BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL

PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting

Saturday, March 27 2010

A B S T R A C T B O O K

Organisation

Prof. Dr. B. Flamion
Prof. Dr. N. Caron
Facultés Universitaires Notre-Dame de la Paix
University of Namur
Molecular Physiology Research Unit (URPHYM)
Laboratory of Physiology and Pharmacology
61, rue de Bruxelles
5000 Namur

BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Spring Meeting
Saturday March 27 2010

Facultés Universitaires Notre-Dame de la Paix
Faculté de Médecine
Auditoire M5
61, rue de Bruxelles
(entrée rue J. Grafé)
5000 Namur

Main Lecture

10.00-11.00 Prof. Dr. Jeremy HUGHES (University of Edinburgh, Queens Medical Research Centre, Edinburgh, United Kingdom)

Inflammatory cells: dual players in tissue injury and repair.

Oral Communications

11.00-11.15 N. NKEJABEGA, R. AXTON, J. WILSON, H. TAYLOR, L. FORRESTER, D. KLUTH, J. HUGHES (Univ. Edinburgh, United Kingdom), supported by N. CARON (FUNDPNamur)
Embryonic stem cell-derived macrophages – a novel approach to developing anti-inflammatory macrophages for cell therapy.

11.15-11.30 D. DE GEYTER, W. STOOP, S. SARRE, R. KOOIJMAN (VUBrussel)
A new preclinical stroke model to study the effects of the neuro-protective peptide IGF-I on focal cerebral ischemia.

11.30-11.45 A-G. CEULEMANS, T. ZGAVC, R. KOOIJMAN, S. HACHIMI-IDRISSI, S. SARRE , Y. MICHOTTE (VUBrussel)
The effect of mild hypothermia on gliosis after an endothelin-1 induced transient focal cerebral ischemia in male Wistar rats.

11.45-12.00 J. PORTELLI, N. AOURZ, D. DE BUNDEL, A. MEURS, I. SMOLDERS, Y. MICHOTTE, R. CLINCKERS (VUBrussel)
Intrastrain differences in seizure susceptibility, pharmacological response and basal neurochemistry of Wistar rats.

12.00-12.15 S. GOURSAUD, M. FOCANT, J. BERGER, J.M. MALOTEAUX, E. HERMANS (UCLouvain)
Reduction of caspase-3 activity upregulates the glutamate transporter GLT-1 in cultured callosal astrocytes from a rat model of amyotrophic lateral sclerosis ($hSOD1^{G93A}$).

12.15-12.30 M. FOCANT, S. GOURSAUD, Y. NIZET, E. HERMANS (UCLouvain)
Regulation of the expression of glutamate transporter GLT-1 splice variants in primary cultures of astrocytes.

12.30-12.40 **Bullet Session**
Oral presentation of poster n°1, 2

12.40-13.30 **Lunch and Poster Session**

Oral Communications

13.30-13.45 K. DECALUWE, S. NIMMEGEERS, H. COPPENS, R. THOONEN, P. BROUCKAERT, J. VAN DE VOORDE (UGent)
In vivo experiments using soluble guanylyl cyclase beta1 His 105 Phe mutant mice: NO-vasodilatation and penile erection fully dependent on activation of sGC.

13.45-14.00 E. PRIEM, R.A. LEFEBVRE (UGent)
Characterization of intrinsic neurogenic excitatory and inhibitory motor responses in porcine distal colon.

14.00-14.15 E. BOCKSTEINS, G. VAN DE VIJVER, T. BRUYNS, P.P. VAN BOGAERT, D.J. SNYDERS (UAntwerpen)
Kv3.x subunits contribute substantially to the delayed rectifier K^+ current (I_K) in small cultured DRG neurons.

14.15-14.30 S. DILLY, C. LAMY,J.-F. LIEGEOIS, V. SEUTIN (ULiège)
Combined experimental and computational approaches to study the action of blockers of small conductance calcium-activated potassium (SK) channels.

14.30-14.45 G. DRION, A. COLLARD, R. SEPULCHRE, V. SEUTIN (ULiège)
SK Channels as regulators of synaptically-induced bursting and neural synchrony.

Posters
(height 120 cm – width 120 cm)

1. M. BARAKA, L. LEEMANS, D. COOMANS, S. STEURBAUT, M. LAUBACH, E. JANSEN, A.G. DUPONT (VUBrussel)
Ethnicity and use of medication in pregnant immigrated women.

2. N. MAENHAUT, C. BOYDENS, J. VAN DE VOORDE (UGent)
Different vasoactive effect of adherent adipose tissue during hypoxia in mice aorta and mesenteric arteries.

3. N. ZANOU[1], Y. IWATA[2], O. SCHAKMAN[1], J. LEBACQ[1], S. WAKABAYASHI[2], P. GAILLY[1] (UCLouvain[1], National Cardiovasc. Center Research Institute Suita, Osaka, Japan [2])
Role of TRPV2 ion channel in the physiopathology of Duchenne muscular dystrophy.

4. J. BERGER, E. HERMANS (UCLouvain)
Neuroinflammation triggers regulations of glutamatergic targets in astrocytes : implication in neurological diseases.

