BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL

PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Saturday, October 16 2010

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

Organisation

Prof. Dr. R. Beauwens
Laboratoire de Physiologie Cellulaire et Moléculaire
Université Libre de Bruxelles
CP 611. Campus Erasme
808, Route de Lennik
B-1070 Bruxelles

BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting
Saturday October 16 2010

UNIVERSITE LIBRE DE BRUXELLES
Faculté de Médecine
Campus Erasme
Auditoire Jules Bordet – Bâtiment F
808, Route de Lennik
1070 Bruxelles

Main Lecture

10.00-10.50 **Prof. Dr. B. NILIUS**
(Departement Fysiologie – Katholieke Universiteit Leuven)

Amazing TRP channels

Oral Communications

11.00-11.15 R. CRUTZEN, V. SHLYONSKY, K. LOUCHAMI, A. BOOM, A. SENER, W.J. MALAISSE, R. BEAUWENS (ULBruxelles)
Hypotonicity-induced insulin release is mediated by hydrogen peroxide.

11.15-11.30 P. LYBAERT, F. LELEUX, G. VEGH, S. MEURIS, P. LEBRUN (ULBruxelles)
K_{ATP} channels in spermatozoa: expression in different species and involvement in calcium influx.

11.30-11.45 A. MASSIE, A. SCHALLIER, S-W. KIM, R. FERNANDO, H. BECK, D. DE BUNDEL, K. VERMOESEN, S. BANNAI, M. CONRAD, N. PLESNILA, H. SATO, Y. MICHOTTE (VUBrussel)
Dopaminergic neurons of system xc-deficient mice are highly protected against 6-OHDA induced toxicity.

11.45-12.00 G. CALJON, V. CAVELIERS, T. LAHOUTTE, I. SMOLDERS, P. DE BAETSELIER, Y. MICHOTTE, S. MUYLDERMANS, S. MAGEZ, R. CLINCKERS (VUBrussel)
Analysis of BBB permeability for nanobodies using microdialysis.

12.00-12.15 M. VIRREIRA, R. CRUTZEN, X. DE DEKEN, A. BOOM, R. BEAUWENS (ULBruxelles)
Expression of DUOX in glomerular podocytes and its overexpression by puromycin aminonucleoside.

12.15-12.45 **Bullet Session**
Oral presentations of posters 1,2,3,4,5,6,7,8

12.45-14.15 **Lunch**
Poster Session
General Assembly of the Society

Oral Communications

14.15-14.30 P. CORNU, S. STEURBAUT, M. AUDENAERT, L. HUYGHENS, A.G. DUPONT (VUBrussel)
Intensive care stay as a risk factor for unintentional change of chronic medication at hospital discharge.

14.30-14.45 W. VERMEULEN, J.G. DE MAN, S. NULLENS, P.A. PELCKMANS, B.Y. DE WINTER, T.G. MOREELS (UAntwerpen)
Colonoscopy to assess the time course of inflammation after TNBS colitis in rats.

14.45-15.00 A. SOLIS, A.A. SANCHEZ-TUSIE, G. CHURCHILL, G.A. DE BLAS, A. DARSZON, C. TREVINO (Univ. Mexico & Univ. Oxford) (presented by P. LYBAERT, ULBruxelles)
Characterization of the signaling pathway of the acrosome reaction in human sperm.

15.00-15.15 L. BORDE, H. AMORY, A. LEROUX, A. AL HAIDAR, I. BORDET, C. SANDERSEN (ULiège)
Myocardial depression as a component of endotoxic shock in horses: preliminary results from an echocardiographic study.

15.15-15.30 S. WENINGER, J.H. DE MAEYER, R.A. LEFEBVRE (UGent & Movetis, Turnhout).
Influence of selective phosphodiesterase (PDE) subtype inhibitors on the inotropic response to 5-HT_4 receptors in porcine left atrium.

15.30-15.45 I. DE MEYER, W. MARTINET, D.M. SCHRIJVERS, J.-P. TIMMERMANS, H. BULT, G.R.Y. DE MEYER (UAntwerpen):
Imiquimod induces selective macrophage autophagy in rabbit atherosclerotic plaques via Toll-like receptor 7.

