, A. TUTUNARU, V. MAROLF, J. DUPONT, D. SERTAIN, C. SANDERSEN (ULiège):
   Cis-atracurium neuromuscular block and antagonization with neostigmine for phacoemulsification in dogs.

10. I. JADOT, A.E. DECLÈVES, B. MARTIN, O. BOTTON, J. NORTIER, N. CARON (UNamur, UMons, ULBruxelles)
    Imbalance between vasoactive substances during the development of experimental aristocholochic acid nephropathy.

11. B. MARTIN, C. WILKIN, I. JADOT, O. BOTTON, A-E DECLÈVES AND N. CARON (Univ. Namur, Univ. Mons)
    Effect of gender on obesity-associated renal dysfunction and involvement of adipokines.

12. P. GEUBELLE, J. GILISSEN, S. DILLY, L. POMA, N. DUPUIS, C. LASCHET, D. ABBOUD, E. GOFFIN, T. DRAPIER, F. JOURET, B. PIROTTE, J. HANSON (ULiège, Univ. Bordeaux, France)
    Identification and pharmacological characterization of succinate receptor ligands as pharmacological tools.

Oral Communications

14.00-14.15 B. VAN DER VEKEN, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen)
Inhibition of glycolysis reduces intraplaque angiogenesis in a mouse model of advanced atherosclerosis.

14.15-14.30 A. KURDI, B. VAN DER VEKEN, M. DE DONCKER, H. NEELS, G.R.Y. DE MEYER, W. MARTINET (UAntwerpen, ZNA Stuivenberg Antwerpen)
Everolimus attenuates growth of established plaques and improves survival in mice via macrophage autophagy-dependent and independent mechanisms.

14.30-14.45 A. LELOUP, C. VAN HOVE, G. DE MEYER, G. DE KEULENAER, P. FRANSEN (UAntwerp)
The pressure-dependency of aortic stiffness is modulated by vascular smooth muscle tone.

14.45-15.00 Z. VERMEULEN, A-S HERVENT, L. VANDEKERCKHOVE, L. DUGAUCQUIER, V. SEGERS, G. DE KEULENAER (UAntwerpen)
Neuregulin-1 suppresses the inflammatory response during myocardial fibrosis through direct inhibitory effects on macrophages.

15.00-15.15 N. DUPUIS, C. LASCHET, D. FRANSSEN, M. SZPAKOWSKA, J. GILISSEN, P. GEUBELLE, A. SONI, A-S PARENT, B. PIROTTE, A. CHEVIGNÉ, J-C TWIZERE, J. HANSON (ULiège, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg)
Activation of the orphan G protein-coupled receptor GPR27 by surrogate ligands promotes β-arrestin 2 recruitment.

15.15-15.30 V. MAROLF, C. LUYET, C. SPADAVECCHIA, U. EICHENBERGER, U. RYTZ, H. ROHRBACH (supported by C. SANDERSEN, ULiège), Vetsuisse Faculty Bern, Lindenhof Hospital Bern, St. Anne Clinic Lucerne, Suisse)
Use of a perineural coiled catheter at the sciatic nerve in dogs after tibial plateau levelling osteotomy – preliminary observations.

Coffee – Tea

ABSTRACTS

Legend
O = Oral communication
P = Poster

O-01 (10.45-11.00)

PROGRESS IN THE ROLE OF TRPV4 AS AN OSMO- AND MECHANOSENSOR IN SYSTEMIC OSMOREGULATION AND RENAL FUNCTIONS

F. Seghers, X. Yerna, O. Schakman, P. Gailly

Université catholique de Louvain, B-1200, Brussels, Belgium

TRPV4 is a mammalian polymodal cation channel activated by osmotic and mechanical stimuli, by heat, and by endogenous or exogenous lipids. Its osmo- and mechanosensitivity, along with its pattern of expression – including kidney, bladder, blood vessels, lung, nerve endings and central osmosensing nuclei, raise the hypothesis of its involvement in the transduction of osmotic and mechanical stimuli in several physiological processes. In particular, TRPV4 is thought to participate to the control of systemic osmoregulation, a homeostasis system involving central osmosensing nuclei that regulate thirst and vasopressin secretion, and kidney function. We observed an impaired water consumption and vasopressin release in neuron specific TRPV4 KO mice submitted to a salt overload. Besides, in order to study the role of TRPV4 in renal function, we have characterized the localization of TRPV4 along the nephron and compared the urinary phenotype of wild-type and TRPV4 KO mice. We showed that TRPV4 is, at least partially, responsible for the sensitivity of juxtaglomerular cells to blood pressure and appears to regulate renin secretion. In the proximal and distal tubules, TRPV4 is essentially localized at the basolateral and the apical membranes respectively. There, it seems to respond respectively to osmotic and stretch stimuli, or to shear stress. Its involvement in tubular physiological functions is under study.

O-02 (11.00-11.15)

THE CALCIUM PERMEABLE MECHANOSENSITIVE PIEZO1 AS CELLULAR SENSOR IN ENDOMETRIAL EPITHELIAL CELLS.

A. Hennes[1], K. Held[1,2], K. De Clercq[1], T. Voets[2], and J. Vriens[1]

[1] Laboratory of Experimental Gynecology and Obstetrics, KU Leuven, Herestraat 49 box 611, Leuven, B-3000, Belgium, [2] Laboratory of Ion Channel Research, KU Leuven, Herestraat 49 box 611, Leuven, B-3000, Belgium

Embryo implantation is one of the most crucial and important steps during reproduction, and is characterized by several defining interactions between the blastocyst and the receptive endometrium. Nevertheless, it still remains unclear how signals from the blastocyst can be detected by the luminal endometrial epithelial cells and transmitted towards the stromal bed for optimal decidualization and implantation. Recently, the mechanosensitive epithelial sodium channel ENaC was proposed as an important signal mediator in the process of embryo implantation. Activation of ENaC via serine proteases such as trypsin results in membrane depolarization and calcium influx via voltage-dependent calcium channels (VDCC), ultimately leading to the signalling cascade necessary for stromal decidualization. As we aim at characterizing the role of ion channels as signal tranducers during the implantation process in endometrial epithelial cells, primary endometrial epithelial cells from mouse and humans were isolated and assessed for their functional ion channel expression pattern. Since decidualization is highly dependent on calcium influxes, we aimed at both confirming the hypothesis of ENaC as signal mediator, as previously described in literature, and at investigating other mechanosensitive, calcium permeable ion channels in primary cells. Our functional analyses showed no evidence for the presence of VDCC in our cells. Moreover, trypsin induced transient calcium influxes, independent of ENaC channel activation, via the PLC pathway and intracellular store depletion. Subsequently, other calcium permeable mechanosensitive ion channels were evaluated. Upon application of the Piezo1 activator Yoda1 or a mechanical stimulus, high calcium influxes were induced, indicating the functional presence of Piezo1 in endometrial epithelial cells and a possible role for piezo1 as key player in the signalling pathway during decidualization and implantation.

