BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting
Friday April 19th 2013

Palace of the Academies
Rue Ducale / Hertogsstraat 1
1000 Brussels

Main Lecture

10.00-11.00 Prof. Dr. Andrea VOLTERRA (Department of Fundamental Neurosciences, University of Lausanne, Switzerland).

Astrocytes as active synaptic partners in physiology and disease.

Oral Communications

11.00-11.15 S.G.A. VAN NEERVEN, P. PANNAYE, E. HERMANS, R. DEUMENS (RWTH Aachen, Germany, UCLouvain).
A novel, pro-regenerative role of epineurial fibroblasts: proof from in vitro experiments.

11.15-11.30 F. DEMOL, R. KOOIJMAN, A. MASSIE, J. DE KEYSER, C. JENSEN (VUBrussel).
The role of astrocytic beta2-adrenergic receptors in the pathophysiology of multiple sclerosis.

11.30-11.45 B. MICHOT, V. KAYSER, M. HAMON, S. BOURGOIN (INSERM U894 Paris, France, UCLouvain).
Anti-allodynic effects of tapentadol in rats with ligatures of the infraorbital nerve versus the sciatic nerve.

11.45-12.00 I. KOPLJAR, AJ. LABRO, J. TYTGAT, DJ. SNYDERS (UAntwerpen, KULeuven).
Gambierol inhibits Kv channels through a novel mechanism of gating modification.

12.00-12.15 D. GHOSH, G. OWSIANIK, P. VANDEN BERGHE, A. SEGAL, J. VRIENS, T. VOETS (KULeuven).
Characterization of the trafficking of human Transient Receptor Potential Melastatin 8 (hTRPM8) channel by Total Internal Reflection Fluorescence (TIRF) microscopy.

12.15-12.30 J. HANSON, N. FERREROS, B. PIROTTE, G. GEISSLINGER, S. OFFERMANNS (Max Planck Institute for heart and lung research Bad Neuheim, Germany, ULiège, Goethe-University Frankfurt, Germany).
Are lipoxin A_4 and its receptor (ALX/FPR2) mismatched?

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

Posters (height 120 cm – width 100 cm)

1. T. DEMUYSER, S. GOURSAUD, E. BENTEA, J. VAN LIEFFERINGE, E. MERCKX, I. SMOLDERS, A. MASSIE, E. HERMANS (VUBrussel, UCLouvain).
Glutamate uptake is affected in the central nervous system of system x_c^- -deficient mice.

2. J. COPPENS, J. PORTELLI, Y. MICHOTTE, I. SMOLDERS (VUBrussel).
The orexin pathway is not involved in the attenuation of limbic seizures by Des-acyl ghrelin.

3. D. ENGEL, V. SEUTIN (ULiège).
Hyperpolarization-activated cation channels influence synaptic integration properties in substantia nigra dopamine neurons.

4. A. DIMIZIANI, J. DAMBLON, E. HERMANS, R. DEUMENS (UCLouvain).
Does spinal cord inflammation play a role in peripheral nerve regeneration? Implications for acute and chronic pain.

5. L. LAMBOT, S.N. SCHIFFMANN, D. GALL (ULBruxelles).
Distinct target selectivity of fast-spiking interneurons in the regulation of striatal output pathways.

6. J-F. DE BACKER, S. MONLEZUN, M. ZOLI, O. VALVERDE, D. GALL, O. DE BACKER, S.N. SCHIFFMANN, A. DE KECHOVE D'EXAERDE (ULBruxelles, Univ. Modena & Regio Emilia, Italy, Univ. Pompeu Fabra, Spain, FUNDPNamur).
Involvement of MAGED1 in motor behaviour.

7. A. STERNOTTE, E. HERMANS (UCLouvain).
A metabolic hypothesis in amyotrophic lateral sclerosis.

8. K. HELD, S. KERSELAERS, P. CHALTIN, T. VOETS, J. VRIENS (KULeuven).
Mode of action and in vivo application of TRPM3 antagonists.

9. T. D'HOOGHE, R. VAN BREE, D. PETERSE, A. FASSBENDER, J. VRIENS (KULeuven).
Functional characterization of TRP channels in human endometrium.

10. J. STAS, I. KOPJLAR, A.J. LABRO, S. PEIGNEUR, A.J. ZAHARENKO, J. TYTGAT, D.J. SNYDERS (UAntwerpen, KULeuven, Univ. São Paulo, Brazil).
Bcg 31.16, a peptide toxin derived from the sea anemone Bunodosoma cangicum, modulates the gating properties of K_v1 channels.

11. D.P. BISCHOP, D. ORDUZ, L. LAMBOT, S.N. SCHIFFMANN, D. GALL (ULBruxelles).
Control of neuronal excitability by calcium binding proteins: a new mathematical model for striatal fast-spiking interneurons.

12. B. BOUTIN, N. TAJEDDINE, V. BUTOESCU, P. GAILLY (UCLouvain).
Androgen deprivation modulates calcium homeostasis of hormone-resistant prostate cancer cells and confers resistance to apoptosis.

13. P.E. PORPORATO, V.L. PAYEN, C.J. DE SAEDELEER, T. COPETTI, O. FERON, P. SONVEAUX (UCLouvain).
Lactate stimulates angiogenesis, prevents ischemic skeletal muscle atrophy, and accelerates wound healing.

14. M. ROMERO-PEREZ, E. LEON-GOMEZ, I. LOBYSHEVA, G. RATH, J.M. DOGNÉ, O. FERON, C. DESSY (UCLouvain, FUNDPNamur).
Effects of BM-573 on endothelial function and increased blood pressure at early stages of atherosclerosis.

15. S. SEBAA, Ph. COURTOIS, Z. BOUCHERIT-OTMANI, M. AHARIZ (ULBruxelles, Univ. Abou Bekr Belkaïd, Tlemcen, Algeria).
H_2O_2 consumption by Candida albicans from dentures.

16. S. CETIK, E. HUPKENS, A. SENER (ULBruxelles).
A tentative model for D-glucose turn-over in human saliva.

17. V. SHLYONSKY, F. DUPUIS, D. GALL (ULBruxelles) .
A low cost, open-source lipid bilayer setup for hands-on learning of biophysics.

Oral Communications

14.00-14.15 L. VANDEKERCKHOVE, A-S HERVENT, G.W. DE KEULENAER (UAntwerpen).
Neuregulin-1 attenuates the development of dilated cardiomyopathy but not atherosclerosis in type 1 diabetic mice.

14.15-14.30 J. CRAPS, J. VANDERSTRAETEN, I. LOBYSHEVA, J-L. BALLIGAND, P. SONVEAUX, P. GILON, M-C. MANY, B. LENGELE, I.M. COLIN, A–C. GERARD (UCLouvain).
Involvement of nitric oxide in iodine deficiency-induced microvascular remodeling in thyroid gland: role of NOS3 and ryanodine receptors.

14.30-14.45 J. VANDERSTRAETEN, J. CRAPS, I.M. COLIN, A-C. GÉRARD (UCLouvain).
The effects of iodine deficiency on stomach, salivary glands and mammary glands vascularization.

14.45-15.00 N. DRAOUI, O. SCHIKE, X. DROZAK, A. MARCHAND, P. CHALTIN, P. SONVEAUX, O. RIANT, O. FERON (UCLouvain).
Development of new inhibitors of lactate transporters: from the in vitro screening procedure to the in vivo validation of the therapeutic strategy.