15.45-16.30 **Poster Session**
Coffee – Tea

Posters

(dimensions: height 120 cm – width 100 cm)

1. F. NSUADI, C. EL KHATTABI, J. FONTAINE, G. BERKENBOOM, J. LAMI, S. POCHET (ULBruxelles, Univ. Kinshasa)
   Vasorelaxant activity in isolated rat aorta of extracts from plants used in congolese traditional medicine.

2. N. LABRANCHE, C. EL KHATTABI, J. FONTAINE, G. BERKENBOOM, S. POCHET (ULBruxelles)
   Effects of diesel exhaust microparticles on vascular endothelial function.

3. L. DEWACHTER, B. RONDELET, C. DEWACHTER, F. KERBAUL, X. KANG, S. BRIMIOULLE, R. NAEIJE (ULBruxelles)
   Right ventricular failure in prolonged overcirculation-induced pulmonary hypertension: role of apoptosis and inflammation.

4. C. DEWACHTER, L. DEWACHTER, B. RONDELET, P. FESLER, S. BRIMIOULLE, F. KERBAUL, R. NAEIJE (ULBruxelles)
   Acitvation of apoptotic pathways in experimental acute afterload-induced right ventricular failure.

5. A. ROBBE, J. CARPENTIER, A. LEGRAND (UMons)
   Bleomycin aerosolization: the best route of administration for pulmonary fibrosis model improvement?

6. R. EL TAHRY, R. RAEDT, L. MOLLET, V. DE HERDT, T. WYCKUYS, A. VAN DYCKE, A. MEURS, F. DEWAELE, D. VAN ROOST, P. DOGUET, J. DELBEKE, W. WADMAN, K. VONCK, P. BOON (UGent, UCLouvain, Neurotech sa. Louvain La Neuve & Univ. Amsterdam)
   A novel implantable vagus nerve stimulation system (ADNS-300) for combined stimulation and recording of the vagus nerve pilot trial at Ghent University Hospital.

7. A. AVILA, L. NGUYEN, J-M. RIGO (UHasselt, ULiège)
   Glycine receptor activation influence early cortical development.

8. D. DE GEYTER, S. SARRE, R. KOOIJMAN (VUBrussel)
   A qualitative and quantitative comparison between normotensive and hypertensive rats after stroke.

9. N. SWINNEN, C. RIGATO, B. BRÔNE, P. LEGENDRE, JM. RIGO (UHasselt, Univ. Pierre et Marie Curie et UPMC Univ. Paris)
   Maternal inflammation affects embryonic microglia.

10. S. STEURBAUT, P. CORNU, E. BERGHMANS, I. HUBLOUE, A.G. DUPONT (VUBrussel)
Medication history reconciliation by a pharmacy student in patients admitted to the emergency department.

11. S. DILLY, C. LAMY, D. SNYDERS, J.-F. LIÉGEOIS, V. SEUTIN (ULiège, UAntwerpen)
Block of SK channels by the sigma agonist 1,3-di-*o*-tolyl-guanidine: evidence for a novel site of action for SK blockers.

12. P. VAN LOO, R. RAEDT, L. MOLLET, T. WYCKHUYS, M. KOOL, B. LAMBRECHT, R. VANHOLDER, K. VONCK, P. BOON (UGent)
Increase in hippocampal uric acid after kainic acid induced seizures is suppressed by allopurinol.

13. N. BAEYENS, C. DE MEESTER, N. MOREL (UCLouvain)
EBP50 modulates migration and cytokinesis in vascular smooth muscle cells.

14. A. MARTINSEN, N. BAEYENS, N. MOREL (UCLouvain)
Regulation of agonist-evoked calcium entry by Rho kinase in rat aorta and cultured aortic smooth muscle cells.

15. N. BULUR , W.J. MALAISSE, P. LYBAERT, M. VIRREIRA, A. NOVIALS, A. BOOM, R. BEAUWENS, A. SENER (ULBruxelles & Idibaps, Ciberdem, Univ. Hosp. Barcelona)
Expression of GLUT-2 and the electrogenic Na^+- HCO_3^--cotransporter NBCe1 in human endocrine pancreas.

16. N. ZANOU, Y. O. SCHAKMAN, A. DIETRICH, L. BIRNBAUMER, P. GAILLY (UCLouvain, Univ. Marburg, NIHS North Carolina)
Lack of TRPC1 channels impairs skeletal muscle regeneration.