O-03 (11.15-11.30)

ROLE OF TRPC1 ION CHANNEL IN HIPPOCAMPAL NEURONS

S. Lepannetier, S. Tempesta, F. Seghers, O. Schakman, P. Gailly

Université catholique de Louvain, B-1200, Brussels, Belgium

Transient receptor potential-canonical 1 (TRPC1) is a non selective cation channel (PCa/PNa ~ 1). It is involved in store-operated Ca^{2+} entry in cooperation with Orai1 channel and activated by STIM1, a sensor of Ca^{2+} contents in the endoplasmic reticulum. However, several pieces of evidence suggest that TRPC1 can be activated independently of store depletion in response to agonists. Using a LacZ reporter mouse, we found that TRPC1 is abundantly expressed in neurons from the CA1-CA3 regions and to a lesser extent from the dentate gyrus region of the hippocampus. We compared cellular culture of hippocampal neurons from *Trpc1*$^{-/-}$ mice to wild type mice. Interestingly, stimulation with dihydroxyphenylglycine (DHPG), an agonist of group I metabotropic glutamate receptors (consisting of mGluR1 and mGluR5) induced a store-independent entry of Ca^{2+} that was very much reduced in hippocampal neurons from *Trpc*1$^{-/-}$ mice. Extracellular electrophysiological recordings showed that LTP in Schaffer collaterals-CA1 synapses was diminished in *Trpc1*$^{-/-}$ mice when induced with a mild stimulus (theta burst consisting of 4 stimuli at 100 Hz repeated at 5 Hz during 1s). Cognition tests such as Y-shape modified maze and contextual fear-conditioning, realized on 4-8 months old mice revealed a deficit of memory. Moreover, we noted that the specific hippocampal over-expression of the immediate early gene zif268 related to new environment learning was blunted in *Trpc1*$^{-/-}$ mice. We conclude that TRPC1 channels are activated by mGluR stimulation and play a role in neuronal plasticity.

O-04 (11.30-11.45)

NOVEL REGULATION MECHANISM OF THE NOCICEPTOR TRPM3 BY REDOX REAGENTS

K. Held[1,2], A. Janssens[2], T. Voets[2] and J. Vriens[1]

[1]Laboratory of Experimental Gynaecology, KU Leuven, 3000 Leuven, Belgium,
[2]Laboratory of Ion Channel Research, KU Leuven, 3000 Leuven, Belgium.

Oxidative stress describes the occurrence of reactive oxygen species (ROS) that cannot be balanced by antioxidant defenses of the body. Oxidative stress can occur in every cell of the body and is linked to an increasing number of diseases. For instance, ROS were indicated to play an important role in chronic pain conditions. Interestingly, recent reports showed that ROS-induced cysteine modifications can modulate members of the transient receptor potential (TRP) channel family. TRPM3, is a member of the TRP superfamily that is highly expressed in sensory neurons where it is involved in the detection of painful stimuli and the development of heat hyperalgesia. TRPM3 can be activated by multiple stimuli including chemicals such as pregnenolone sulfate (PS) as well as physical signals such as heat and hypo-osmolarity. Interestingly, we recently discovered that redox reagents can modulate the activity of TRPM3. Patch clamp experiments and Ca^{2+}-imaging showed that application of Dithiothreitol (DTT, reducing agent) on heterologously expressed TRPM3 resulted in reduced sensitivity to PS as well as an increased sensitivity to the antifungal drug clotrimazole (Clt). Excitingly, this effect was reversible upon treatment with the oxidizing agent H_2O_2. Furthermore, data obtained in Ca^{2+}-imaging show similar pharmacological changes occurring in endogenously expressed TRPM3 in sensory neurons of the dorsal root ganglia (DRG) and trigeminal ganglia (TG). Finally, we were able to identify an extracellular cysteine, located in the putative pore-loop of TRPM3 (C1067), that critically contributes to the mechanism of redox sensing. Mutation of C1067 to an alanine resulted in a channel phenotype that resembles wild type TRPM3 following DTT treatment, showing insensitivity to PS and activation by Clt. Our findings indicate for the first time that TRPM3 can be modulated by redox reagents due to cysteine modifications and proposes an important role of TRPM3 in pain conditions that lead to ROS production.

O-05 (11.45-12.00)

ENHANCED FUNCTION OF HUMAN AND MURINE PANCREATIC BETA-CELLS BY STIMULATING TRPM5

K. Philippaert[1], P. MacDonald[2], P. Light[2], R. Vennekens[1]

[1]Laboratory of Ion Channel Research, KU Leuven, Belgium [2]Alberta Diabetes Institute, University of Alberta, Edmonton, Canada

Diabetes occurs when the pancreatic β-cells in the body secrete insufficient insulin to maintain normal blood glucose levels. Patients developing type II diabetes often have a predisposed genetic risk that surfaces due to an environmental trigger such as obesity. A lot of the mutations that show an increased risk in the development of diabetes result in a reduced excitability and secretory potential of the β-cells. TRP channels are depolarizing cation channels, and several members of the TRP family, including TRPM5, are functionally expressed in the β-cells. It was recently shown that the potentiation of the TRPM5 ion channel with stevioside in murine islets leads to increased β-cell activity. The administration of stevioside to mice could prevent the onset of high-fat diet induced diabetes. Due to differences in the architecture and function between murine and human islets, the findings in mice are only indicative for their relevance in humans. We used human islets isolated from donor pancreas for research purposes. We show human islets express several TRP channels, including TRPM5. In functional measurements, following the intracellular calcium in human islets, we could show that the potentiation of TRPM5 with stevioside leads to increased activity of the human β-cells. This indicates that the TRPM5 potentiation that prevents the onset for type II diabetes in mouse models is translatable to human tissue. This makes the targeting of TRPM5 a potential new way for the development of anti-diabetic therapies in patients.

O-06 (12.00-12.15)

INHIBITION OF CYCLIC GMP EXPORT BY MULTIDRUG RESISTANCE PROTEIN 4: A NEW STRATEGY TO TREAT ERECTILE DYSFUNCTION?