5. D. ORDUZ, P. BISCHOP, B. SCHWALLER, S.N. SHIFFMANN, D. GALL (ULBruxelles)
Calcium binding protein parvalbumin regulates the electroresponsiveness of striatal fast spiking interneurons.

6. M. BOL, M. DE BOCK, E. DE VUYST, N. WANG, E. DECROCK, J. MONSALVO, K. DECALUWE, B. VANHEEL, J. VAN DE VOORDE, L. LEYBAERT (UGent) Hemichannel involvement in Ca^{2+} dynamics and contractililty of smooth muscle cells in acutely isolated small mesenteric arteries.

A B S T R A C T S

O-01

EMBRYONIC STEM CELL-DERIVED MACROPHAGES - A NOVEL APPROACH TO DEVELOPING ANTI-INFLAMMATORY MACROPHAGES FOR CELL THERAPY

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Renal ischemia/reperfusion injury (IRI) is the major cause of acute renal failure (ARF) and is still associated with high morbidity and mortality. The enzyme hemeoxygenase-1 (HO-1) is upregulated in response to various cell stresses such as hypoxia. HO-1 metabolises haem-containing proteins derived from injured cells etc to carbon monoxide (CO) and biliverdin (rapidly converted to bilirubin by biliverdin reductase). CO inhibits platelet aggregation and exerts anti-apoptotic effects whilst bilirubin is a powerful anti-oxidant. Our recent work suggests that deficient upregulation of HO-1 plays a key role in the increased susceptibility of aged mice to ARF following renal IRI (manuscript under review at JASN). Also, the administration of bone marrow-derived macrophages (BMDM) induced to overexpress HO-1 by adenoviral transduction is protective in murine renal IRI (revised manuscript under review, Molecular Therapy). This project has adopted an embryonic stem (ES) cell approach to develop ES cell-derived macrophages (ESDM) overexpressing HO-1 for therapeutic use in experimental models of ARF.

An ES cell line (E14 IV) was induced to form embryoid bodies and the non-adherent cells harvested and cultured in the presence of M-CSF and IL-3 for 7 days to form ESDM. The phenotype of ESDM was compared to BMDM and bone marrow derived dendritic cells (BMDC) by assessment of morphology, expression of cell surface markers (F4/80, CD11b, CD11c and MHC Class II) by flow cytometry and evaluation of their capacity to phagocytose fluorescent latex beads. ES cells with constitutive overexpression of HO-1 were generated by cloning HO-1 cDNA into a pCAG vector, which randomly integrated into the ES cell genome. HO-1 expression level was determined by western blot analysis and ESDM generated from selected HO-1^{HI} and HO-1^{LOW} expressing ES cell clones.

We successfully generated functional ESDM *in vitro*. ESDM were characterised by a large mononuclear cell morphology. ESDM exhibit a cell surface phenotype comparable to BMDM rather than BMDC and are F4/80^{HI}CD$11b^{HI}$CD$11c^{LOW}$MHC class II^{LOW}. ESDM are significantly phagocytic and readily ingest latex beads. However, cell count and microscopic analysis indicated that constitutive overexpression of HO-1 in ES cells using the pCAG system resulted in the generation of very few ESDM. Current work is examining whether the detrimental effect of HO-1 overexpression is upon ES cell self renewal or the process of macrophage differentiation.

Our work demonstrates that functional macrophages may be generated from ES cells. Since the randomly inserted pCAG vector may inadvertently silence key genes such as those involved in ES cell self-renewal and/or macrophage differentiation, our future work will use a more precise and targeted tetracycline inducible system that integrates at the HPRT locus. Also, studies of ESDM localisation to injured kidneys are in progress.