17. K. VEYS, E. BOCKSTEINS, D.J. SNYDERS (UAntwerpen)
Molecular composition of the delayed rectifier K-current in single adult mouse DRG neurons.

18. P. BISCHOP, C. ROUSSEL, D. ORDUZ, S.N. SCHIFFMANN, D. GALL (ULBruxelles)
Dynamic control of neuronal firing threshold by calcium buffering : a new role for calcium binding proteins.

O-01

HYPOTONCITY-INDUCED INSULIN RELEASE IS MEDIATED BY H_2O_2

Crutzen R., Shlyonsky X., Louchami K., Boom A., Sener A., Malaisse W.J., Beauwens R.

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NAD(P)H oxidase-derived H_2O_2 was recently proposed to act, in several cells, as the signal mediating the activation of volume-sensitive Cl^- channels (VSAC) under a variety of pathophysiological conditions. The present study aims at investigating whether a similar situation prevails in β cells derived BRIN-BD11 cells exposed to hypotonicity, a condition previously demonstrated to increase insulin secretion. Exogenous H_2O_2 also stimulated insulin secretion with a threshold concentration close to 38 µM and a maximal response at about 100 µM. The inhibitors of volume-sensitive anion channels 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB) and niflumic acid decreased the secretory response to exogenous H_2O_2, as well as to hypotonicity. In patch clamp experiments, exogenous H_2O_2 was observed to induce cell membrane depolarization that was also inhibited by NPPB, as previously reported for hypotonicity. Exposure of the BRIN-BD11 cells to a hypotonic medium cause a detectable increase in intracellular reactive oxygen species (ROS) that was abolished by diphenylene iodinium chloride (DPI), a NAD(P)H oxidase inhibitor. DPI as well as other NAD(P)H oxidase inhibitors such as phenyl arsine oxide and plumbagin nearly totally inhibited the insulin release provoked by exposure of the BRIN-BD11 cells to an hypotonic medium. Measurements of cell volume under hypotonic conditions showed that DPI like NPPB completely blocked the cell volume regulatory decrease otherwise observed within 15-20 min. On the other hand exogenous H_2O_2 did not change cell volume. Taken together, these data suggest that NAD(P)H oxidase-derived H_2O_2 appears the key intracellular messenger leading to opening of VSAC, hence membrane depolarization and the insulin secretory response observed in BRIN-BD11 cells to extracellular hypotonicity.

O-02

K_{ATP} CHANNELS IN SPERMATOZOA: EXPRESSION IN DIFFERENT SPECIES AND INVOLVEMENT IN CALCIUM INFLUX

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Laboratory of Experimental Hormonology[1] and Pharmacology[2], Université Libre de Bruxelles, B-1070 Brussels, Belgium.

ATP-sensitive K^+ (K_{ATP}) channels have been described in a variety of cell types. They exhibit the unique property to couple intracellular metabolic changes to membrane electrical activity. Closure of K_{ATP} channels results in membrane depolarization leading to the opening of voltage-dependent calcium channels and subsequent calcium entry. K_{ATP} channels consist of hetero-octameric complexes constituted by 4 pore-forming subunits (Kir6.x) and 4 regulatory sulfonylurea receptor subunits (SURx). K_{ATP} channels subunits have been described in mouse spermatogonia and spermatozoa. The study was designed to ascertain the presence of K_{ATP} channels in spermatozoa from various mammalian species and to investigate their potential role in modulating calcium influx.
Kir6.2, Kir6.1 and SUR2 subunits were detected on rat, mouse, dog, stallion and man spermatozoa. Western blot analysis confirmed the expression of Kir6.1 and Kir6.2 subunits in protein extracts from murine spermatozoa. mRNAs for Kir6.2, Kir6.1 and SUR2 subunit were detected by RT-PCR technique on mouse total RNA extracts. Cytosolic calcium measurements showed an increase in $[Ca^{2+}]_i$ in response to various hypoglycaemic sulfonylureas (glibenclamide, gliclazide, tolbutamide). The absence of extracellular calcium abolished this response. The addition of Ni^{2+} also counteracted the tolbutamide-induced $[Ca^{2+}]_i$ increase. Moreover, the tested sulfonylureas provoked a significant increase in the acrosome reaction of capacitated spermatozoa. This study reports the expression of K_{ATP} channel subunits on spermatozoa from various mammalian species. Our observations further suggest the existence of functional K_{ATP} channels involved in the control of spermatozoa membrane potential and, as a consequence, of calcium influx and acrosome reaction.