15.00-15.15 M. ALHOUAYEK, J. MASQUELIER, T. TIMMERMAN, D.M. LAMBERT, G.G. MUCCIOLI (UCLouvain).
The endocannabinoid 2-arachidonoylglycerol controls macrophage activation through its oxidative metabolite prostaglandin D_2-glycerol (PGD_2-G).

15.15-16.00 **ROUND TABLE**

“Where to go next in physiological and pharmacological sciences”

Prof. Dr. Rick ALDRICH (Member of the National Academy of Sciences of the United States of America).

Introduction by Prof. Dr. Vincent SEUTIN (Secretary general of the Belgian Society of Physiology and Pharmacology)

Coffee - Tea

ABSTRACTS

Legend
O = Oral communication nummered
P = Poster numbered

O-01 (11.00-11.15)

A NOVEL, PRO-REGENERATIVE ROLE OF EPINEURIAL FIBROBLASTS: PROOF FROM *IN VITRO* EXPERIMENTS

S.G.A. van Neerven[1], P. Pannaye[1], E. Hermans[2], R. Deumens[2]

[1]Institute of Plastic Surgery, Reconstructive and Hand Surgery, RWTH Aachen University, Germany; [2]Institute of Neuroscience, Université Catholique de Louvain, Brussels.

A poor regenerative response of the peripheral nervous system to injury leads to long-term deficits in functional outcome as well as neuropathic pain. While Schwann cells are strong promoters of regeneration, fibroblasts have up till now been incriminated with impediment of regeneration due to their active role in the formation of scar tissue that entraps regrowing axons. Recent data, however, point to a sofar unknown role of fibroblasts in the regulation of Schwann cell behavior. We here explored this concept further by performing *in vitro* experiments focusing on epineurial fibroblasts, which only gain access to neuronal cells and Schwann cells upon severe nerve injury. Our data show that culture medium conditioned by highly enriched cultures of adult epineurial fibroblasts (1) supports migratory activity of adult Schwann cells, and (2) promotes neurite outgrowth from dorsal root ganglion neurons. Since these two factors are hallmarks of peripheral nerve regeneration, these data signify a pro-regenerative character of epineurial fibroblasts. We also found that a high degree of stretch, which is perhaps the most frequent cell stressor during nerve injury, did not further change this regenerative characteristic of epineurial fibroblasts. We conclude that epineurial fibroblasts may be supportive of peripheral nerve regeneration in conditions of severe nerve injury in which these cells get into contact with axons and Schwann cells. This insight sheds a different light on fibroblasts which have traditionally been regarded as impeditors of peripheral nerve regeneration. Further studies are now needed to investigate the consequences of such pro-regenerative effects of epineurial fibroblasts in the injured nerve.

O-02 (11.15-11.30)

THE ROLE OF ASTROCYTIC BETA2-ADRENERGIC RECEPTORS IN THE PATHO-PHYSIOLOGY OF MULTIPLE SCLEROSIS

F. Demol[1], R. Kooijman[2], A. Massie[3], J. De Keyser[1], C. Jensen[1]

[1] Department of Neurology, University Hospital Brussels, Center for Neuroscience, Vrije Universiteit Brussel, Brussel, Belgium; [2]Department of Pharmacology, Center for Neuroscience, Vrije Universiteit Brussel, Brussel; [3]Department of Pharmaceutical Biotechnology and Molecular Biology, Center for Neuroscience Vrije Universiteit Brussel, Brussel, Belgium.

Multiple sclerosis (MS) is a chronic progressive disease of the central nervous system characterized by demyelination of axons. Research has shown that astrocytic beta2-adrenergic receptors (β2AR) are undetectable in MS patients. This deficiency may reduce astrocytic glycogenolysis necessary for axonal energy metabolism, and the release of brain derived growth factor (BDNF), leading to progressive axonal degeneration that underlies the progressive disability seen in subjects with MS. The aim of our research is to investigate the hypothesis that absence of the astrocytic β2AR plays a key role in white matter axonal degeneration. Therefore, the functional consequences of astrocytic β2AR deficiency were examined in an inducible astrocytic β2AR knock-out (KO) mice model. To induce the KO, intraperitoneal tamoxifen injections (2x 1mg/day during 5 days) were given to 8 weeks old GLAST::CreERT2+/- ADRB2 floxed+/+ mice. One week, 2 weeks, 4 weeks, 6 months and 12 months after the KO induction, mice were phenotyped and compared with sham-induced mice and tamoxifen-treated control mice (GLAST::CreERT2+/- ADRB2 floxed -/- and GLAST::CreERT2-/- ADRB2 floxed +/+) (n=10 in each group). The blinded phenotyping was based on the modified SHIRPA method, the swim ability test and the rotarod test. All experiments after 1, 2, 4 weeks and 6 months are completed. Experiments 12 months after induction are still running. So far none of the experimental groups showed significant differences in appearance, behavior or motor skills at the different time points. Over time there were differences due to ageing of the mice, such as a decrease in latency time on the rotarod, but all of these differences were comparable between the groups. Furthermore, significant differences between males and females of the same groups were found in accordance with literature. In conclusion no relevant significant differences were found between astrocytic β2AR KO mice and there controls. No relevant significant differences in behavior were found between astrocytic β2AR KO mice and there controls. Magnetic resonance imaging to assess axonal integrity using diffusion tensor imaging will be the next step.

O-03 (11.30-11.45)

ANTI-ALLODYNIC EFFECTS OF TAPENTADOL IN RATS WITH LIGATURES OF THE INFRAORBITAL NERVE VERSUS THE SCIATIC NERVE

B. Michot[1,2], V. Kayser[1], M. Hamon[1], S. Bourgoin[1]

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Convergent data showed that neuropathic pain has specific characteristics and responds differently to alleviating drugs at cephalic versus extracephalic level. In order to assess whether the limited effectiveness of drugs at cephalic versus extra-cephalic level, or *vice versa*, might be linked to drugs action at a single molecular target, we investigated whether tapentadol, which is both a noradrenaline (NA) reuptake inhibitor and a μ-opioïd receptor agonist, would be more effective than single targeted drugs. Accordingly, we also tested whether the effects of tapentadol could be mimicked by the combination of the selective NA reuptake inhibitor reboxetine and morphine. Rats underwent unilateral constriction injury (CCI) to the infraorbital nerve (ION; cephalic territory) or the sciatic nerve (SN; extracephalic territory). Two weeks later, the effects of tapentadol, morphine, reboxetine, combination of the latter two drugs or their vehicle were tested on mechanical allodynia in ipsilateral vibrissae territory (CCI-ION rats) or hindpaw (CCI-SN rats). Threshold pressures to trigger nocifensive responses were markedly less in nerve-ligated rats compared to sham-operated animals, as expected of nerve lesion-induced allodynia. Acute as well as subchronic (twice daily for 4 days) administration of tapentadol markedly reduced mechanical allodynia in both CCI-SN and CCI-ION rats. In contrast, morphine and reboxetine were only moderately active or inactive when administered alone, but their combination produced supra-additive effects with marked allodynia alleviation in both CCI-ION and CCI-SN rats. These findings showed that combined μ-opioïd receptor activation and NA reuptake inhibition exert anti-allodynic effects at both cephalic and extra-cephalic levels, and support the idea that multitargeted drugs such as tapentadol are especially indicated for a better treatment of neuropathic pain whatever its location in the body.