C. Boydens[1], B. Pauwels[1], L. Vanden Daele[1], J. Van de Voorde[1]

[1]Department of Pharmacology, Ghent University, Ghent, 9000, Belgium

Elevation of cyclic guanosine monophosphate (cGMP) levels is a key event for normal penile erection. Intracellular cGMP concentrations are regulated by degradation enzymes (phosphodiesterases, PDEs) as well as by active transport across the plasma membrane by multidrug resistance protein (MRP) 4 and 5. This study evaluated the functional effect of MRP 4 inhibition and the role of MRP 4-mediated cGMP export in mouse corpora cavernosa. Isometric tension of mouse corpora cavernosa was measured after cumulative addition of MK-571, an inhibitor of MRP 4, or sildenafil, a PDE-5 inhibitor. In addition the effect of MRP 4 inhibition on cGMP-(in)dependent relaxations was studied. In vivo intracavernosal pressure (ICP) and mean arterial pressure (MAP) measurements were performed after intracavernosal injection of MK-571. MK-571 and sildenafil both relaxed the corpora cavernosa concentration dependently with sildenafil being the most potent relaxing compound. Furthermore, MK-571 enhanced relaxing responses to cGMP-dependent substances even under in vitro diabetic conditions. In contrast cGMP-independent relaxations were not altered by MRP 4 inhibition. Intracavernosal administration of MK-571 significantly increased ICP, with minimal effect on MAP. cGMP analysis revealed that MRP 4 inhibition was accompanied with increased cGMP levels. This study demonstrates that inhibition of MRP 4 increases basal and stimulated levels of cGMP leading to corpora cavernosa relaxation and penile erection. Therefore it is suggested that, in addition to degradation of cGMP, export of cGMP by MRP 4 substantially contributes to regulating the cGMP levels in mouse corpora cavernosa. As a consequence, MRP 4 might be a valuable alternative target for the treatment of (diabetic) erectile dysfunction.

O-07 (12.15-12.30)

EFFECTS OF CHEMERIN ON RAT PULMONARY ARTERY SMOOTH MUSCLE CELL PROLIFERATION, RESISTANCE TO APOPTOSIS AND MIGRATION

A. Hanthazi[1], P. Jespers[1], G. Vegh[1], C. Dubois[2], JY. Springael[2], I. Dewachter[1], K. Mc Entee[1]

[1]Laboratoire de Physiologie et Pharmacologie, [2]IRIBHM, Université libre de Bruxelles (ULB), Brussels 1070, Belgium

Deleterious vascular remodeling observed in pulmonary arterial hypertension (PAH) includes proliferation, resistance to apoptosis and migration of pulmonary artery smooth muscle cells (PASMCs) leading to progressive pulmonary artery obliteration. Adipokines such as leptin and adiponectin have been respectively described as deleterious or protective in PAH. Recently, another adipokine chemerin has been shown to induce the proliferation of myoblasts. In the present study, we therefore hypothesized that chemerin alone or added to endothelin-1 (ET-1), a major mediator of PAH, might be involved in pulmonary vascular remodeling**.** Primary cultures of rat pulmonary and aortic SMCs were performed by the explant technique. Proliferation was tested by bromo-deoxyuridine incorporation and migration by the Transwell migration assay, both after 24-hour incubation with increasing concentrations of chemerin (from 5.10^{-9} to 10^{-7}M) with or without ET-1 (10^{-7}M). Apoptosis was induced by staurosporine and quantified by detection of Annexin V/propidium iodide-positive cells using flow cytometry after 24-hour incubation with increasing concentrations of chemerin. In cultured PASMCs, chemerin (from 10^{-8}M) added to ET-1 induced cell proliferation, while chemerin or ET-1 alone did not. No proliferative effect of chemerin, ET-1 or chemerin + ET-1 was observed in aortic SMCs. Chemerin alone (from 5.10^{-9}M) increased pulmonary artery and aortic SMC migration, while a potentiating effect was observed with ET-1. Chemerin induced a resistance to apoptosis in PASMCs, but not in aortic SMCs. This study shows that in primary cultures of rat PASMCs, chemerin alone or in association with ET-1 induces proliferation and migration and leads to resistance to apoptosis of these cells. The proliferative and apoptosis response is specific to the pulmonary circulation.

O-08 (14.00-14.15)

INHIBITION OF GLYCOLYSIS REDUCES INTRAPLAQUE ANGIOGENESIS IN A MOUSE MODEL OF ADVANCED ATHEROSCLEROSIS

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

Laboratory of Physiopharmacology, University of Antwerp

Intraplaque (IP) neovascularization is a critical factor in atherosclerotic plaque rupture. Recent studies in cancer research have demonstrated that proliferating endothelial cells generate up to 85% of their ATP from glycolysis. Because 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) plays a critical role in glycolysis, we investigated whether PFKFB3 inhibition affects IP neovascularization and plaque stability in $ApoE^{-/-}$ mice containing a heterozygous mutation in the fibrillin-1 gene ($Fbn1^{C1039G+/-}$), a model of advanced atherosclerosis with IP neovascularization. $ApoE^{-/-}$ $Fbn1^{C1039G+/-}$ mice were fed a western diet (WD). PFKFB3 inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO, 50 µg/g, i.p.) or solvent was administered starting either after 4 weeks WD (2x/week, 10 weeks, preventive regimen) or after 16 weeks WD (4x/week, 4 weeks, curative regimen). 3PO reduced IP neovascularization and haemorrhages by 50% (preventive regimen) and 38% (curative regimen). This compound had no effect on smooth muscle cell content in the plaques (preventive: 7±1% vs. 7±1% in control; curative: 9±1% vs. 8±1% in control). Similarly, the collagen deposition in the plaques was not affected (preventive: 16±4% vs. 16±1% in control; curative: 25±1% vs. 26±2% in control). However, 3PO tended to decrease the macrophage content (preventive: 8±2% vs. 14±4%; curative: 5±1% vs. 7±1%). Plasma VEGF-A levels decreased significantly (curative: 838±449 vs. 2871±653 pg/ml, p=0.027) and cardiac function improved after 10 weeks of treatment (fractional shortening 31±4% vs. 23±3%, p=0.013; LVIDd 4.6±0.2mm vs. 4.0±1mm, p=0.032, LVIDs 3.7±0.1mm vs. 2.8±0.3mm, p=0.009). Inhibition of PFKFB3 by 3PO significantly represses IP angiogenesis and haemorrhages in mice, demonstrating its potential in preventing plaque rupture.