O-02

A NEW PRECLINICAL STROKE MODEL TO STUDY THE EFFECTS OF THE NEUROPROTECTIVE PEPTIDE IGF-I ON FOCAL CEREBRAL ISCHEMIA

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Introduction: Stroke is the first cause of morbidity in the European Union. The only approved acute therapy is stimulation of reperfusion by treatment with tissue-type plasminogen activator. The ischemic cascade comprises, among others, excitotoxic effects, neuroinflammation and cell death by necrosis or apoptosis. The inflammatory response may contribute to injury and can exacerbate tissue damage. Insulin-like growth factor (IGF)-I is a pleiotropic factor that stimulates the proliferation and differentiation of oligodendrocytes, myelinisation, synaptogenesis and the survival of neurons and glial cells. In addition, IGF-I may be neuroprotective in animal models of focal cerebral ischemia. Since IGF-I modulates cytokine expression in the immune system, it could thus also be possible that IGF-I influences inflammatory responses in the brain. For successful translation to clinical studies the Stroke Therapy Academic Industry Round Table (STAIR)-criteria are essential. Two important criteria are testing of drugs in conscious animals with a co morbidity factor such as hypertension. *Aims:* To study the mechanisms by which IGF-I exerts its neuroprotective effects, a preclinical relevant rat model will be used. Optimisation of the Et-1 model was necessary to study the effects of IGF-I in conscious Spontaneous Hypertensive Rats (SHRs) and in their controls, the Wistar Kyoto (WKY) rats. A second study addresses the effects of IGF-I in a lipopolysaccharide (LPS) rat model for neuroinflammation and the in vitro effects of IGF-I on cytokine production by astrocytes. *Material and methods:* Using a stereotactic frame, Et-1 was applied in the vicinity of the middle cerebral artery of control and SHRs. A dose-range finding study was carried out applying 120-240 pmol Et-1. Motor/sensory functions were measured 1, 6 and 24 hours after the insult using the Neurological Deficit Score. The infarct size was assessed by cresylviolet staining. LPS was administered in the striatum of Wistar CRL rats. For these studies, the activation of microglia and astrocytes in the striatum was investigated by immunohistochemistry using antibodies directed against ED-1 and glial fibrillary acidic protein (GFAP). The effects on IL 8 secretion were determined in vitro using ELISA. *Results:* A dose of 120 pmol Et-1 had no effect in WKY rats; doses of 180 and 200 pmol induced an appropriate infarct size and motor/sensory deficits but only a dose of 200 pmol produced an infarct in both striatum and cortex. The dose of 240 pmol showed a high mortality rate. For the SHRs there was a significant increase in infarct size using a dose of 180 (33%, $p < 0.01$) and 200 (26%, $p < 0.05$) pmol Et-1 compared to the WKY rats. In the LPS model preliminary experiments showed that IGF-I increased the number of activated microglia (ED-1^+ cells), but not to a significant degree. In vitro IGF-I stimulated the secretion of IL-8 by astrocytes . *Conclusion:* Using a dose of 200 pmol Et-1 in SHR rats we will further investigate the possibility to use IGF-I as a neuroprotective agent in stroke, and the possible role of immunomodulatory effects of IGF-I in neuroprotection.

O-03

THE EFFECT OF MILD HYPOTHERMIA ON GLIOSIS AFTER AN ENDOTHELIN-1 INDUCED TRANSIENT FOCAL CEREBRAL ISCHEMIA IN MALE WISTAR RATS

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Microglia contribute to the inflammatory response after ischemic stroke. Mild hypothermia (33°C) is a promising neuroprotective strategy and this study investigated whether such neuroprotection in the endothelin-1 (Et-1) rat model for transient focal cerebral ischemia, is associated with changes in gliosis. Therefore, Et-1 was infused near the middle cerebral artery in male Wistar rats which were subjected to 2 hours of mild hypothermia, started 20 minutes after Et-1 injection. They were compared with normothermic rats (37°C). Besides assessing functional outcome and infarct volume, the activation of microglia and astrocytes was evaluated using immunohistochemistry for CD-68 and glial fibrillary acidic protein (GFAP) respectively, both at the level of the core (striatum) and the penumbra (cortex). All parameters were determined up to one week after the insult. Et-1 administration caused neurological deficit and a reproducible infarct size. Both parameters were significantly reduced by hypothermia. There was a gradual increase in CD-68 expression up to 1 week after stroke onset. Hypothermia resulted in an attenuated expression of CD-68 compared to normothermic rats 8, 24 hours and 1 week after the insult. Surprisingly, at 3 days after the insult, hypothermia resulted in a 5-fold increase of CD-68 expression in both the core and the penumbra. GFAP expression peaked 1 day after the insult, after which it gradually decreased. Hypothermia significantly attenuated this response both in the core and the penumbra at 24 and 72 hours after the insult, but had no effect on the expression of GFAP at other time points. These data suggest that the beneficial effects of hypothermia after stroke on infarct volume and functional outcome may, at least partly, be mediated by inhibition of astrogliosis and microglial activation.

O-04

INTRASTRAIN DIFFERENCES IN SEIZURE SUSCEPTIBILITY, PHARMACOLOGICAL RESPONSE AND BASAL NEUROCHEMISTRY OF WISTAR RATS

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Reliable well-characterised animal models of seizures are necessary in order to better understand the underlying pathophysiological mechanisms as well as to screen potential anticonvulsant drugs. We currently use the focal pilocarpine model as an acute limbic seizure model. Due to breeding problems at the vendor, and apparent changes in pilocarpine-induced seizure susceptibility, we were forced to change breeding locations and vendors over a period of 2 years. Male Wistar rats were either purchased from 2 breeding locations of Charles River Laboratories (France and Germany), or obtained from Harlan Laboratories (The Netherlands). In the present retrospective study we evaluated the impact of these vendor changes on ketamine dosing to establish anaesthesia, on pilocarpine-induced seizure susceptibility, and on basal extracellular hippocampal noradrenaline, dopamine, serotonin, γ-amino butyric acid, and glutamate levels of all pilocarpine-treated rats included in our studies. Significant differences were present in all of the parameters analysed. This study clearly illustrates that intrastrain differences do exist from one vendor/breeding location to another, or even between rats from the same breeding location.