O-04 (11.45-12.00)

GAMBIEROL INHIBITS KV CHANNELS THROUGH A NOVEL MECHANISM OF GATING MODIFICATION

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Ciguatoxins are marine ladder-shaped polyether toxins that cause ciguatera fish poisoning. These toxins potentiate voltage-gated sodium (Nav) channels through receptor site 5. Several ciguatoxins also affect Kv channels and we showed previously that the polyether toxin gambierol inhibits Kv3.1 channels via a lipid-exposed binding site, located outside the K^+ permeation pathway. However, the mechanism by which gambierol inhibits Kv channels remained unknown. Using gating and ionic current analysis to investigate how gambierol affected S6 gate opening and voltage-sensor domain (VSD) movements, we show that gambierol is a gating modifier that binds with high affinity to the resting (closed) channel conformation. The voltage dependence of activation was shifted by >120 mV in the depolarizing direction, precluding channel opening in the physiological voltage range. The (early) transitions between the resting and the open state were monitored with gating currents, and provided evidence that strong depolarizations allowed VSD movement up to the activated-not-open state. However, for transition to the fully open (ion conducting) state, the toxin first needed to dissociate. A tetrameric concatemer with only one high-affinity binding site still displayed high toxin sensitivity, suggesting that interaction with a single binding site prevented the concerted step required for channel opening. We propose a mechanism whereby gambierol anchors the channel´s gating machinery in the resting state requiring more work from the VSD to open the channel. This mechanism is quite different from the action of classical gating modifier peptides (e.g., hanatoxin). Therefore, polyether toxins open new opportunities in structure-function relationship studies in Kv channels and in drug design to modulate channel function.

O-05 (12.00-12.15)

CHARACTERIZATION OF THE TRAFFICKING OF HUMAN TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (HTRPM8) CHANNEL BY TOTAL INTERNAL REFLECTION FLUORESCENCE (TIRF) MICROSCOPY

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Transient Receptor Potential (TRP) channels form a superfamily of channels that can be considered as multiple signal integrators. TRP channels play vital roles in perception of all major classes of external stimuli, like thermosensation, chemosensation and mechanosensation. These channels also help individual cells with the ability to sense changes in the local environment, such as alterations in osmolarity. There is very little knowledge of the kinetics and molecular mechanism regarding the intracellular trafficking of TRP channels. Till date, only a handful of proteins are known to interact with TRP channels and to influence their trafficking. However, it is becoming increasingly clear that the dynamic modulation of the number of active TRP channels in the plasma membrane and intracellular organelles represents an important mechanism to regulate channel activity. A better knowledge of the fundamentals of TRP channel trafficking are therefore essential to our understanding of the role of these channels in various physiological/pathophysiological processes. We focused our study on TRPM8 - a cation channel activated by cold and the cooling compounds menthol and icilin. With the help of TIRF Microscopy- a state of art high resolution microscopic system, which allows the detection of individual fluorophores within 100 nm of the cell surface, we present for the first time a detailed depiction of TRPM8-vesicles in the near-membrane field. TRPM8 is present in intracellular structures with diverse morphological characteristics. We observed that a large fraction of TRPM8 resides in highly mobile late endosomal and lysosomal vesicles whereas less than 1% of TRPM8 resides in clathrin and caveolin coated vesicles or early endosomal vesicles. We found that TRPM8 vesicle movement is dependent on microtubular tracks while actin filaments could be involved in near membrane movements. Calcium entry as a result of TRPM8 activation seems to alter the dynamics of TRPM8 protein movement. Our results fetched significant insights regarding the movement pattern and habitation of TRPM8 within the intracellular compartment and further study need to be persisted to unveil its physiological importance.

O-06 (12.15-12.30)

ARE LIPOXIN A_4 AND ITS RECEPTOR (ALX/FPR2) MISMATCHED?

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Lipoxin A_4 (LXA_4) has been described as an anti-inflammatory mediator which exerts its effects through the formyl peptide receptor FPR2, also known as lipoxin receptor (ALX). However, there has been a controversy whether or not cells expressing FPR2/ALX, such as neutrophils, respond to LXA_4. We, therefore, systematically examined the ability of the human and murine forms of the receptor to respond to LXA_4. We show that both receptor orthologues responded to the FPR2/ALX peptide agonist WKYMVM when expressed heterologously. In contrast, LXA_4 from different sources neither increased $[Ca^{2+}]_i$ and extracellular-signal-regulated kinase (ERK) phosphorylation, nor did it induce a decrease in cAMP levels or a translocation of β-arrestin. Also, several LXA_4 analogues were found to be unable to signal through FPR2/ALX. We conclude that FPR2/ALX is not activated by LXA_4 and that the molecular mechanism by which LXA_4 functions still needs to be identified.

O-07 (14.00-14.15)

NEUREGULIN-1 ATTENUATES THE DEVELOPMENT OF DILATED CARDIO-MYOPATHY BUT NOT ATHEROSCLEROSIS IN TYPE 1 DIABETIC MICE

L. Vandekerckhove[1], A-S. Hervent[1], G.W. De Keulenaer[1]

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Neuregulin-1 (NRG-1) is an endothelium-derived growth factor with cardioprotective properties in conditions of cardiac injury and overload. NRG-1 has also been shown to suppress the development of atherosclerotis. Therapeutic effects of rhNRG-1 in heart failure are currently tested in phase III clinical trials in humans. In this study, we analyzed the cardiac and anti-atherosclerotic effects of rhNRG-1 in a model of type I diabetes-associated cardiomyopathy and atherosclerosis. Apolipoprotein-E deficient mice (ApoE-/-, age 16 weeks, n=34) were treated with the beta-cell toxin streptozotocin (60 mg/kg) or buffer solution for 5 consecutive days. Diabetic mice (glycemia > 300 mg/dl, n=24) were daily treated with NRG-1 (20 µg/kg/d, IP) or saline during 14 weeks. Mice were then analyzed by transthoracic echocardiography and invasive hemodynamic recordings, next sacrificed for histochemical and molecular analyses of the heart and the arteria brachiocephalica. *In absence of rhNRG-1*, diabetic mice displayed distinct arterial atherosclerotic plaques and a dilated heart. The left ventricle (LV) was hypocontractile (reduced preload-recruitable stroke work and reduced end-systolic elastance) and hypercompliant (increased end diastolic elastance). Histological and molecular analyses showed no signs of myocardial fibrosis and apoptosis. *rhNRG-1 treatment* induced a significant activation of ErbB2/4 receptors in the LV myocardium. NRG-1 did not affect glycemia, cholesterol levels or body weight in diabetic mice. RhNRG-1 did not affect the morphology and size of the arterial atherosclerotic plaques. However, rhNRG-1 completely prevented LV dilatation, and all systolic and diastolic parameters of LV function returned to levels measured in non-diabetic control mice. Histological and molecular analyses showed again no signs of myocardial fibrosis or apoptosis. In this model of type I diabetes and atherosclerosis, rhNRG-1 prevents the development of dilated cardiomyopathy, independently of effects on glycemia, atherosclerosis, myocardial fibrosis or myocardial apoptosis.

O-08 (14.15-14.30)

INVOLVEMENT OF NITRIC OXIDE IN IODINE DEFICIENCY-INDUCED MICRO-VASCULAR REMODELING IN THYROID GLAND: ROLE OF NOS3 AND RYANODINE RECEPTORS.