O-09 (14.15-14.30)

EVEROLIMUS ATTENUATES GROWTH OF ESTABLISHED PLAQUES AND IMPROVES SURVIVAL IN MICE VIA MACROPHAGE AUTOPHAGY-DEPENDENT AND INDEPENDENT MECHANISMS

A. Kurdi,[1] B. Van Der Veken,[1] M. De Doncker,[2] H. Neels,[2] G.R.Y. De Meyer,[1] W. Martinet[1]

[1]Laboratory of Physiopharmacology, Department of Pharmaceutical Sciences, University of Antwerp, Antwerp, Belgium; [2]Laboratory for TDM and Toxicology, ZNA Stuivenberg, Antwerp, Belgium

Inhibition of the mechanistic target of rapamycin (mTOR) is a promising approach to halt atherogenesis in different animal models. This study aims to evaluate whether the mTOR inhibitor everolimus stabilizes atherosclerosis, prevents myocardial infarctions and improves survival in a mouse model of advanced atherosclerosis. Furthermore, the role of macrophage autophagy in plaque stabilization was studied. $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice (n=24) were fed a Western diet (WD) for 12 weeks. Subsequently, mice were treated with everolimus (1.5 $mg \cdot kg^{-1}$ daily) or vehicle for another 12 weeks while WD continued. Despite hypercholesterolemia, everolimus reduced lesion size in the proximal ascending aorta as compared to vehicle-treated controls ($495x10^3$ vs. $786x10^3$ µm, p=0.014). This effect was associated with a reduction of circulating $Ly6C^{high}$ monocytes (15 vs. 28% of total leukocytes, p=0.046), a depletion of plaque macrophages (1.9 vs. 3.7%, p=0.035) and lack of intraplaque neovascularization. Moreover, everolimus reduced hypoxic brain damage and improved cardiac function by reducing coronary plaque formation and myocardial infarction, which led to improved survival (100 vs. 69% of animals, p=0.038). $ApoE^{-/-}$ mice with autophagy-deficient macrophages showed that impaired plaque progression by everolimus was not dependent on macrophage autophagy. However, it was essential for reducing apoptosis, lipid content and macrophage burden in the plaque. Everolimus counters atherosclerosis and its complications in $ApoE^{-/-}Fbn1^{C1039G+/-}$ mice, even when administered at a later stage of the disease. Everolimus-induced macrophage autophagy is partially responsible for plaque stabilization but not for inhibition of plaque growth.

O-10 (14.30-14.45)

THE PRESSURE-DEPENDENCY OF AORTIC STIFFNESS IS MODULATED BY VASCULAR SMOOTH MUSCLE TONE

A. Leloup[1], C. Van Hove[2], G. De Meyer[1], G. De Keulenaer[1], P. Fransen[1]

[1]University of Antwerp, Department of Pharmaceutical Sciences, Laboratory of Physiopharmacology Universiteitsplein 1, B-2610 Antwerp, Belgium, [2]University of Antwerp, Faculty of Medicine and Health Sciences, Laboratory of Pharmacology, Campus Drie Eiken, Universiteitsplein 1, B-2610 Antwerp, Belgium

Stiffening of the large arteries is a hallmark of the aging process and the root cause of increased cardiovascular morbidity and mortality. The mechanisms of arterial stiffening are complex and incompletely understood but it is generally assumed that not only passive, but also endothelial and vascular smooth muscle cell (VSMC) components are important. We used the Rodent Oscillatory Tension Set-up to study Arterial Compliance (ROTSAC) to investigate the role of VSMCs in the mouse aorta. This set-up is an in-house developed organ bath that allows the acquisition of biomechanical parameters at physiological pressure and frequencies. At normal frequency (10 Hz) and pressure (80-120 mmHg), the Peterson modulus (Ep, a diameter-independent measure of aortic stiffness) was 293±4 mmHg (n=5). Upon α1-adrenergic stimulation with 1 µM phenylephrine (PE), Ep increased by 30% to 381±33 mmHg. However, when in PE-stimulated segments NO production was blocked with 300 µM L-NAME, Ep increased further by 80% to 527±9 mmHg, confirming the important role of basal NO production in maintaining low VSMC tone. At very high pressure (180-220 mmHg), Ep was increased 6-7-fold to 1923±148 mmHg. Interestingly, when VSMC tone was increased with PE, Ep decreased to 1060±52 mmHg. Even more interesting, Ep did not change significantly upon addition of L-NAME (1011±33 mmHg). These observations confirm that the NO pathway in the mouse aorta is – at least *in vitro* – a major determinant of its bio-mechanical properties. The effect of VSMC tone on Ep at high pressure is completely opposite from the effect at low to normal pressure. This suggests a physiological role for aortic VSMC tone in maintaining optimal hemodynamics, instead of merely a pathological phenomenon typically associated with vascular aging.

O-11 (14.45-15.00)

NEUREGULIN-1 SUPPRESSES THE INFLAMMATORY RESPONSE DURING MYOCARDIAL FIBROSIS THROUGH DIRECT INHIBITORY EFFECTS ON MACROPHAGES

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The neuregulin-1 (NRG-1)/ErbB system is an endothelium-controlled paracrine system modulating cardiac performance, adaptation and regeneration. The function of this system is generally explained by the activation of ErbB4/ErbB2 signaling in cardiomyocytes. However, recent reports indicate that fibroblasts are equally responsive to NRG-1 and that NRG-1 treatment has anti-fibrotic effects in the left ventricle (LV). Still, NRG-1's underlying mechanisms of cardiac protection remains poorly understood. In this study, we further explore the role of NRG-1/ErbB signaling during myocardial fibrosis. The underlying mechanism of NRG-1 was studied in cultured primary fibroblasts using cDNA micro-array analysis with the Illumina MouseRef8 v2.0 Expression BeadChip. Myocardial inflammation and fibrosis were induced by treatment of 8-week-old male mice (n=10 per group) with angiotensin II (ATII) for respectively 7 days and 4 weeks using micro-osmotic pumps (1000 ng.kg-1.day-1). NRG-1/ErbB4 signaling was either activated by co-treatment with recombinant human (rh)NRG-1 (β-isoform, 20 µg.kg-1.day-1, daily i.p. injections) or inhibited in inflammatory cells by myeloid-specific deletion of ErbB4 (by crossing LysM-Cre+/+ mice and ErbB4F/F mice). Anti-fibrotic effects of NRG-1 were confirmed in a mouse model of ATII-induced myocardial fibrosis. Micro-array analysis on NRG-1-treated fibroblasts elucidated that NRG-1, apart from suppressing pro-fibrotic genes, also down-regulates important inflammatory pathways. In vivo experiments in a mouse model of ATII-induced inflammation demonstrated that NRG-1 attenuates macrophage density and cytokine expression in the myocardium, which may indirectly attenuate LV fibrosis and remodeling. Subsequently, we investigated the direct role of NRG-1/ErbB signaling on macrophages and discovered that NRG-1 activated the ErbB4 receptor in cultured macrophages, inhibited important signaling pathways and decreased the synthesis of pro-inflammatory cytokines. In addition, deleting the ErbB4 receptor in macrophages using a myeloid-specific ErbB4 knock-out (ErbB4F/FLysM-Cre+/-), resulted into an intensified fibrotic response to ATII in the myocardium. Interestingly, these observations were reproducible in skin and lung, extending the anti-inflammatory and anti-fibrotic effects of the NRG-1/ErbB system beyond the heart and opening a new therapeutic window for NRG-1 as a drug therapy. This study unmasked the anti-inflammatory effects of NRG-1 during myocardial fibrosis through ErbB4-mediated inhibitory effects on macrophages. These observations also endorse a pluricellular action of NRG-1 during cardiac protection.