O-05

REDUCTION OF CASPASE-3 ACTIVITY UPREGULATES THE GLUTAMATE TRANSPORTER GLT-1 IN CULTURED CALLOSAL ASTROCYTES FROM A RAT MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (HSOD1^{G93A})

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Amyotrophic lateral sclerosis (ALS) is typically characterized by a dramatic loss of lower motor neurons in spinal cord and brainstem. Nevertheless, upper motor neuron dysfunction is also reported in ALS and frequently related to a deficit of transcallosal connections associated to a reduced volume of the corpus callosum. Besides, impairment of the astroglial glutamate transporter GLT-1 associated with accumulation of extracellular glutamate is demonstrated in ALS and related excitotoxicity is likely to participate in the progression of the disease. At the molecular level, the caspase-3-mediated cleavage of GLT-1 leading to a selective and functional inhibition of the transporter was evidenced in spinal cord samples from a transgenic mice model of ALS. We herein characterised the expression and activity of GLT-1 and caspase-3 in cultured callosal astrocytes isolated from a transgenic rat strain expressing an ALS-related mutated form of human superoxide dismutase 1 (hSOD1^{G93A}). Quantitative RT-PCR and Western-blotting studies revealed that the expression of GLT-1 was higher in the cells prepared from the transgenic animals in comparison to the wild-type rats. However, specific measurements of D-[^{3}H]-aspartate uptake velocity failed to evidence differences in the activity of this transporter. Measures of uptake were also performed in the presence of a selective caspase-3 inhibitor (Ac-Asp-Met-Gln-Asp-aldehyde) or in cells exposed to the Peptide Histidine Isoleucine (PHI) which belongs to VIP/PACAP neuropeptide family and which decreases caspase-3 activity. Reducing the activity of this apoptotic enzyme, which is highly detected in callosal astrocytes from hSOD1^{G93A} rats, was found to upregulate the GLT-1 activity, exclusively in cells from transgenic animals. Together, these findings reinforce the hypothesis of an involvement of caspase-3-mediated impairment of glutamate uptake in the pathogenesis of ALS.

O-06

REGULATION OF THE EXPRESSION OF GLUTAMATE TRANSPORTER GLT-1 SPLICE VARIANTS IN PRIMARY CULTURES OF ASTROCYTES

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Glutamate transporters are key actors in the clearance of this excitatory neurotransmitter from the synaptic cleft in the central nervous system (CNS). So far, 5 subtypes of glutamate transporters have been described: Glutamate transporter-1 (GLT-1), Glutamate-Aspartate transporter (GLAST), Excitatory Amino Acid Carrier 1 (EAAC1) and Excitatory Amino Acid Transporters 4 and 5 (EAAT4, EAAT5). These transporters are present both on the surface of neuronal and glial cells. Nevertheless, most of the glutamate uptake is supported by GLT-1 and GLAST which are principally present on the surface of astrocytes, suggesting a key role for those cells in glutamate homeostasis in the CNS. Indeed, the persistence of elevated glutamate levels in the synaptic cleft leads to excitotoxic damages to neuronal cells. A few years ago, alternative splicings of the GLT-1 transporter have been described. These splicings lead to the generation of several transporter variants with distinct intracellular amino or carboxy-terminals or to transporter isoforms lacking internal strectches of the peptide sequence. In the literature, a variety of biochemical and pharmacological agents are known to upregulate or downregulate the activity of the GLT-1 promoter or the production of GLT-1a, the originally cloned isoform. However, little is known regarding the influence of these drugs on the alternative splicing of GLT-1. In this study, we focused our interest on the splicing of GLT-1 carboxy-terminus which generates the best characterized GLT-1a and GLT-1b isoforms. We have previously reported on an altered expression of these isoforms in a model of amyotrophic lateral sclerosis where excitotoxicity appears to play a key role. We incubated primary cultures of astrocytes during 3, 7 or 10 days in the presence of selected cytokines, growth factors or agonists of glutamate targets and evaluated the relative gene expression of these two isoforms by quantitative PCR. We have also developed a set of monoclonal antibodies that specifically recognize the two isoforms and these original tools will be used for investigating the expression profile of GLT-1 isoforms in astrocytes exposed to defined environments.

O-07

IN VIVO EXPERIMENTS USING SOLUBLE GUANYLYL CYCLASE BETA$_1$ HIS 105 PHE MUTANT MICE: NO-VASODILATATION AND PENILE ERECTION FULLY DEPENDENT ON ACTIVATION OF SGC