O-03

DOPAMINERGIC NEURONS OF SYSTEM X_C^- DEFICIENT MICE ARE HIGHLY PROTECTED AGAINST 6-OHDA INDUCED TOXICITY

Massie A.[1], Schallier A.[1], Kim S-W.[2], Fernando R.[3], Kobayashi S.[4], Beck H.[5], De Bundel D.[1], Vermoesen K.[1], Bannai S.[4], Smolders I.[1], Conrad M.[6], Plesnila N.[2], Sato H.[4],
Michotte Y.[1]

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Malfunctioning of system x_c^-, responsible for exchanging intracellular glutamate for extracellular cystine, can cause oxidative stress as well as excitotoxicity, both important phenomenons in the pathogenesis of Parkinson's disease. Although cystine that is imported via system x_c^-, is reduced to cysteine which is the rate-limiting substrate in the synthesis of glutathione, deletion of xCT ($xCT^{-/-}$), the specific subunit of system x_c^-, did not result in decreased glutathione levels in striatum. Accordingly, no signs of increased oxidative stress could be observed in striatum or substantia nigra of $xCT^{-/-}$ mice. In sharp contrast to the expectations, $xCT^{-/-}$ mice were less susceptible to 6-OHDA-induced neurodegeneration in the substantia nigra pars compacta. This reduced sensitivity to a Parkinson's disease inducing toxin, might be related to the significantly reduced striatal extracellular glutamate levels that were observed in mice lacking xCT. The current data point towards system x_c^- as a possible target for the development of new pharmacotherapies for the treatment of Parkinson's disease and emphasizes the need to continue the search for specific ligands for system x_c^-.

O-04

ANALYSIS OF BLOOD-BRAIN BARRIER PERMEABILITY FOR NANOBODIES USING MICRODIALYSIS.

Caljon G.[2,3,5], Caveliers V.[4], Lahoutte T.[4], De Baetselier P.[2,3], Van Den Abbeele J.[5], Smolders I.[1], Michotte Y.[1], Muyldermans S.[2,3], Magez S.[2,3], Clinckers R.[1]

[1] Department of Pharmaceutical Chemistry and Drug Analysis, Research Group Experimental Pharmacology, Vrije Universiteit Brussel, Brussels, Belgium. [2] Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium. [3] Department of Molecular and Cellular Interactions, VIB, Ghent, Belgium. [4] In Vivo Cellular and Molecular Imaging, ICMI, Vrije Universiteit Brussel, Brussels, Belgium.[5] Department of Animal Health, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium.

Nanobodies are considered as very promising antigen-binding moieties for molecular imaging and therapeutic purposes due to their favorable pharmacological and pharmacokinetic properties. However, the possibility for monovalent Nanobodies to reach targets in the central nervous system (CNS) remains to be demonstrated. We have assessed the blood-brain barrier permeability for Nb_An33, a Nanobody against the Trypanosoma brucei Variant-specific Surface Glycoprotein (VSG), in healthy rats and rats that are in the encephalitic stage of African trypanosomiasis and suffer from acute brain inflammation. To assess the integrity of the blood-brain barrier, brain disposition of 2-Methoxy-IsoButyl-Isonitrile (MIBI) and Evans Blue was monitored in both experimental groups of animals. Perfusion of the different compounds was analyzed by intracerebral microdialysis, Single Photon Emission Computed Tomography (SPECT) or a combination of both methodologies. This enabled the quantification of unlabeled and (99m)Tc-labeled Nanobodies using respectively a sensitive VSG-based Nanobody-detection ELISA, radioactivity measurement in collected microdialysates and SPECT image analysis. As the ELISA analysis provided direct evidence for antigen-binding potential, this approach allowed to differentiate between active Nanobody and fragments that might be generated during the (99m)Tc-labeling process or in vivo by proteolytic processing. The combined read-out methodologies illustrate that, while brain disposition of Nb_An33 upon intravenous injection is at the limit of detection in healthy rats, inflammation-induced damage to the blood-brain barrier significantly increases (approximately 20 times) the Nanobody perfusion efficiency. Despite the enhanced brain disposition, calculated intrahippocampal Nb_An33 concentrations remained very low (131 ± 63 ng/ml with a dose of 4 mg/kg), likely resulting from the observed high clearance rates of Nanobodies in general. Collectively, we illustrate the advantage of complementing SPECT analyses with intracerebral microdialysis in brain disposition studies and suggest that it is of interest to evaluate the blood-brain barrier penetrating potential of monovalent Nanobodies in models of CNS-inflammation. However, the pharmacokinetic properties that are considered favorable for peripheral imaging and therapeutic approaches might be key factors that prelude efficient application of Nanobodies to target the CNS.