J. Craps[1], J. Vanderstraeten[1], I. Lobysheva[2], J-L. Balligand[2], P. Sonveaux[2], P. Gilon[3], M-C. Many[1], B. Lengelé[1], IM. Colin[1] and A–C Gérard[1]

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Iodine deficiency (ID) induces microvascular remodeling via a ROS-HIF-1α-VEGF pathway in thyrocytes. Among ROS, Nitric oxide (NO) is a key regulator of ID-induced vasodilation. Our goal was to study the involvement NO in the ROS-HIF-1α-VEGF pathway and the role of calcium in Nos3 activation by ID. ID was induced *in vitro* (PCCL-3, FRTL-5, human thyrocytes primary cultures) and in mice (LID/perchlorate). The role of NO was assessed by using L-NAME (NO synthase inhibitor), and SNAP (NO donor). Nos3 phosphorylation on serine 1177 (activation, pSNos3) and on threonine 495 (inactivation, pTNos3) and HIF-1α protein were detected by WB, VEGF mRNA by QRT-PCR, and VEGF protein by WB and immunohistochemistry. NO production was measured *in vitro* by electron paramagnetic resonance (EPR). The thyroid blood flow was measured by laser Doppler. *In vitro*, ryanodine was used to inhibit (10µM) or activate (1nM) the RYR calcium channels. Ca^{++} involvement was studied by the use of Thapsigargin (releases Ca^{++} by inhibiting endoplasmic reticulum Ca^{++}-ATPase). Both *in vitro* and *in vivo*, Nos3 was activated by ID (increased pSNos3 and decreased pTNos3) and ID-induced VEGF mRNA and protein were inhibited by L-NAME. *In vitro*, ID increased NO release, and L-NAME inhibited ID-induced HIF-1α protein while SNAP increased VEGF mRNA. *In vivo*, LID/perchlorate-induced thyroid blood flow as well as VEGF mRNA and protein expression were inhibited by L-NAME. RYR inhibition decreased ID-induced HIF-1α, pSNos3 and VEGF expression while their activation increased pSNos3. Thapsigargin mimics ID by inducing VEGF production. In conclusion, NO plays a major role in ID-induced microvascular remodeling through an activation of pNos3 dependent on RYR activation.

O-09 (14.30-14.45)

THE EFFECTS OF IODINE DEFICIENCY ON STOMACH, SALIVARY GLANDS AND MAMMARY GLANDS VASCULARIZATION

J. Vanderstraeten, J. Craps, I.M. Colin, A.C. Gérard

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Despite the efforts to introduce salt iodization in iodine insufficient countries, iodine deficiency (ID) remains a global problem. Belgium is particularly concerned, with 66.9% of its population being iodine deficient in 2012. Beside the well-known effects of ID on foetal development and its implication in thyroid diseases, it has also been linked to breast and stomach disorders. Therefore, the aim of our work is to study the impact of ID on three organs which take up iodine: stomach, salivary and mammary glands. Eight week CD-1 mice (group 1) and 4 month previously lactating CD-1 females (group 2) were fed with iodide deficient diet and perchlorate in water (NaClO4, a sodium/iodide symporter (NIS) inhibitor) during one to six days. As it has previously been observed in our laboratory that thyroid cells can react to ID by secreting VEGF resulting in an increased thyroid blood flow, VEGF expression was studied by immunochemistry in the three organs and blood flow was measured with a laser Doppler in mammary and salivary glands before sacrifice at day 0, 1, 2, 4 and 6. VEGF expression was strongly enhanced in the mammary glands stroma and duct cells in group 2 at day 2 but not in group 1. Moreover, a significant increase in blood flow was observed at day 2 in group 1 (114% of control blood flow) and 2 (131% of control). Unlike the thyroid (previous experiment) and mammary glands, the blood flow decreased in salivary glands from day 1 to day 4 in group 1 (85% of controls'), while it tended to increase in group 2. Differences were noticed between males and females. However, no differences were observed in VEGF expression between control and ID mice. In gastric mucosa, VEGF expression was strongly enhanced at day 1 and day 2 and then dropped at day 4 and 6. From these data, we can conclude that cells other than thyroid cells can react to ID by inducing microvascular changes, trying to adapt iodide inflow.

O-10 (14.45-15.00)

DEVELOPMENT OF NEW INHIBITORS OF LACTATE TRANSPORTERS: FROM THE *IN VITRO* SCREENING PROCEDURE TO THE *IN VIVO* VALIDATION OF THE THERAPEUTIC STRATEGY

N. Draoui[1,2], O. Schike[2], X. Drozak[2], A. Marchand[3], P. Chaltin[3], P. Sonveaux[1], O. Riant[2], O. Feron[1]

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Proliferating cancer cells consume glucose at a high rate and release lactate. This adaptive metabolism provides tumor cells with major anabolic intermediates to support tumor growth. In addition, lactate, the end-product of glycolysis, can be re-captured by cancer cells and after reconversion into pyruvate, can feed the TCA cycle. Lactate (monocarboxylate) transporters (MCT) therefore represent potential targets to limit tumor growth and invasiveness. The aim of this work was to identify new potent and selective MCT inhibitors to target tumor metabolism and to validate their efficacy *in vivo*. In the first part of this study, we have developed and implemented a stepwise *in vitro* screening procedure on human cancer cells to guide the selection of new MCT inhibitors. Different families of compounds were synthesized and characterized with conventional analytical approaches (patent pending). We first evaluated the efficacy of our compounds by comparing their cytotoxic potential in either glucose- or lactate-containing medium. This primary assay led us to identify hit compounds which exhibit selective inhibition of cell proliferation in lactate conditions but fail to exert toxic effects in the control glucose conditions. The secondary assay confirmed that hit compounds functionally block lactate flux and helped us to gain insights on the structure-activity relationship of our compound families. Finally, we set up a third assay based on the measurement of the flux of [^{14}C]-lactate during short periods of time (minute range) to validate our hits as *bona fide* MCT inhibitors. In the second part of the work, two hits were selected for the *in vivo* proof of principle of their antitumor effects in nude mice s.c. injected with tumor cells derived from distinct human cancers. Each hit compound was daily administered (i.p.) and consistently led to significant growth delays in human cervix and colorectal carcinoma models but not in tumors derived from bladder carcinoma cells. This distinct sensitivity could be related to the presence (at the surface of tumor cells) or the lack of functional expression of MCT transporters, respectively. In MCT-expressing tumors, growth inhibition by conventional chemotherapy (eg, cisplatin) was also increased in the presence of our hit compounds, leading to a significant reduction in post-treatment tumor recurrence. Altogether, these data confirm the rationale to interfere with lactate transport in tumors and validate a new family of MCT inhibitors as an efficient anticancer strategy.

O-11 (15.00-15.15)

THE ENDOCANNABINOID 2-ARACHIDONOYLGLYCEROL CONTROLS MACROPHAGE ACTIVATION THROUGH ITS OXIDATIVE METABOLITE PROSTAGLANDIN D_2-GLYCEROL (PGD_2-G)