O-12 (15.00-15.15)

ACTIVATION OF THE ORPHAN G PROTEIN-COUPLED RECEPTOR GPR27 BY SURROGATE LIGANDS PROMOTES β-ARRESTIN 2 RECRUITMENT

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G protein-coupled receptors are the most important drug targets for human diseases. An important number of them remain devoid of confirmed ligands. GPR27 is one of these orphan receptors and has recently been linked to insulin secretion. However, the absence of endogenous or surrogate ligands for GPR27 complicates the examination of its biological function. Our aim was to to identify GPR27-specific surrogate agonists. In order to select an optimal screening assay, we investigated GPR27 ligand-independent activity. Both in G protein-mediated pathways and in β-arrestin 2 recruitment, no ligand-independent activity could be measured. We observed a recruitment of β-arrestin 2 to a GPR27V_2 chimera in the presence of membrane-anchored β-adrenergic receptor kinase 1 (GRK2). Therefore, we optimized a firefly luciferase complementation assay to screen against this chimeric receptor. We identified two compounds sharing a *N*-phenyl-2,4-dichlorobenzamide scaffold, which were selective for GPR27 over its closely related family members GPR85 and GPR173. The specificity of the activity was confirmed with a NanoBiT® β-arrestin 2 assay, imaging of GFP-tagged β-arrestin 2 and PathHunter® β-arrestin 2 Assay. Interestingly, no G protein activation was detected upon activation of GPR27 by these compounds. Our study provides the first selective surrogate agonists for the orphan GPR27.

O-13 (15.15-15.30)

USE OF A PERINEURAL COILED CATHETER AT THE SCIATIC NERVE IN DOGS AFTER TIBIAL PLATEAU LEVELLING OSTEOTOMY – PRELIMINARY OBSERVATIONS

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The analgesic effects of peripheral nerve blocks can be prolonged with the placement of perineural catheters allowing repeated injections of local anaesthetics in humans. The objectives of this study were to evaluate the clinical suitability of a perineural coiled catheter (PCC) at the sciatic nerve and to evaluate pain during the early post-operative period in dogs after tibial plateau levelling osteotomy. Pre-operatively, a combined block of the sciatic and the femoral nerves was performed under sonographic guidance (ropivacaine 0.5%; 0.3 mL/kg per nerve). Thereafter, a PCC was placed near the sciatic nerve. Carprofen (4 mg/kg intravenously) was administered at the end of anaesthesia. After surgery, all dogs were randomly assigned to receive four injections of ropivacaine (group R; 0.25%, 0.3 mL/kg) or NaCl 0.9% (group C; 0.3 mL kg) every 6 h through the PCC. Pain was assessed by use of a visual analogue scale (VAS) and a multi-dimensional pain score (4Avet) before surgery (T-1), for 390 min (T0, T30, T60, T120, T180, T240, T300, T360 and T390) as well as 1 day after surgery (Day 1). Methadone (0.1 mg/kg) was administered each time the VAS was ≥40 mm or the 4Avet was ≥5. At T390 dogs received buprenorphine (0.02 mg/kg). Data were compared using Mann–Whitney rank sum tests and repeated measures analysis of variance. Regardless of group allocation, 55% of dogs required methadone. VAS was significantly lower at T390 ($P = 0.003$), and at Day 1 ($P = 0.002$) and so was 4Avet at Day 1 ($P = 0.012$) in group R than in group C. Bleeding occurred in one dog at PCC placement and PCC dislodged six times of 47 PCCs placed. Minor complications occurred with PCC but allowed four repeated administrations of ropivacaine or saline over 24 h in 91.5% of the cases.

P1

DEVELOPMENT AND OPTIMIZATION OF A FURA-2 BASED HIGH-THROUGHPUT SCREENING PROCEDURE FOR THE SIMULTANEOUS IDENTIFICATION OF AGONISTS AND ANTAGONISTS OF THE Ca^{2+} PERMEABLE CATION CHANNEL TRPV2

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TRPV2 is a calcium permeable non-selective cation channel with a very broad expression pattern throughout the body. TRPV2 has been proposed to be involved in a number of physiological processes like axonal outgrowth, maintenance of cardiac structure and insulin secretion. Its function, however, remains controversial since mice lacking TRPV2 do not show a strong defect, and pharmacological effectors necessary to study this channel are rather non-specific. Currently, 2-Aminoethoxydiphenyl borate (2-APB), probenecid and cannabinoids like cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (Δ^9-THC) are being used as TRPV2 activators, and ruthenium red (RR) is used as an antagonist. These compounds are known to activate or inhibit other TRP channels, which can lead to complications in interpreting experiments in physiological systems coexpressing several TRP channels. Therefore, the development of a screening method to identify TRPV2 modulators in a high-throughput screening fashion should open new scientific and therapeutic perspectives for a channel with a potential implication in various physiological processes. In this study, we aim to develop a Fura-2 based high-throughput calcium mobilization assay that enables simultaneous identification of agonists and antagonists for TRPV2. We have identified Tetrahydrocannabivarin (THCV) as an adequate positive control to identify antagonists in this assay, rather than 2-APB. Our first preliminary results demonstrate that the dual agonist/antagonist assay format with THCV as positive control is indeed feasible. Screening compound libraries with this format could be useful in the identification of tool compounds to probe the physiological role of endogenous TRPV2.