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The nitric oxide/cyclic guanosine phosphate pathway plays a pivotal role in vasodilatation and as such also in penile erection. Because of its central role in this molecular pathway, sGC represents a very attractive and promising new target for the development of new treatments for hypertension and/or erectile dysfunction. The cloning of sGC from various species has revealed that the protein is a heterodimer composed of a larger α and a smaller β subunit, which are both required for catalysis. Although two α and two β isoforms have been characterised so far, only the sGC$\alpha_1\beta_1$ and the sGC$\alpha_2\beta_1$ isoforms have been shown to occur in vivo. To establish the functional role of sGC and its different isoforms in the mechanism of vasodilatation and penile erection we performed in vivo studies on β_1 His 105 Phe transgenic mice (sGC$\beta_1^{ki/ki}$ mice) and their littermates. Different agents were injected either intravenously or intracavernosally and the changes in mean arterial pressure (MAP) and intracavernosal pressure (ICP) were recorded in the anesthetized mice. Intravenous and intracavernosal injection of exogenous NO (SNP – Spermine/NO) resulted in a decrease of MAP and an increase in ICP respectively in the wild-type controle mice. These responses are however completely abolished in sGC$\beta_1^{ki/ki}$ mice. Intravenous administration of L-NAME which induced an increase in MAP in sGC$\beta_1^{+/+}$ mice, had no effect when injected in sGC$\beta_1^{ki/ki}$ mice. Stimulation of the nervus cavernosus induced frequency-dependent increases in ICP in sGC$\beta_1^{+/+}$ mice but again no response could be observed in sGC$\beta_1^{ki/ki}$ mice. Responses to the sGC-independent agents forskolin and 8-pCPT-cGMP which were injected intracavernosally did not differ between the sGC$\beta_1^{ki/ki}$ mice and the sGC$\beta_1^{+/+}$ mice. These studies indicate that the NO-dependent vasodilatation and NO-dependent induction of penile erection is fully dependent on sGC. By comparing the results from this study with results obtained from a previous study using sGC$\alpha_1^{-/-}$ mice (were a remaining response could be observed to both exogenous and endogenous NO) we provide strong evidence for the contribution of the less abundantly expressed sGC$\alpha_2\beta_1$ isoform in the mechanism of vasodilatation and penile erection. The unaltered responses to sGC-independent agents confirm the specificity of the impaired sGC-related responses observed in sGC$\beta_1^{ki/ki}$ mice.

O-08

CHARACTERIZATION OF INTRINSIC NEUROGENIC EXCITATORY AND INHIBITORY MOTOR RESPONSES IN PORCINE DISTAL COLON

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In human colon, 5-HT_4 receptors have been described on excitatory cholinergic nerves but also on inhibitory nitrergic nerves underlying the colonomimetic effect of 5-HT_4 receptor agonists. Therefore intrinsic neurogenic excitatory and inhibitory motor responses in porcine distal colon were characterized in order to evaluate this organ as a possible model for 5-HT_4 receptor agonism. After removal of the mucosa, circular smooth muscle strips were obtained from the distal colon of young male pigs (Seghers – breed Line 12; 10-12 weeks; 20-25 kg) and mounted in isometric conditions between 2 platinum electrodes under a load of 2g in 5 ml organ baths with Krebs Henseleit solution containing 4.10^{-6}M guanethidine to avoid noradrenergic influences. Electrical field stimulation (EFS) was applied with 10s trains at 0.25ms, 4Hz and a voltage range of 5-50V. Responses were measured at the end of the 10s stimulation train. At basal tone, EFS (10-50V) only induced off-contractions. In the combined presence of the NO synthase inhibitor N^G-L-arginine methyl ester (L-NAME, 3.10^{-4} M) and the SK channel blocker apamin (5.10^{-7}M), EFS systematically induced voltage-dependent on-contractions. These on-contractions were abolished by the neuronal conductance blocker tetrodotoxin (TTX, 3.10^{-6} M), largely reduced by the muscarinic receptor antagonist atropine (10^{-6}M) and not influenced by a combination of tachykinin receptor antagonists (NK_1, 10^{-5}M FK888; NK_2, 10^{-6}M MEN10627; NK_3, 3.10^{-7}M SB222200) illustrating activation of cholinergic neurones. To study relaxant responses, EFS (5-50 V) was performed in the continuous presence of atropine (10^{-6}M) in strips contracted with 10^{-7}M substance P. This yielded voltage-dependent relaxations, maximum being reached at 15 or 20V. The responses with EFS at 5 to 15 V were abolished by TTX. Neither L-NAME nor apamin alone influenced the relaxations but L-NAME plus apamin abolished the relaxations with EFS at 5-10V and reduced them at higher voltages. A moderate reduction was also observed with the P2Y1 receptor antagonist MRS2179 (10^{-5}M). These results suggest a redundant action of NO and ATP as inhibitory neurotransmitters. In *conclusion*, neurogenic pure cholinergic responses can be obtained in pig distal colon, but not nitrergic ones as ATP seems to compensate when NO is switched off.

O-09

KV3.X SUBUNITS CONTRIBUTE SUBSTANTIALLY TO THE DELAYED RECTIFIER K^+ CURRENT (I_K) IN SMALL CULTURED DRG NEURONS

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Voltage-gated potassium (Kv) channels serve a wide range of functions in both excitable and non-excitable cells. In neurons these include the regulation of the resting membrane potential and control of the shape, duration and frequency of action potentials. The large number of Kv subunits presents a challenge to determine the molecular composition of the native currents. We attempted to identify the Kv subunits underlying the delayed rectifier current (I_K) in cultured small mouse dorsal root ganglia (DRG) neurons. Using extracellular Stromatoxin (ScTx) and intracellular Kv2.1 antibodies we recently reported that approximately 60% of the I_K current in these DRG neurons is carried by both homotetrameric Kv2.1 and heterotetrameric Kv2.1/silent Kv channel complexes. The 40% of I_K remaining after ScTx (100 nM) pretreatment, was reduced with 1 mM extracellular TEA (n = 6) indicating that this part of the I_K current could be represented by the Kv subunits Kv1.1, Kv3.1, Kv3.2 and/or Kv3.3, and possibly a fraction of KCNQ2 and KCNQ2/3 channels, which underlie the M-current in small DRG neurons. Using channel specific toxins we determined the contribution of each channel to the remaining 40% of I_K. Furthermore, we detected the presence of Kv3.1, Kv3.2 and Kv3.3 mRNA using RT-PCR in freshly isolated DRG. These observations support a substantial role of at least the Kv3.x subunits in small DRG neurons which are visceral and somatic sensory neurons that conduct information about temperature, pressure and touch.