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Macrophages are key players in innate and adaptive immune responses. Their role during an inflammatory process is to eliminate the threat and protect the body from noxious agents. However, under persistence of the pro-inflammatory phase or when macrophages trigger an altered response, inflammation becomes chronic and thus deleterious. Therefore pro-inflammatory macrophages responses must be controlled in chronic inflammatory settings. The endocannabinoid 2-arachidonoylglycerol (2-AG) exerts anti-inflammatory actions in various settings, classically through activation of the cannabinoid receptors CB_1 and CB_2. Here, using two macrophage cell lines in culture, we show that 2-AG controls macrophage activation (LPS, 100ng/mL). Indeed, 2-AG counteracts the LPS-induced production of pro-inflammatory cytokines, such as interleukin (IL)-1beta (IC_{50} = 6 microM), IL-6 and tumor necrosis factor-alpha, as well as nitric oxide production. Interestingly, in both cell lines analyzed, we show, using receptor-selective antagonists (SLV319 and SR144528), that the effects of 2-AG are cannabinoid receptors independent. Moreover, this response seemed to be 2-AG specific, since other fatty acid glycerols (e.g. 2-oleoylglycerol) had no effect on the LPS-induced response. Thus, considering that LPS induces cyclooxygenase (COX)-2 expression and that 2-AG could also be oxidized by COX enzymes, similarly to arachidonic acid, to give protanglandin-glycerols (PG-G), we sought to investigate the biological effects of these PG-G. In the same setting, PGD_2-G reduced the LPS-induced expression of pro-inflammatory cytokines and nitric oxide production, whereas PGE_2-G and PGF_{2alpha}-G strongly exacerbated the LPS-induced macrophage response. The effect of PGD_2-G is not due to its hydrolysis into PGD_2, since the latter had no effect in the same setting, or to the activation of the PGD_2 receptors DP_1 and DP_2. Additionally, we show through RT-qPCR analysis, that in the cell lines used, prostaglandin D synthase is more expressed than prostaglandin E synthase. This is corroborated by the fact that these cells produce significantly more PGD_2 than PGE_2 as assessed by HPLC-MS. Thus 2-AG could be oxidized by COX-2 into PGH_2-G and then preferentially give rise to PGD_2-G in this setting. *In conclusion*, we describe here a novel pathway through which the endocannabinoid 2-AG can control inflammation.

P-01

GLUTAMATE UPTAKE IS AFFECTED IN THE CENTRAL NERVOUS SYSTEM OF SYSTEM X_C^- - DEFICIENT MICE

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We recently characterized system x_c^- (cystine/glutamate antiporter, xCT as specific subunit) as the major source of extracellular glutamate in the hippocampus/striatum by showing significantly lower dialysate glutamate levels in system x_c^--deficient mice (xCT^-) compared to wildtype littermates ($xCT^{+/+}$). In order to exclude that this difference in glutamate levels is linked to compensatory changes in expression of the glutamate transporters, we measured their expression in hippocampal/striatal protein extracts of $xCT^{-/-}$ mice and wildtype littermates by Western blot. No difference was observed. However, glutamate uptake activity might be affected without changes in expression levels. Therefore, in this study, we conducted a D-[^{3}H]-aspartate uptake assay on synaptosomes prepared from different brain structures of the $xCT^{-/-}$ and $xCT^{+/+}$ mice. Freshly dissected brain tissue was subjected to the functional assay. The involvement of the different transporters was analysed by measuring the D-[^{3}H]-aspartate uptake in the presence and absence of specific GLAST or GLT-1 inhibitors (WAY213613 and UCPH101, respectively). Our data show that there is no genotype-dependent effect on D-[^{3}H]-aspartate uptake in prefrontal cortex, striatum and midbrain. On the other hand we show that uptake of aspartate is significantly higher in the hippocampus and lower in the spinal cord of $xCT^{-/-}$ versus $xCT^{+/+}$ mice. Both changes are due to a change in the activity of the GLT-1 transporter. These results contribute to the characterization of our transgenic model and will be subject for further research.

P-02

THE OREXIN PATHWAY IS NOT INVOLVED IN THE ATTENUATION OF LIMBIC SEIZURES BY DES-ACYL GHRELIN

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Once considered inactive, des-acyl ghrelin is now implicated in a number of biological functions. Unlike ghrelin, des-acyl ghrelin is unable to activate the ghrelin receptor and recently one study implied that intracerebroventricular des-acyl ghrelin administration affected food intake via the orexin pathway. Recently, des-acyl ghrelin has been proposed to have beneficial effects on limbic seizures. In this study, we further characterized the role of des-acyl ghrelin in seizures using the focal pilocarpine model for limbic seizures. In this study we used the in vivo rat model for pilocarpine-induced limbic seizures. Intrahippocampal administration of des-acyl ghrelin, the dual orexin receptor antagonist almorexant, or co-administration of des-acyl ghrelin and almorexant was performed in rats for 2h prior pilocarpine administration directly in the hippocampus. Rats were monitored following pilocarpine perfusion, and seizure behavior grades were evaluated according to a modified Racine's scale. We noted that while des-acyl ghrelin attenuated pilocarpine-induced limbic seizures at different concentrations, almorexant did not affect seizure severity. To determine whether des-acyl ghrelin utilizes the orexin pathway for its anticonvulsant effect, des acyl ghrelin was co-administered with almorexant. Dual receptor blockade did not prevent des-acyl ghrelin's anticonvulsant effect. We confirmed that des-acyl ghrelin attenuates limbic seizures and established that the orexin pathway is not involved. This is also first evidence that simultaneous antagonism of hippocampal orexin receptors does not affect seizure severity. This study highlights the need of identifying the mechanism of action of des-acyl ghrelin in epileptic seizures.

P-03

HYPERPOLARIZATION-ACTIVATED CATION CHANNELS INFLUENCE SYNAPTIC INTEGRATION PROPERTIES IN SUBSTANTIA NIGRA DOPAMINE NEURONS

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Hyperpolarization-activated channels (I_h or HCN channels) are widely expressed in principal neurons and in some interneurons. Their contribution to neuronal signaling includes the modulation of various parameters such as passive and active membrane properties, intrinsic resonance, synaptic integration and synaptic plasticity. One of the most characteristic function of I_h is to influence synaptic integration along dendrites by shortening the width of postsynaptic potentials (PSPs) and dampening the summation of trains of input. Herewith, PSPs arriving at the soma have similar width, regardless of their origin. Interestingly, this effect has been correlated to a nonuniform density of I_h in dendrites of cortical and hippocampal pyramidal neurons. In substantia nigra (SN) dopamine neurons, the presence of I_h is recognized, but its distribution along the somatodendritic axis and its function in synaptic integration are fully unknown. In contrast to pyramidal neurons, the axon of dopamine neurons often originates from a dendrite dividing the dendritic compartement in axon- and non-axon bearing dendrites. In addition, dopamine neurons express different I_h channel subunits in comparison to pyramidal neurons. We used cell-attached patch-clamp recordings to map the distribution of I_h in dopamine neurons along its somatodendritic axis and showed that I_h exhibits a nonuniform density along the somatodendritic axis. Using simultaneous whole-cell somatic and dendritic recordings we determined the effect of the current on single and multiple synaptic inputs, and observed that I_h shortens PSP duration and minimizes PSP summation.

P-04

DOES SPINAL CORD INFLAMMATION PLAY A ROLE IN PERIPHERAL NERVE REGENERATION? – IMPLICATIONS FOR ACUTE AND CHRONIC PAIN

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Therapeutic suppression of spinal cord inflammation has been shown to reduce early nerve injury-associated pain. Nevertheless, inflammation is also fundamental to regenerative responses, and successful nerve regeneration leads in turn to resolution of nerve injury pain. We here investigated whether modulation of spinal cord inflammation in a rat model of nerve injury affects nerve regeneration and/or pain chronification. Hereto, we used a glial modulator, propentofylline (PPF), or vehicle, which were daily administered to the intrathecal space for the first 10 days after either spared nerve crush injury (SNI) or sham surgery. Animals undergoing sham surgery were used as control. For a period of seven weeks, animals were tested for pain sensitivity using the von Frey hair filament test, and sciatic nerve-dependent motor function using the static sciatic index. Preliminary data show that PPF-treatment attenuates early SNI-evoked pain hypersensitivity, but has no obvious effect on the evolution of motor recovery. Additional data are currently being obtained and will be presented at the meeting. Together, these experiments should help to define the relevance of manipulating inflammation in the pharmacological treatment of neuropathic pain.