P2

INVOLVEMENT OF STORE-OPERATED CALCIUM ENTRY IN CISPLATIN TOXICITY

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Cisplatin (CDDP) is one of the principal chemotherapeutic agents used for the first-line treatment of non-small cell lung cancer (NSCLC), with deceptive results. After an initial success leading to partial therapeutic responses, chemotherapy-resistant tumour cells are selected, imposing successive changes in the chemotherapeutic regimen. CDDP induces mitochondrial membranes permeabilization (MMP) and release of mitochondrial intermembrane proapoptotic factors in NSCLC cells. Mitochondrial Ca^{2+} overload can trigger these events. The entry of Ca^{2+} consecutive to ER depletion, a phenomenon called store-operated calcium entry (SOCE), might be also involved in this process. Indeed, it has been shown that SOCE activation induced long lasting increase in mitochondrial Ca^{2+} concentration. Moreover, mitochondrial uptake buffers $[Ca^{2+}]_i$ increase consecutive to SOCE activation, thereby maintaining a low Ca^{2+} concentration at the subplasmalemmal domains, that in turn sustains SOCE by preventing Ca^{2+}-dependent inactivation of the Ca^{2+} entry channels. All these data prompted us to explore the role of SOCE in CDDP-induced cell death. In this study, we showed that depletion of STIM1 or TRPC1, two mediators of SOCE in NSCLC cells, dramatically inhibited CDDP-induced MMP and plasma membrane permeabilization in A549 cells. Moreover, caspase-3 activation and PARP cleavage induced by CDDP treatment were also reduced in STIM1- or TRPC1-depleted cells. In contrast, genetic inhibition of SOCE was unable to inhibit DNA damage and p53 accumulation induced by CDDP. In many models, autophagy is described as an inhibitor of cell death and several clinical trials test the opportunity to combine chemotherapy with pharmacological inhibitor of autophagy. We then investigated whether SOCE inhibition activated autophagy but neither STIM1 siRNA nor TRPC1 siRNA had an effect on the autophagic flux in NSCLC cells. As ER stress may be triggered by ER Ca^{2+} emptying and may trigger unfolded protein response (UPR) that can protect cells against cytotoxic insults, we measured the activation of markers of ER stress after SOCE inhibition. We observed that both STIM1 and TRPC1 siRNAs were able to trigger the ER stress. This effect was exacerbated in the presence of CDDP. We then suggest that SOCE inhibition, by altering ER Ca^{2+} homeostasis, triggers ER stress that in turn protects NSCLC cells against the cytotoxic effect of CDDP.

P3

MOLECULAR INSIGHTS INTO hERG POTASSIUM CHANNEL BLOCKADE BY LUBELUZOLE

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Drug-induced block of 'Human ether-a-go-go-related Gene' (hERG) K^+ channels is the main reason of long QT syndrome, a disorder of cardiac repolarization which may lead to sudden death due to ventricular fibrillation. Lubeluzole, a neuroprotective compound, has been associated with the acquired long QT syndrome and ventricular arrhythmias; however its effects on the hERG K^+ channel have not been described to date. Therefore, we studied in detail the interaction of lubeluzole (racemic mixture and single isomers) and its moieties on hERG channel heterologously expressed in mammalian HEK cells. We found that lubeluzole and its enantiomer are highly potent inhibitors of hERG current with an IC_{50} value of 12.9 ± 0.7 nM (no stereoselectivity observed). Block occurred during activation of the channels and was not observed for the closed state of the channel. Steady-state activation and inactivation were shifted to more negative potentials and inactivation time was accelerated. Mutations in the binding site reported for other hERG channel blockers (Y652A and F656A) reduced the potency of lubeluzole. To go further in details, we synthesized a series of lubeluzole congeners designed to explore the role of lipophilicity and electronic distribution. For each compound we determined the relative hERG affinity, to evaluate how specific modification of drug structure affected hERG binding interaction. Main structure-activity relationship results will be presented.

P4

CELLULAR LOCALIZATION AND FUNCTION OF TRPM3 CHANNELS IN MOUSE RESISTANCE ARTERIES

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The vascular tone is determined by a complex interplay of vasodilator and vasoconstrictor stimuli that modulate the contractile state of vascular smooth muscle cells (VSMCs). Activation of the cation channel Transient Receptor Potential Melastatin 3 (TRPM3) has been shown to induce contraction of VSMCs in aorta. However, the contribution of this channel to the vascular tone in resistance arteries remains unknown. Real-time qPCR and immunochemistry experiments showed *Trpm3* expression in mesenteric arteries isolated from wild type (WT) C57BL/6J mice. Myography experiments carried out in intact pressurized mesenteric arteries from WT mice showed that the TRPM3 agonist PS induces vasodilation at concentrations above ~5 µM, with a concentration-dependency featuring two distinct increasing phases. In contrast, PS only induced vasodilation above 10 µM following a single Hill-type behavior in preparations from *Trpm3* knockout (KO) mice. Recordings in WT arteries in the presence of the CGRP receptor antagonist BIBN 4096 recapitulated the results of *Trpm3* KO preparations, indicating that the TRPM3-mediated effect of PS entails CGRP release from perivascular nerve endings. The effect of 10 µM PS was inhibited to about 50% by the combination of potassium channel blockers (500 nM paxilline, 10 µM correolide and 50 nM stromatoxin). Electrophysiological recordings in freshly isolated mesenteric VSMCs, revealed that basal currents are not sensitive to PS (10 µM and 30 µM) and that PS has no effect on potassium currents. Our data indicates that activation of TRPM3 channels in perivascular sensory nerves induces CGRP release, which leads to activation of potassium channels in smooth muscle cells, resulting in dilation of mesenteric arteries. These findings reveal a potential role of TRPM3 in vascular tone regulation, and support the recent notion that this channel may play roles in neurogenic inflammation.

P5

GPR101 ORPHAN RECEPTOR: A NOVEL CAUSE OF GROWTH HORMONE DEREGULATION

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GPR101 is an orphan (without known ligand) G-protein coupled receptor. Recently, a clinical study showed that GPR101 is strongly associated X-linked acrogigantism syndrome (XLAG), which is a genetic rare disorder caused by Xq26.3 microduplications and characterized by an abnormal growth hormone (GH) hypersecretion. Considering the GPR101 involvement in X-LAG on one hand, and the lack of pharmacological tools to investigate its function and/or to correct its defects on the other hand, we propose a research program to study this receptor functions and its role in GH regulation. GPR101 is characterized by a very high level of constitutive activity. Therefore, we analysed GPR101 constitutively activated signalling pathways. We confirmed an increase of cAMP levels and observed a strong association with arrestin pathway. We completed our study with an examination of receptor coupling to other pathways and G proteins. With immunohistochemistry and FACS analysis, we determined the receptor precise cellular localization and constitutive trafficking. Furthermore, we applied targeted mutagenesis to modulate the receptor constitutive activity in order to understand the receptor function at a molecular level. These GPR101 mutants will help us to understand the role of this receptor in GH regulation and/or to treat people suffering from pituitary dysfunction. This information is an absolute prerequisite to link molecular pharmacology of GPR101 with physiological functions.