O-05

EXPRESSION OF DUOX IN GLOMERULAR PODOCYTES AND ITS OVER-EXPRESSION BY PUROMYCIN AMINONUCLEOSIDE

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DUOX 1 and 2 are two NADPH oxidases directly producing H_2O_2 (instead of superoxide anion for the other NADPH oxidases). They were first cloned from the thyroid but since then, their expression was also found in the airways and gastro-intestinal tract. The present study aimed at examining their possible expression in kidney epithelial cells. Using immunohistochemical technique on rat sections, the renal glomerulus was labeled by an anti-DUOX antibody with a pattern similar to that of anti-synaptopodin antibody, highly suggesting that the labeled cells were the podocytes. RT-PCR confirmed the expression of transcripts of DUOX 1 in rat renal cortex. By RT-PCR, cultured human podocytes were also observed to express DUOX 1 (and 2) and this expression increased more than 3-fold by treatment with puromycine aminonucleoside (PAN). Experiments are on their way in PAN-treated rats to examine whether DUOXes are indeed overexpressed in podocytes and whether this possible overexpression precedes the albuminuria and glomerulonephritis as we surmise a possible pathogenic role of increased hydrogen peroxide in this nephritis. The temporal relationship with the expression of other podocyte's proteins, nephrin, TRPC6 and Notch will be specifically addressed.

O-06

INTENSIVE CARE STAY AS A RISK FACTOR FOR UNINTENTIONAL CHANGE OF CHRONIC MEDICATION AT HOSPITAL DISCHARGE

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Background and Objective: Earlier research at our institution revealed a high incidence of drug discrepancies (DD) at the intensive care unit (ICU). Whether some of these changes were intentional (however not documented) or not was not yet investigated. The objective of the study was to determine the number of unintentional changes. Only unintentional changes were considered as discrepancies. *Design*: Observational, prospective cohort study. At hospital admission, the medication history was documented by a pharmacist and was compared with the physician's medication history with special focus on chronic drugs. The administered medication on the ICU (last 24h), on the hospital ward subsequently following the ICU stay and the medication scheme in the discharge letter were all compared with the pharmacist-acquired medication history. If there was a non-documented modification of the chronic medication, the responsible physician was contacted to determine whether the change was intentional or not. *Setting*: A cardiosurgical intensive care unit of a Belgian university hospital (UZ Brussel). *Main outcome measures:*The percentage of patients with DD, as well as the incidence and the type of DD in chronic medication. Also the number of intentional however not documented changes was investigated. *Results*: The study group consisted of 36 patients. At hospital admission 30 patients (83%) had one or more DD in the medication history. There were a total of 99 DD for 179 detected chronic preadmission drugs (median of 2 DD per patient, range 0-10). For the comparison at hospital admission, every difference between the physician's and pharmacist's medication history was considered as unintentional and thus DD. The two most frequent types of DD were erroneous listed drugs in the physician's medication history (25% of DD) and omission of drugs (21% of DD). Comparison of the administered chronic medication on the ICU (last 24h) with the preadmission drugs revealed 26 patients (72%) with DD. A total number of 65 DD were detected (median of 1 DD per patient, range 0-7). The most common type of DD was omission or unintentional stop of a chronic drug (58% of DD). Of these 38 DD, 19 were drugs that were already missing in the physician's medication history and the other 19 were unintentionally stopped at the ICU. There were also 48 intentional but not documented changes. On the hospital ward subsequently following the ICU stay, 28 patients (78%) had one or more DD. In total, 68 DD were detected (median of 2 DD per patient, range 0-7). The most common type of DD was an unintentional stop of a chronic drug (32% of DD) that was listed by the physician at hospital admission. There were also 45 intentional but not documented changes. Comparison of the discharge letter revealed 116 DD for 165 preadmission drugs and 30 patients (91%) had one or more DD (3 patients without discharge letter). The most common type of DD was again the unintentional stop of a chronic drug (37% of DD) that was listed by the physician at hospital admission. *Conclusions*: Acquiring complete and correct medication histories at hospital admission is a prerequisite for adequate pharmacotherapy during hospitalization. Intentional medication changes during hospitalization should be well documented to avoid discrepancies during further transition moments. At transition moments, structural medication reconciliation with respect towards the original preadmission medication is crucial to reduce the number of potential adverse drug events that can arise from drug discrepancies.