P-05

DISTINCT TARGET SELECTIVITY OF FAST-SPIKING INTERNEURONS IN THE REGULATION OF STRIATAL OUTPUT PATHWAYS

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The *striatum* is the main input *nucleus* of the basal *ganglia.* It receives excitatory inputs from the *cortex* and the *thalamus* that predominantly target the spines of medium-sized spiny neurons (MSNs), the mainneuronal cell-type and the projection neurons of the *striatum*. MSNs are segregated into two subpopulations based on projection targets and a variety of molecular markers. Although the actions of these two pathways are complex, a simplified model of the basal *ganglia* circuit proposes that the so-called « direct pathway » facilitates directed movements via the dMSNs, while the so-called « indirect pathway » terminates or suppresses movements via the iMSNs. Imbalances in the activity of both pathways are hypothesized to underline numerous movement disorders, including Parkinson disease, Huntington's disease, and dystonia. So, the output of the *striatum* depends on which MSNs are stimulated but also on their firing rates, which is mainly controlled through feedforward inhibition from local interneurons. Most inhibition is mediated by parvalbumin (PV)-expressing fast-spiking interneurons (FSIs). They provide inhibition to both direct and indirect pathway MSNs and are also interconnected with each other via electrical and chemical synapses. To understand how activity is coordinated within the *striatum*, it is essential to highlight the functional connectivity between the different neuronal types. In this study we focus on the GABAergic microcircuits of the mouse *striatum* by investigating the target selectivity of striatal FSIs using optogenetic techniques, enabling us to selectively stimulate FSIs with pulses of 470 nm blue light. This approach is simultaneously combined with electrophysiological measurements of inhibitory post-synaptic currents of MSNs using patch clamp recordings in acute brain slices.

P-06

INVOLVEMENT OF MAGED1 IN MOTOR BEHAVIOUR

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Melanoma antigen-encoding gene D1 (MAGED1) belongs to the large family of MAGE genes, first discovered as tumour markers. However MAGED1 is expressed in healthy tissues during embryogenesis and adulthood, including a strong expression in the central nervous system. To unravel the functions of MAGED1, we use a knockout mice model for MAGED1. We show that these mice display a diminution of spontaneous motor activity and a deficit in motor coordination. We hypothesize that those phenotypes can be due to a defect in *basal ganglia* functions. Indeed this system is known to be involved in the regulation of motor behaviour. We focus on the striatum, the main input structure of the *basal ganglia* system. First, using whole cell patch-clamp recordings in acute brain slices, we show a diminution of excitability of projection neurons of the striatum of MAGED1 knockout mice. Second, immunolabelling experiments show that MAGED1 knockout mice display a diminution in GABAergic interneurons density in the striatum and cerebral cortex. However this diminution is not correlated with a modification of the spontaneous GABAergic currents as recorded in striatal projection neurons. Third, we cross floxed MAGED1 mice with mice expressing the Cre recombinase under the control of the Dlx5/6 promoter, specific of telencephalic GABAergic neurons. The characterization of the resulting mice is running. Taken together our data show that MAGED1 is important for motor control and that MAGED1 inactivation alters striatal structure and physiology. We also plan to investigate the role of *nuclei* projecting to the striatum in phenotypes displayed by MAGED1 knockout mice.

P-07

A METABOLIC HYPOTHESIS IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is an adult disease characterized by a selective loss of motor neurons, resulting in progressive paralysis and death within 3-5 years. In several inherited cases, the disease is caused by point mutations in the gene encoding for superoxide dismutase 1 (SOD1) which promotes its aggregation, leading to biochemical damages, including mitochondrial dysfunction. Alteration of mitochondrial membrane potential and/or respiratory chain leads to ATP depletion and these metabolic dysfunctions could contribute to motor neuron death in patients and animal models of ALS. In motor-neurons, the combination of altered axonal transport with mitochondrial dysfunction likely leads to considerable decrease in ATP levels at distant neuromuscular junctions. Commonly know as the fuel gauge of mammalian cells, AMP-activated protein kinase (AMPK) is a key enzyme in the control of cellular ATP and energy homeostasis. In this study, we have investigated the consequences of ablating AMPK in mice overexpressing the mutated human SOD1 (hSOD1G93A), a commonly used experimental model of ALS, by breeding AMPK knock out mice with these hSOD1G93A mice. While we did not detect any difference in the lifespan of AMPK(-/-) / hSOD1G93A mice as compared to hSOD1G93A mice, we observed substantial alterations in the progression of the disease in selected behavioural tests. In particular, analysis of the gait (catwalk) which enables early detection of muscle weakness revealed that the onset was delayed by up to 30 days while the progression of the disease after onset was accelerated. Further studies will be conducted to establish the importance of ATP- in the disease, and to validate this enzyme as a putative pharmacological target in ALS and in other neurodegenerative diseases involving mitochondrial dysfunction.

P-08

MODE OF ACTION AND *IN VIVO* APPLICATIONS OF TRPM3 ANTAGONISTS

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According to the American Pain Society, pain is one of the most common symptoms why patients search medical attention. But currently available drugs do not guarantee adequate pain relief and are often linked to unwanted effects. The investigation of new drug targets and new analgesic agents, without undesirable effects, is therefore of major importance. A recent achievement in pain research was the identification of TRP channels as analgesic targets. TRPV1, an ion channel expressed in sensory neurons, was already fully validated as a target for pain treatment. Unfortunately, the TRPV1 antagonist development is strongly hampered by the appearance of hyperthermia in clinical trials. Recently, we have identified TRPM3 as a potential novel analgesic target since it is expressed in sensory neurons and is involved in the detection of noxious heat. The validation of TRPM3 as a potential target for the development of novel analgesia. To reach our goals we plan: 1) to characterize the structure-function relationships of TRPM3, 2) to identify new potent and selective TRPM3 antagonists and 3) to investigate their potential as therapeutic drugs. A high throughput screening was performed for identifying TRPM3 antagonists. Three different hit groups (CIM028885, CIM567411 and CIM046247) were selected based on IC_{50} values (respectively, 128 nM, 130 nM, and 241 nM), selectivity, ADME properties, toxicology and chemical design. Moreover, preliminary results indicate that the core structures of the selected hit groups exhibit significant selectivity towards TRPM3 compared to several related temperature sensitive TRP channels (e.g. TRPV1, TRPM8 and TRPA1). First analysis of the *in vivo* activity of selected compounds from all three inhibitor classes on TRPM3-related processes, showed significant (> 60%) reduction of the nociceptive response in mice after intraplantar PS injection. The obtained results validate TRPM3 as a potential target for the development of novel analgesia. The current project may provide a basis for the development of small molecule TRPM3 antagonists for use as analgesics in humans.

P-09

FUNCTIONAL CHARACTERIZATION OF TRP CHANNELS IN HUMAN ENDOMETRIUM

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Human endometrium is functionally dependent on its responsiveness towards female reproductive hormones**,** with substantial remodeling during the proliferative and secretory phases of the menstrual cycle, and plays a pivotal role in blastocyst implantation. Enhanced Ca^{2+} dynamics play an important role in the decidualization process in human endometrial stromal cells. The regulation of endometrial function is of major basic and clinical importance. Indeed, inappropriate endometrial function can lead to unsuccessful pregnancies and gynecological pathologies like endometriosis. Endometriosis is a common, chronic gynecological disease defined by the ectopic presence of endometrial glands and stroma, and is associated with infertility and pelvic pain. The study aims to investigate the functional role of Transient Receptor Potential (TRP) channels in human endometrium in health and in a specific gynaecological disease, endometriosis. The first part of the study exploring the expression level of TRP channels during different phases of the menstrual cycle, showed enhanced expression of TRPC4 and TRPV2. The second study, comparing the expression level of TRP channels in the endometrium between endometriosis and non-endometriosis patients, showed significant upregulation of TRPC4 expression in endometrium during menstruation phase form endometriosis patients compared to non-endometriosis patients. Enhanced expression level of the Ca^{2+} permeable TRPC4 channel could suggest an important role of TRPC4 during the process of decidualization. In addition, Ca^{2+} signaling by TRPC4 activation is involved in cell-cell adhesion processes (Graziani, et al.). Increased TRPC4 expression in endometriosis patients specifically during the menstrual phase may suggest a possible involvement of TRPC4 in the ectopic adhesion of endometrium in endometriosis patients.