P6

TARGETED MUTAGENESIS OF ORPHAN GPCRS OF THE SREB FAMILY

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The super-conserved receptor expressed in the brain (SREB) subfamily is composed of three receptors, GPR27 (SREB1), GPR85 (SREB2) and GPR173 (SREB3). SREB is the most evolutionarily conserved GPCR subfamily, which have the double particularity to be mainly expressed in the central nervous system and to be highly conserved throughout vertebrate evolution. For instance, GPR85 share 100% amino acid sequence homology between human and rat orthologues. This high level of conservation indicates that the functions of the SREB family are essential in vertebrates. Very few ligands have been proposed for SREB and they are still considered orphan. Some studies have suggested a connection between GPR27 and insulin production, whereas GPR85 is thought to be involved in determining brain size, modulating diverse behaviors, and potentially in vulnerability to schizophrenia. GPR173 seems to modulate migration and maturation of GnRH neurons during development. Our research project proposes to investigate the molecular pharmacology of SREB by characterizing precisely SREB signaling pathways in a ligand independent manner. We investigated their functions by using a well-established receptor property: constitutive activity. This characteristic is commonly used to study receptor function. However the intensity of the basal activation varies between receptor, ranging from almost inactive to full activation. Therefore, we generated mutants of the receptors to identify region involved in basal activity (constitutively active mutants). Several constitutively active mutants of class A GPCR have been described in the literature. Key amino acids for SREB activation have been hypothesized from known activating mutations and the corresponding residues in SREB have been mutated by targeted mutagenesis.

P7

CX43 HEMICHANNEL OPENING IN VENTRICULAR CARDIOMYOCYTES NECESSITATES SIMULTANEOUS RYANODINE RECEPTOR ACTIVATION AN ELEVATION OF INTRACELLULAR CA^{2+}, AND IS MODULATED BY CAMKII AND P38MAPK

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Connexins typically assemble to form gap Junctions that promote the electro-chemical coupling between cells. Nevertheless, connexins also exist in the form of unapposed hemichannels that function as non-selective and large conductance pores in the membrane. Connexin hemichannels are closed under resting condition, but open in response to increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), strong depolarization and mechanical stress. Connexin 43 (Cx43) is the most prevalent connexin in cardiomyocytes and we hypothesize that the opening of only a few channels per cardiomyocyte are sufficient to alter the electrical excitability and provoke significant Ca^{2+} entry. We here aimed to investigate the mechanisms that lead to $[Ca^{2+}]_i$ -dependent Cx43 hemichannel opening in cardiomyocytes. Mouse ventricular cardiomyocytes were studied under whole-cell voltage-clamp at the resting potential of -70mV with simultaneous $[Ca^{2+}]_i$ recording using Fluo-4 as indicator. We recorded spiking unitary current in response to activation of ryanodine receptor (RyR) with two different agonists, caffeine and 4-CmC that are known to trigger the release of Ca^{2+} from the sarcoplasmic reticulum. These agonists triggered spiking (short lived) unitary current activity with a single channel conductance of ~200pS, which is typical for Cx43 hemichannels. In line with this, Cx43 hemichannel activity was suppressed in cardiomyocytes isolated from conditional Cx43 knock down animals. To our surprise, elevating $[Ca^{2+}]_i$ by applying buffered solutions with increased $[Ca^{2+}]$ via the patch pipette did not elicit any hemichannel response. However, applying solutions with increased $[Ca^{2+}]_i$ significantly promoted Cx43 hemichannel activity triggered in response to RyR agonists, indicating that both RyR activation and $[Ca^{2+}]_i$ elevation are necessary to trigger hemichannel opening. Most notably, Cx43 hemichannel colocalized with RyR at the level of intercalated discs. Interestingly, RyR/$[Ca^{2+}]_i$ -triggered hemichannel activity was significantly reduced by inhibiting both CaM-dependent kinase II (CaMKII) and p38MAPK with AIP and SB202190 respectively. We conclude that $[Ca^{2+}]_i$ does not directly trigger hemichannel opening in ventricular cardiomyocytes, but necessitates the simultaneous activation of RyRs along a signaling cascade that includes CaMKII and p38MAPK. We hypothesize that opening of the large conductance Cx43 hemichannels may have a significant effects on electrical and $[Ca^{2+}]_i$ signaling in the cardiomyocyte propagation and might lead to cardiac arrhythmogenesis.

P8

COMPARISON OF DETOMIDINE-KETAMINE AND MIDAZOLAM-KETAMINE FOR SEDATION IN YUCATAN PIGS

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Pigs, compared to other laboratory animals, are particularly effective in translational medicine because of their genetic similarities. Furthermore, skin tissue and heart valves from pigs are compatible to humans. Regenerative medicine research uses this species as the main laboratory animal. One of the major ways to refine laboratory animal use is by using sedation and general anaesthesia to limit stress and pain. Our study aimed to rate and to compare two different sedative protocols. Seven Yucatan healthy laboratory pigs received both protocols, with a seven days washout period. The sedative protocols consisted of intramuscular midazolam 0.5 mg/kg plus ketamine 10 mg/kg and detomidine 0.1 mg/kg plus ketamine 10 mg/kg, respectively. Sedation, respiratory and heart rate, mean arterial pressure and SpO2 were assessed every five minutes for 20 minutes. The presence or absence of cyanotic membrane, muscular tremor, nystagmus, pedalling and salivation were recorded. The possibility to introduce a venous catheter on the lateral ear vein without producing a spontaneous head shake was also assessed. This study proves the efficiency of both protocols in producing fast and profound sedation with spare side effects. Both protocols have shown similarities in the sedation and recovery scores suggesting equipotent effects of midazolam and detomidine at this dose rates.

P9

CIS-ATRACURIUM NEUROMUSCULAR BLOCK AND ANTAGONIZATION WITH NEOSTIGMINE FOR PHACOEMULSIFICATION IN DOGS

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Neuromuscular blocking is required for centralisation of the ocular globe in ophthalmologic procedures. Cis-atracurium is a non-depolarizing neuromuscular blocking agent that was associated with less cardiovascular depression. Whether an effective dose for phacoemulsification surgery has an effect over the incidence of hypotension systolic arterial pressure (SAP <65 mmHg) remains unknown. Cis-atracurium (0.15 mg/kg intravenously) was administered in thirty-three adult dogs (12 ± 8 kg) under isoflurane anaesthesia and volume-controlled ventilation for phaco-emulsification surgery. Neuromuscular function was assessed by a calibrated train-of-four (TOF) monitor with surface electrodes placed over the tibia plateau for stimulation (every 15 seconds) of the peroneal nerve, and recording electrode placed over the dorsal aspect of the paw. The SAP was assessed using the Doppler technique. Neuromuscular blockade (TOF=0%) started at (mean ± SD) 2.0 ± 1.2 minutes after cis-atracurium administration and lasted 29.4 ± 9.8 minutes. An incremental dose of 0.05 mg/kg of cis-atracurium administered in twenty-one dogs had an onset time of 1.4 ± 0.6 minutes and lasted 17.3 ± 7.9 minutes. Intravenous administration of neostigmine (10 µg/kg) and glycopyrrolate (10 µg/kg) resulted in reversal time of 4.5 ± 3.4 minutes from TOF=62 ± 28% to TOF=100%. No hypotension was observed throughout in any dog. Cis-atracurium produced an effective neuromuscular blocking effect that was fully antagonised with neostigmine, and that was not associated with hypotension.