O-07

COLONOSCOPY TO ASSESS THE TIME COURSE OF INFLAMMATION AFTER TNBS COLITIS IN RATS

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Animal models of colitis are widely used to study the pathogenesis of inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS). However techniques allowing sequential assessment of colonic inflammation over time, without the need to sacrifice the animal, are required. This study evaluated in vivo colonoscopy to follow the evolution of colitis in rats in comparison with the more commonly used post-mortem macroscopic, microscopic and biochemical assays of inflammation. Colitis was induced in rats by intrarectal instillation of trinitrobenzene sulphonic acid (TNBS). Using a baby upper gastrointestinal endoscope, the severity of colitis was monitored at days 3, 10, 28 and 56 after the induction of colitis. Inflammation was scored by colonoscopy based on the degree of ulceration, extent of inflammation, mucosal bleeding, oedema and stenosis. During follow-up, rats were randomly selected for post-mortem macroscopic and microscopic histology and myeloperoxidase (MPO) assessment of the colon. Colonoscopy showed signs of severe mucosal inflammation in the distal colon 3 days after induction of TNBS colitis. Subsequently, colitis subsided at days 10 and 28 with complete remission at day 56. During the acute phase of inflammation, endoscopic findings were consistent with the post-mortem inflammatory parameters (macroscopic and microscopic histopathology, MPO colonic activity). A strong correlation between endoscopy and macroscopy remained even during the chronic phase of inflammation.

O-08

CHARACTERIZATION OF THE SIGNALING PATHWAY OF THE ACROSOME REACTION IN HUMAN SPERM

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Although the Acrosome Reaction (AR) is a fundamental exocytic event for fertilization, the signaling pathway involved in this process is not completely understood. The physiological induction of the AR triggers a biphasic Ca^{2+} influx; however, the molecular entities involved in this process are not clearly defined. A model of the signaling pathway during the AR that implies the participation of the Exchange Protein directly Activated by cAMP (Epac) has been proposed. We speculate that Epac might activate the enzyme (CD38) that catalyzes the production of nicotinic acid adenine dinucleotide phosphate (NAADP), which in turn activates the intracellular Two Pore Channels (TPC), releasing a small amount of Ca^{2+} that would convey the signal to other channels (IP_3R and RyR) releasing more Ca^{2+}, and amplifying the response. In the present work we employ the EPAC specific cAMP analogue (8-pCPT-2'-O-Me-cAMP) to trigger the Ca^{2+} rise in the presence or absence of specific channel inhibitors to confirm or rule out the participation of these components in the proposed signaling pathway. We would characterize the process by 1) Measuring the intracellular Ca^{2+} change in sperm loaded with a fluorescent Ca^{2+} indicator and 2) Evaluating the percentage of sperm that undergo the AR in different conditions. Our preliminary results suggest that the TPC and IP_3R are implicated in the signaling pathway of the AR trigger by Epac.