O-10

COMBINED EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO STUDY THE ACTION OF BLOCKERS OF SMALL CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM (SK) CHANNELS

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Small conductance calcium-activated potassium channels (SK) are widely expressed throughout the central nervous system (CNS) and underlie medium duration afterhyperpolarizations in many types of neurons. Three subtypes of SK channels, SK1, SK2 and SK3, have been identified so far in different parts of the brain. Blocking SK channels might be beneficial in the treatment of several CNS disorders such as depression, Parkinson's disease and cognitive disorders. Until now, the precise site of interaction between these channels and their blockers has not yet been elucidated. In this context, molecular modeling is a theoretical approach that can quickly provide ideas on the binding mode of SK blockers. We first performed homology modeling of the S5-H5-S6 portion of the channels on the basis of the crystal structure of the KcsA potassium channel (Zhou et al. Nature. 2001, 414, 43-48). The binding sites of *N*-methyl-laudanosine (NML) (Scuvée-Moreau et al. J. Pharmacol. Exp. Ther. 2002, 302, 1176-83), a non-selective and non-peptidic ligand, and apamin (Blatz et al. Nature. 1986, 323, 718-20), an octadecapeptide with a preference for the SK2 subtype, were subsequently explored by docking analysis. Different amino-acids were suggested to interact with the two blockers. The docking of NML revealed a binding site in the turret region, far from the pore. The docking of apamin identified a very large binding site that includes a portion of the site of NML. In order to confirm the predicted binding sites, site-directed mutagenesis was used. The first mutant channels tested in electrophysiological experiments by the patch clamp technique validated some of the theoretical data. Using this strategy, we hope to get a better understanding of the mechanism of action of SK blockers and eventually find strategies to obtain subtype-selective blockers

O-11

SK CHANNELS AS REGULATORS OF SYNAPTICALLY-INDUCED BURSTING AND NEURAL SYNCHRONY

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Although the central nervous system is composed of neurons with variable electro-physiological phenotypes, several groups of cells show qualitative similarities in their electrical behavior. For example, pacemaker neurons such as dopaminergic and serotoninergic neurons are spontaneously active in vitro and exhibit two distinguished firing pattern in vivo, namely single-spike and burst firing. Importantly, the firing pattern of these cells strongly affects behavioral parameters, through the modulation of neurotransmitter release. Therefore, the understanding of the mechanisms underlying this switch of firing pattern may be of critical interest. It has been shown that the blockade of small-conductance calcium-activated potassium (SK) channels has a common effect on the firing pattern of these cells, as well as many others (e.g. GnRH neurons, deep cerebellar nucleus neurons, subthalamic nucleus neurons, mitral cells of the olfactory bulb), increasing irregularity and/or bursting. On the basis of these experimental results, we have developed a minimal computational model that is general enough to apply to different types of neurons without including their specific details. Its two main components are I) the dynamics of generation of action potentials, sustained by sodium and delayed-rectifier potassium channels II) the calcium dynamics, involving L-type calcium channels and calcium pumps. In addition, stochastic activation of excitatory synaptic inputs is used to model the afferences. Using this model, we propose a common mechanism which may underlie both the control of neuronal firing and synchrony of pacemaker cells. We propose that SK channels, which act as filters against excitatory inputs through the regulation of the calcium balance in the cell, act as “isolators” against synaptically-induced bursting, and oppose the emergence of a collective rhythm entrained by a common synaptic input.

P-01

ETHNICITY AND USE OF MEDICATION IN PREGNANT IMMIGRATED WOMEN

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Background and Objectives. Pregnant immigrated women might be expected to behave differently with respect to drug use because of differences associated with socio-cultural and religious factors and with customs and beliefs. Therefore, the aim of this study is to compare the behavioral pattern relating to drug use during pregnancy in Western and immigrated women. *Design.*A structured questionnaire was designed to collect information on demographic characteristics of the participants as well as on their drug use pattern (whether prescribed by obstetrician or self-administered). Pregnant women who gave consent to enrollment in the study were asked to fill in the questionnaire during the third trimester of their pregnancy. If necessary, complementary information was collected from the patient's medical record. *Setting.* The study was conducted at the University Hospital of Brussels which is one of the medical referral centers in Brussels, with a large number of immigrants. *Main outcome measures.* Quantitative data analysis was done using SPSS 17.0 program. *Results.* The analyses included 350 patients categorized into 3 categories according to ethnicity (Western group, Arabic & Turkish and Other origins group).The most common classes of medications used were analgesics, anti-emetics, hormones, respiratory medications and antibiotics. 47.4% of the pregnant women used contraceptives before pregnancy, 35.1% of them used medication & 80.3% used food supplements during pregnancy. Higher levels of education were associated with greater use of medication (p = 0.019). Similarly, higher socio-economic status was associated with use of medication; pregnant women in families in which both partners are working used more medication. Western women used medication (42%) more than Arabic women (27%) (p = 0.033). Moreover Western women used folic acid (67%) more than non Western ethnicities (47%) & (51%) in (Arabic & Turkish group) and in other origin women (p = 0.003) There was no significant difference in use of contraceptives. *Conclusion*. There are some differences between Western& non Western pregnant women in drug intake behaviour but more international collaborative studies are warranted to confirm the role of immigration. Educational level, origin & household income were associated with the use of medication during pregnancy. There is a need for doctors to understand ethnicity related characteristics in order to improve the quality of prescribing in order to decrease DRPs.