P-10

Bcg 31.16, A PEPTIDE TOXIN DERIVED FROM THE SEA ANEMONE *BUNODOSOMA CANGICUM*, MODULATES THE GATING PROPERTIES OF K_V1 CHANNELS

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Natural peptide toxins are a rich source of channel modulators that act on the extracellular face of K_v channels, either at the external pore mouth or at the voltage-sensing domain (S3-S4 linker). As such, natural toxins provide us with highly selective and potent molecular probes to unravel the structure and function of K_v channels. Toxins binding to the external pore mouth partly or completely block the K^+ permeation while those binding to the VSD inhibit channel function by modifying channel gating. The *Shaker*-related K_v1 channels are targeted by several peptide toxins that block the outer pore mouth but no gating modifiers that shift the voltage-dependence of channel opening have been identified before. Furthermore, the K_v1.5 channel - an important target for the treatment of atrial fibrillation - has no known external peptide pore blockers, presumably due to the presence of a positively charged arginine residue in the outer pore mouth (R379, equivalent to Shaker T449). Bcg31.16 is a recently discovered peptide neurotoxin derived from the sea anemone *Bunodosoma cangicum* that inhibited several K_v1 subunits (potency in nM range). Bcg31.16 caused a concentration-dependent depolarizing shift in the voltage-dependence of channel opening; with 300 nM the shifts amounted to +35 mV and +12 mV for K_v1.3 and K_v1.5, respectively. The voltage-dependence of C-type inactivation displayed similar shifts, as well as the voltage-dependence of the gating kinetics. No significant effect on K_v2.1 was obtained at 1 µM. Thus, Bcg31.16 is a new gating modifier toxin of the K_v1 family and a novel peptide toxin to inhibit the K_v1.5 channel.

P-11

CONTROL OF NEURONAL EXCITABILITY BY CALCIUM BINDING PROTEINS: A NEW MATHEMATICAL MODEL FOR STRIATAL FAST-SPIKING INTERNEURONS

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Calcium binding proteins, such as parvalbumin (PV), are abundantly expressed in distinctive patterns in the central nervous system but their physiological function remains poorly understood. Notably, at the level of the striatum, where PV is only expressed in the fast spiking (FS) interneurons. FS interneurons form an inhibitory network modulating the output of the striatum by synchronizing medium-sized spiny neurons (MSN). So far the existing conductance-based computational models for FS neurons did not allow the study of the coupling between PV concentration and electrical activity. In this study, we propose a new mathematical model for the striatal FS interneurons that includes apamin-sensitive small conductance Ca^{2+} -dependent K^+ channels (SK) and the presence of a calcium buffer. Our results show that a variation in the concentration of PV can modulate substantially the intrinsic excitability of the FS interneurons and therefore may be involved in the information processing at the striatal level.

P-12

ANDROGEN DEPRIVATION MODULATES CALCIUM HOMEOSTASIS OF HORMONE-RESISTANT PROSTATE CANCER CELLS AND CONFERS RESISTANCE TO APOPTOSIS

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Treatment of advanced prostate cancer relies on pharmacological or surgical androgen deprivation. However, after a few months or years, the tumor relapses despite the absence of androgenic stimulation: a state referred to as hormone-refractory prostate cancer (HRPCa). Studying the mechanisms involved in androgen-independent phenotype development in a cell line derived from a HRPCa, we found that androgen depletion induced important modifications in Ca^{2+} homeostasis without causing cell death: both thapsigargin- and ionomycin-induced releases of Ca^{2+} from the endoplasmic reticulum (ER) were diminished, as well as store-dependent entry of Ca^{2+}; accordingly, using a ER-targeted Ca^{2+} probe, we observed that Ca^{2+} concentration in the ER was strongly reduced; Mn^{2+}-induced quenching of fura-2 demonstrated that Ca^{2+} influx through plasma membrane was also decreased. Moreover, we noticed a strong phosphorylation of IP3R1 at Ser1756 after androgen depletion. Phosphorylation of this residue by cyclic AMP-dependent protein kinase A (PKA) is known to regulate sensitivity of IP3R1 to IP3. Inhibition of PKA prevented androgen deprivation-induced IP3R1 phosphorylation and Ca^{2+} store content decrease. Similar inhibition was observed after a siRNA-mediated depletion of IP3R1. Interestingly, despite the well-known anti-apoptotic effects of IP3R knockdown, such inhibition of IP3R1 phosphorylation restored androgen deprivation-induced cell death. In parallel, we measured the expression of numerous genes involved in Ca^{2+} homeostasis (SERCA pumps, ryanodine receptor, ion channels…). We observed that, in these conditions, the expression of two TRP ion channels was modified: TRPM8 was ~100 times less expressed and TRPC1 was ~10 times more expressed. We found that TRPC1 overexpression participated in Ca^{2+} store content decrease. We are currently investigating the possible implication of ER calcium store content in resistance to apoptosis of HRPCa-derived cells.

P-13

LACTATE STIMULATES ANGIOGENESIS, PREVENTS ISCHEMIC SKELETAL MUSCLE ATROPHY, AND ACCELERATES WOUND HEALING

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Wounds notoriously accumulate lactate as a consequence of both anaerobic and aerobic glycolysis following microcirculation disruption, immune activation and increased cell proliferation. A number of evidence from our work and the work of others suggests that lactate actively participates in the healing process through the activation of several molecular pathways collectively promoting angiogenesis (*i.e.*, endothelial cell migration, tube formation, the recruitment of circulating vascular progenitor cells and vascular morphogenesis). Whether these activities of lactate may be exploited therapeutically has never been demonstrated. In this study, we showed that, by inducing reparative angiogenesis and independently from its use as an energetic fuel, lactate improves reperfusion and opposes muscular atrophy in a mouse model of ischemic hindlimb injury. Conversely, impairing lactate flux with inhibition of monocarboxylate transporter 1 (MCT1, a transporter expressed at the plasma membrane of endothelial cells where it facilitates lactate uptake) strongly deregulated this process, further stressing out the role of lactate in physiopathology. We therefore decided to develop lactate as a wound-accelerating treatment. Lactate in the drinking water failed to increase plasma lactate levels in mice. We developed another strategy and found, using intravital microdialysis, that subcutaneous implants of poly-*D*,*L* lactide-co-glycolide (PLGA) allow sustained local and systemic lactate release. Implants made of poly-*L*-lactide (PLA) did not. As expected from our preliminary experiments using lactate-containing Matrigel, PLGA promoted angiogenesis and accelerated the closure of excisional skin wounds in different mouse strains. This polymer is FDA-approved for other applications, emphasizing the possibility of exploiting PLGA therapeutically to improve wound healing.