P10

IMBALANCE BETWEEN VASOACTIVE SUBSTANCES DURING THE DEVELOPMENT OF EXPERIMENTAL ARISTOLOCHIC ACID NEPHROPATHY

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Aristolochic Acid (AA) nephropathy (AAN) is a pertinent example of tubulo-interstitial nephritis characterized by an early phase of acute kidney injury (AKI) leading to progressive fibrosis and chronic kidney disease (CKD). It is now greatly recognized that endothelial cell activation as well as imbalance between vasoactive substances widely contributes to the transition from AKI to CKD. Therefore, in the present study, we aimed to characterize the potential imbalance between vasoactive substances such as nitric oxide (NO), endothelin-1 (ET-1), angiotensin II (Ang II) and urotensin II (UT II) in the successive phases of AAN. To do so, C57BL/6J male mice were randomly subjected to daily i.p. injection of vehicle or AAI (3,5mg/kg) for 4 days. Mice were euthanized, 5, 10 and 20 days after the beginning of AAI injections. AA-treated mice developed marked renal injury and histopathological features of AAN were reproduced. Early phase of AKI observed at day 5 was characterized by necrosis of proximal tubular epithelial cells and proteinuria. Later phase of CKD developed at day 20 as attested by tubular atrophy and massive interstitial fibrosis. Oxidative stress and inflammatory cell infiltration were also characterized concomitantly to the progression from AKI to CKD. Regarding the vasoactive factors, our results revealed that AA-intoxicated mice presented:

(1) Reduced urinary NO and cGMP levels throughout the protocol whereas renal mRNA expression of NO synthases (eNOS, nNOS, iNOS) remained unchanged.
(2) Reduced renal mRNA expression of angiotensinogen (AGT), angiotensin II converting enzyme (ACE) and angiotensin II receptors (AT_1 and AT_2) throughout the protocol.
(3) Increased urinary and plasma ET-1 levels and increased renal mRNA expression of ET-1 and its receptor A (ET_A) whereas renal mRNA expression of receptor B (ET_B) was strongly downregulated.
(4) Increased renal mRNA expression of UT II and UT receptor.

Our findings demonstrated that imbalance between vasoactive substances occurs during AAN progression and could contribute to the transition from AKI to CKD.

P11

EFFECT OF GENDER ON OBESITY-ASSOCIATED RENAL DYSFUNCTION AND INVOLVEMENT OF ADIPOKINES

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Obesity incidence has dramatically increased during the last few years. This disease, characterized by an excessive fat accumulation, has for consequences an alteration of adipose tissue function and a chronic inflammation status, leading to metabolic disturbances. It is also well described that an excess of fat can be considered as a risk factor for kidney disease development. Today, most researches focus on males. However, it is imperative to determine how the sex difference can affect metabolic homeostasis and obesity syndrome. Indeed, several differences have been highlighted between the two sexes. For example, adipose tissue distribution is different in men and women. Moreover, sexual hormones are involved in lipid and glucose metabolism. Adipose tissue has been to shown to play endocrine functions by the secretion of adipokines, such as chemerin, adiponectin, leptin and TNF-α. In obese patients, secretion of these adipokines is impaired. Therefore, in the present study, we investigated the role of adipokines in obesity progression in males as well as females and, specifically, its impact on obesity-induced kidney alterations. To do so, C57BL/6 male and female mice were randomized to a low fat diet (LFD) or a high fat diet (HFD) for 16 weeks. We demonstrated that male mice fed a HFD developed obesity, as illustrated by an increase in body weight, kidney hypertrophy as well as glucose metabolism disorders. Regarding kidney function, we observed that HFD mice tend to develop renal functional impairment as they exhibited proteinuria and a slight increase in albuminuria. These observations were associated with a mesangial matrix expansion in glomeruli and vacuolated tubular cells. We also observed effects of HFD on adipokine concentrations, inflammation and fibrosis process in kidney. Finally, HFD mice presented a moderated oxidative stress. Female mice, on the other hand, seem less affected by HFD according to metabolic data. Moreover, kidney lesions were less important than in male mice. However, female mice exhibited important inflammation, fibrosis and oxidative stress modifications compared with male fed a HFD and LFD. In summary, we demonstrated appearance of obesity as well as associated kidney failure in HFD male and female mice. However, according to our results, gender seems to influence the obtained data, highlighting roles of sexual hormones in obesity physiopathological mechanisms.

P12

IDENTIFICATION AND PHARMACOLOGICAL CHARACTERIZATION OF SUCCINATE RECEPTOR LIGANDS AS PHARMACOLOGICAL TOOLS

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The succinate receptor (SUCNR1 or GPR91) has been described as a metabolic sensor that may be involved in homeostasis. Notwithstanding its implication in important (patho)physiological processes, the function of SUCNR1 has remained elusive because no pharmacological tools were available. We recently publish the discovery of the first family of synthetic potent agonists by screening a library of succinate analogues and analyse of their activity on SUCNR1. In addition, we modelled a pharmacophore and a binding site for the receptor. New agonists were identified based on the information provided by these two approaches. Their activity was studied in various bioassays and *in vivo* impact of SUCNR1 activation was evaluated on rat blood pressure. We identified *cis*-epoxysuccinic acid (cESA) and *cis*-1,2-cyclopropanedicarboxylic acid (cCPDA) as full agonists. Interestingly, cESA was characterized by a 10 to 20 fold higher potency than succinate on the receptor. In addition, as predicted, cESA and cCPDA increased rat blood pressure to the same extent as succinate did. In parallel we work on the pharmacological characterization of previously published "antagonists". Our first result tends to show a non-competitive action and a lack of specificity. We aim to provide and characterize new pharmacological tools for SUCNR1 that should facilitate further research on this understudied receptor.