P-02

DIFFERENT VASOACTIVE EFFECT OF ADHERENT ADIPOSE TISSUE DURING HYPOXIA IN MICE AORTA AND MESENTERIC ARTERIES

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Recent studies propose a paracrine role for perivascular adipose tissue in the regulation of vascular tone. The potential influence of hypoxia on the influence of brown and white adipose tissue was investigated using isometric tension recording of isolated mice aorta and mesenteric arteries with or without adherent adipose tissue. Aorta and mesenteric arteries from male Swiss mice with or without adipose tissue were mounted in a wire myograph for isometric tension recording. Hypoxia (bubbling with 95% N_2, 5% CO_2) relaxed precontracted (NOR, 5 µM) aorta with brown adipose tissue, while a biphasic response was seen in precontracted (NOR, 10 µM) mesenteric arteries with white adipose tissue. Only a minimal vasorelaxing effect was observed in both arteries without adipose tissue. Indomethacin (10 µM) significantly impaired the hypoxic vasocontractile effect in mesenteric arteries. Precontraction with 60 mM K^+ significantly impaired the hypoxic response in both arteries while glibenclamide (30 µM) significantly blocked the hypoxic response in aorta. 8-(p-sulfophenyl)theophylline (0.1 mM) did not influence the hypoxic response in aorta. Also removal of the endothelium did not influence the hypoxic relaxation in aorta, while in mesenteric arteries removal of the endothelium almost completely blocked the hypoxic relaxation. From these results we conclude that in mice aorta hypoxia has a relaxing influence in the presence of adherent brown adipose tissue. This relaxation is at least in part mediated by opening K_{ATP} channels and independent of the endothelium and functional adenosine receptors. These findings are in line with the involvement of the as yet unidentified "adipocyte-derived relaxing factor" (ADRF). In mice mesenteric arteries, hypoxia induces a biphasic response in the presence of adherent white adipose tissue. The hypoxic vasoconstriction is in part mediated by COX metabolites, while the hypoxic vasodilating response is endothelium-dependent and in part mediated by opening K^+ channels.

P-03

ROLE OF TRPV2 ION CHANNEL IN THE PHYSIOPATHOLOGY OF DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy is a severe degenerative disorder of skeletal muscle due to the loss of dystrophin, a cytoskeletal protein associated with the inner cellular membrane of muscle fibres. The absence of dystrophin induces an abnormal influx of calcium through cationic channels in dystrophic muscle fibres. We previously showed that these channels belong to the Transient Receptor Potential (TRP) family and were activated by membrane stretch (SAC: Stretch Activated Channel). Muscles from dystrophin-deficient mice (mdx) typically present an exaggerated susceptibility to eccentric work characterized by an important force drop and an increased membrane permeability consecutive to repeated lengthening contractions. The present study investigates the possible implication of TRPV2, one of the principal candidates for the abnormally regulated Ca^{2+}-entry pathway in mdx fibres. We show that the abnormal influx of Ca^{2+} is reduced in muscle fibres from mdx mice overexpressing a dominant negative mutant of TRPV2 ion channel. Interestingly, dystrophic muscles are also largely protected from eccentric work-induced damage. These observations point out the role of TRPV2 channel in the physiopathology of Duchenne muscular dystrophy.

P-04

NEUROINFLAMMATION TRIGGERS REGULATIONS OF SEVERAL GLUTAMATERGIC TARGETS IN ASTROCYTES : IMPLICATION IN NEUROLOGICAL DISEASES