P-14

EFFECTS OF BM-573 ON ENDOTHELIAL FUNCTION AND INCREASED BLOOD PRESSURE AT EARLY STAGES OF ATHEROSCLEROSIS

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Numerous studies have emphasized the pivotal role of endothelial dysfunction in the development, progression or clinical complications of atherosclerosis. Such dysfunction has been clearly documented in plaque prone vessels of human or in animal models of dyslipidemia, but it remains to be characterized in the resistance vasculature. In the last decade, increasing evidences suggest that dual inhibition of thromboxane synthase (TxAS) and thromboxane receptors (TP-receptors) antagonism may not only have anti-platelet effects but also impact the inflammatory component of atherosclerosis. However, our knowledge about the beneficial effects of this dual inhibition on endothelial function is limited. Therefore the principal aim of this study was to characterize the effects of combined inhibition of thromboxane synthase (TxAS) and antagonism of thromboxane receptors (TP) with BM-573 on endothelial dysfunction in the resistance vasculature of apolipoprotein E-deficient (ApoE-KO) at early stage of atherosclerosis. We evaluated the effects of acute (3 µM, 1hour) and chronic (10mg/L, 8weeks) BM-573 treatment on endothelial function in resistance mesenteric arteries, nitric oxide (NO) bioavailability, oxidative stress and systolic blood pressure from 15 weeks old ApoE-KO mice and their wild-type litter-mate (C57Bl/6J). Mesenteric microarteries (140-160 µm) were used to evaluate endothelial function in a pressure myograph. NO bioavailability and Akt/eNOS pathway were assayed by electron paramagnetic resonance (EPR) spectroscopy and Western blotting, respectively. Vascular reactive oxygen species (ROS) production was also analyzed by EPR and dihydroethidium (DHE) assay. Finally, systolic blood pressure (SBP) and heat rate (HR) were measured by implanted telemetry. ApoE-KO mice present an impaired endothelium-dependent vasodilatory response to Ach in ApoE-KO versus C57Bl/6J ($P<0.001$). This dysfunction arises from an increased production of endothelium-derived contractile factors combined to an altered endothelium-derived vasodilation. BM-573, administrated acutely or chronically prevents the endothelial dysfunction observed in ApoE-KO vasculature. Acute effects of BM-573 result from an improved eNOS/NO pathway through increased phosphorylation of eNOS and its activated regulator Akt. Chronic effects of BM-573 treatment on endothelial function are mainly due to a reduction of oxidative stress and improvement of NO bioavailability. Finally, our results showed that ApoE-KO mice present a significant and time-dependent increase of systolic blood pressure concomitant to a slight decrease of heart rate. We showed that BM-573 was able to prevent systolic blood pressure and heart rate modifications in ApoE-KO mice. In conclusion, our study shows that BM-573 prevents and corrects endothelial dysfunction in resistance arteries via an up-regulation of the eNOS/NO pathway. Together with the previous reports showing a prevention of plaque progression by BM573 in the same mouse model, it provides additional support to the hypothesis that a combined antagonism of TP receptors and TxAS inhibition is a rational therapeutic approach to prevent the vascular deleterious consequences of atherogenesis.

P-15

H_2O_2 CONSUMPTION BY *CANDIDA ALBICANS* FROM DENTURES

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H_2O_2-peroxidase systems are active *in vitro* on *Candida* proliferation in oral biofilms. The presence of H_2O_2 substrate is then critical for antifungal effect. This could be removed by enzymes such as catalase from yeast cells themselves. The present study aims at evaluating the H_2O_2 consumption by blastoconidia cells as a limitation for using H_2O_2 peroxidase systems in denture decontamination from yeasts. Investigations were conducted on a third subculture of *Candida albicans* ATCC 10231 and on clinical samples isolated from 7 dentures. Strains were grown aerobically at 37℃ on Sabouraud-chloramphenicol agar. Yeasts were suspended in phosphate buffer (0.1 M, pH 7 with 0.1 g/L glucose) and adjusted to a concentration of 20 x 10^6 *Candida* cells (blastoconidia count). *Candida albicans* was identified by colony aspect on CHROMagar™ medium, by chlamydoconidia formation on PCB agar and by API yeast identification system. H_2O_2 degradation by yeast suspensions was evaluated spectrophotometrically at 230 nm. Characteristics of the method: analytical range from 5 to 30 mM, coefficient of accuracy and of variation inferior to 1.5 and 5% respectively, initial H_2O_2 concentration of 10.2 mM. H_2O_2 consumption rate by ATCC 10231 *Candida albicans* was 12.1 ± 2.7 nanomoles x min^{-1} per 10^6 blastoconidia (mean ± SD, N = 8). Data from 7 clinical strains ranged from 5.5 to 22.3 nanomoles x min^{-1} per 10^6 cells. H_2O_2 consumption did not vary in 3 serial subcultures (ANOVA, NS, p: 0.8442). Increase of glucose concentration from 0.1 to 10 or 20 g/L multiplied H_2O_2 disappearance rate by a factor of 1.5 and 1.8 respectively. ATCC 10231 *Candida* survival rate in the presence of H_2O_2-KI- peroxidase during 30 minutes was shown effectively dependent of blastoconidia count. Indeed, suspensions with more than 50 x 10^6 blastoconidia need a higher H_2O_2 intake for killing than suspensions with less than 15 x 10^6 blastoconidia. In conclusion, H_2O_2 supply in peroxidasic systems has to overpass its consumption by *Candida* cells themselves.

P-16

A TENTATIVE MODEL FOR D-GLUCOSE TURN-OVER IN HUMAN SALIVA

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The aim of the present study is to propose a tentative model for D-glucose turn-over in human saliva. The whole saliva and the saliva from submandibulary/sublingual glands, were collected by use of the Salivette™. The saliva glucose concentration was measured by hexokinase method, saliva bacteria glycolysis by use of D-[5-^{3}H] glucose, and the saliva ATP content by luciferase method. The concentration of glucose amounted to 43.9 ± 6.3 (n=29), 104.0 ± 12.4 (n=27) µM in whole saliva and submandibulary/sublingual saliva respectively. The rate of D-glucose utilization by oral bacteria at a physiological concentration of D-glucose in saliva (50 µM) was estimated at 0.047 ± 0.003 (n=11) nmol/min per 10^{6} bacteria. Resting salivary D-glucose turn-over rate, as calculated from the amount of glucose secreted from saliva which comes from parotid and submandibulary and sublingual glands represented 214.6 ± 19.1 %/min. In order for salivary D-glucose production to match bacterial utilization of the hexose, the total number of oral bacteria was estimated at about 2.0 x 10^{9} bacteria, in fair agreement with previously published data. This study, thus, provides support for a tentative model for D-glucose turn-over in human saliva.

P-17

A LOW COST, OPEN-SOURCE LIPID BILAYER SETUP FOR HANDS-ON LEARNING OF BIOPHYSICS

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Although people are generally interested in how the brain functions, neuroscience education is hampered by a lack of low cost and engaging teaching materials. To address this, we developed an open-source lipid bilayer amplifier which is appropriate for use in introductory courses in biophysics or neurosciences. The amplifier is designed using the common lithographic printed circuit board fabrication process and off-the-shelf electronic components. In addition, we propose a specific design for experimental chambers allowing the insertion of commercially available polytetrafluoroethylene film (Teflon®). This device can be used in easy experiments in which students monitor the bilayer formation by capacitance measurement and record unitary currents produced by ionophores like gramicidin A. Used in combination with a low-cost data acquisition board this system provides a complete solution for hands-on lessons, therefore improving the effectiveness in teaching basic neuroscience or biophysics.