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Cross-talks between microglia and astroglia is of key importance in the pathogenesis of several neurological disorders. Upon nervous injury, microglia become activated and release mediators which influence neighbour neuronal and astroglial cells. In particular, astroglial cells are thought to adapt the expression of proteins involved in glutamate handling, a process that could putatively participate in the development or progression of excitotoxic insults. We herein hypothesized that soluble mediators released by activated microglia induce changes in the expression of astroglial metabotropic glutamate receptors (mGluRs), which we previously showed to be implicated in the control of glutamate transmission by astrocytes. In this aim, an *in vitro* model was designed where microglial cells cultivated from neonatal rat cerebral cortex were activated with lipopolysaccharide. After 48 hours, real-time quantitative PCR (q-PCR) were performed to validate the activation state of microglia. In parallel, the conditioned media from these cells were transferred to primary cultures of astrocytes which were then examined after 72 hours. Quantitative PCR were realized on these astroglial cultures to evaluate the effects of this inflammatory environment on the expression of mGluR3 and mGluR5, the two principal subtypes identified on glial cells. We evidenced a robust microglial activation by lipopolysaccharide as shown by increases in transcripts of the pro-inflammatory enzymes iNOS and COX-2, and the cytokines TNFα, IL-1β, or IL-6. The conditioned medium collected from activated microglia was found to induce an opposite regulation of the glutamate receptors on astrocytes, as mGluR3 was upregulated while mGluR5 was downregulated. We conclude that activated microglia release diffusible factors which differentially affect the astroglial expression of mGluR 3 and 5. Because these receptors are thought to contribute to the control of glutamate handling and since these receptors activate distinct signaling cascades, our future objective is to understand the molecular mechanisms supporting these regulations and to investigate the impact on excitotoxic events which affect neurological disorders.

P-05

CALCIUM BINDING PROTEIN PARVALBUMIN REGULATES THE ELECTRO-RESPONSIVENESS OF STRIATAL FAST SPIKING INTERNEURONS

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Intracellular calcium (Cai) regulates a broad spectrum of determinant neuronal processes and therefore must be tighly regulated. Calcium binding proteins (CBP) play a key role in this regulation. Among these, parvalbumin (PV) is particularly enriched in striatal fast spiking interneurons (FSI). FSI exert a powerful feedforward inhibition on striatal projection neurons, orchestrating their fine spike timing. We investigated the role of parvalbumin on the electroresponsiveness of these interneurons by using the amphotericin-perforated patch configuration of the patch-clamp technique. We performed current clamp recordings of single FSI from coronal striatal slices of wild type mice (WT) and PV-knock-out mice (PVKO) aged between 18-25 days-old. Voltage responses obtained by increasing injected current intensities generated slopes of the linear part of current-frequency plots. These slopes reflect the intrinsic FSI excitability. A significant increase ($p<0.05$) has been found in excitability slopes for PVKO mice (0.81±0.1 Hz.pA-1, n=6) vs. WT mice (0.51±0.05 Hz.pA-1, n=6). Furthermore, maximal spiking frequencies are higher in PVKO mice (135.71±9.38 Hz) compared to WT mice (102.66±12.44 Hz , $p<0.05$). Finally, we propose a mathematical model providing a link between our experimental observations about the electrical activity of FSI and a decrease in cytosolic Cai buffering capacity induced by the PV deletion.

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P-06

HEMICHANNEL INVOLVEMENT IN CA^{2+} DYNAMICS AND CONTRACTILITY OF SMOOTH MUSCLE CELLS IN ACUTELY ISOLATED SMALL MESENTERIC ARTERIES

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Intracellular Ca^{2+} mediates a variety of vascular endothelial and smooth muscle cell functions. Smooth muscle cells (SMC) respond to biological activators with oscillatory and propagating rises in $[Ca^{2+}]_i$ that are highly organized in both time and space. Gap junctions (GJs) play a crucial role in the communication between vascular cells and in the synchronization of Ca^{2+} signals thereby tightly controlling the level of vasoconstriction. Before being incorporated into GJs, connexin (Cx) hemichannels reside in the plasma membrane in a closed state. Recent evidence suggests that hemichannels can be opened by various messengers and conditions, thereby forming a pore that allows the passage of ATP and ions. Using confocal microscopy and the Ca^{2+} sensitive dye Fluo-3, we examined the role of hemichannels in dynamic Ca^{2+} responses of SMC in intact acutely isolated small rat mesenteric arteries. Furthermore, we assessed the involvement of these signalling partners in contractile responses of small mesenteric arteries using a wire myograph for isometric tension measurements. Importantly, the experimental conditions were such that vasomotion, characterized by synchronized Ca^{2+} signals, was avoided because in that case gap junctions between SMC and myo-endothelial gap junctions are expected to contribute. Norepinephrine (NOR, 3 µM) induced Ca^{2+} oscillations that were reduced in frequency by 98.4 % ($p< 0.05$) when exposed to carbenoxolone (CBX, 50 µM), a none specific Cx channel inhibitor. Gap27 (200 µM), a Cx mimetic peptide that blocks hemichannel responses (assayed by ATP release and dye uptake) after short incubation, reduced the spiking frequency by 96.4 % ($p< 0.05$). Suramin (200 µM) and PPADS (75 µM), two P2Y receptor antagonists, decreased the spiking frequency by 90.5 % ($p<0.05$) and 96.4% ($p<0.01$) respectively. Apyrase (5 U/ml), an enzyme that rapidly degrades extracellular ATP, reduced the spiking frequency by 71.4 % ($p<0.01$). None of these agents affected the amplitude of the Ca^{2+} oscillations. Both gap27 (56.6 %, $p<0.01$) and CBX (53.4 %, $p<0.05$) reduced the NOR-induced contractions. Incubation with suramin decreased the NOR-induced contractions by 31.6 % ($p<0.001$). Our results suggest a role for Cx hemichannels and purinergic signaling in Ca^{2+} oscillations and contractility.