O-09

MYOCARDIAL DEPRESSION AS A COMPONENT OF ENDOTOXIC SHOCK IN HORSES: PRELIMINARY RESULTS FROM AN ECHO-CARDIOGRAPHIC STUDY

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Cardiovascular consequences of septic shock are well described in humans but have poorly been studied in horses. The endotoxins are known to induce a myocardial depression and a systemic hypotension responsible for a fall of both the cardiac output and the systemic vascular resistance in end-stage endotoxic shock. The aim of this study was to evaluate the impact of endotoxic shock on equine myocardial function. A total of 67 horses including 17 controls and fifty horses admitted in clinic with signs of endotoxic shock were submitted to a doppler echocardiographic exam. A shock score was attributed to each endotoxic horse on the basis of clinical evaluation, non invasive systolic blood pressure and blood tests. Score 1, 2, 3 and 4 included 11, 17, 12 and 10 horses, respectively. Echocardiographic and Doppler parameters were compared between the 5 groups using a multivariable ANOVA analysis. The ejection time (ET), the ET corrected for HR, the mean velocity of circumferential fibre shortening corrected for HR, the aortic velocity time integral and deceleration time, and the stroke volume were significantly lower, whereas the HR and the peak velocity of late diastolic filling of the mitral flow and its velocity time integral were significantly higher in endotoxic than in control horses. Even if the tachycardia, the fall in preload and a probable decrease in afterload influenced the observed changes, the results suggested that a myocardial depression with both an impaired systolic and diastolic left ventricular function could be a component of endotoxic shock in horses.

O-10

INFLUENCE OF SELECTIVE PHOSPHODIESTERASE (PDE) SUBTYPE INHIBITORS ON THE INOTROPIC RESPONSE TO 5 HT_4 RECEPTORS IN PORCINE LEFT ATRIUM

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Inotropic responses to 5-HT_4 receptor activation fade with time in porcine atrium. This has been attributed to the action of phosphodiesterases (PDEs), which hydrolyze the second messenger cAMP in cells. We assessed which PDE subtypes are responsible for this fade by testing selective PDE inhibitors alone and in combination on the inotropic responses elicited by 5-HT and by the 5-HT_4 receptor agonists prucalopride and RS67333. Porcine left atrial pectinate muscles were obtained from young male pigs (Seghers, breed line 12, 10-12 weeks, 15-25 kg), attached to tissue holders equipped with electrodes and mounted into tissue baths filled with aerated Krebs-Henseleit solution preheated to 37℃. Under a resting load of 2 g muscles were continually stimulated with square-wave pulses (0.5 Hz, 5 ms duration, 2-4 V). All inotropic responses were normalized to the response elicited by the β-receptor agonist isoprenaline added at the end of the experiment. The fade of the inotropic response to 5-HT_4 receptor activation could not be prevented by any selective PDE inhibitor alone (PDE2: EHNA; PDE3: Cilostamide; PDE4: Rolipram). The combination of cilostamide and rolipram increased the amplitude and completely prevented the fade of the response to 5-HT and prucalopride, similar to the unselective PDE inhibitor IBMX. Inotropic responses to RS67333 were only observed in the presence of IBMX or cilostamide plus rolipram. The combination of rolipram plus EHNA, but not cilostamide plus EHNA increased the amplitude of the inotropic response to 5-HT but only partially prevented the fade. Our results illustrate that the fade of the response to 5-HT_4 receptor stimulation is mediated by PDE3 and PDE4 subtypes in porcine left atrium.

O-11

IMIQUIMOD INDUCES SELECTIVE MACROPHAGE AUTOPHAGY IN RABBIT ATHEROSCLEROTIC PLAQUES VIA TOLL-LIKE RECEPTOR 7

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Macrophages in atherosclerotic plaques are responsible for weakening of the fibrous cap and may finally cause plaque rupture, whereas smooth muscle cells (SMCs) contribute to the tensile strength of the fibrous cap and consequently to plaque stability. Selective removal of macrophages from plaques via drug-induced macrophage death may be a promising strategy to alter plaque composition favouring plaque stabilisation. Macrophages are part of the innate immunity, express Toll-like receptors (TLRs) to recognise pathogens and induce autophagy as an innate defence mechanism to eliminate intracellular pathogens. Because macrophages but not SMCs express TLR7 in human and mouse plaques, we questioned whether TLR7 ligands can selectively induce autophagy in macrophages. In vitro, the TLR7 ligand imiquimod induced cell death in a concentration-dependent manner in cultured macrophages, but not in SMCs. Transmission electron microscopy (TEM) showed that imiquimod-induced cell death was characterised by an increased number of vesicular structures containing cytoplasmic debris. Moreover, there was an increased turnover of microtubule-associated protein 1 light chain 3 (LC3-I) from the cytoplasm to LC3-II in autophagosomes, as demonstrated by western blotting. Accordingly, imiquimod stimulated the formation of autophagosomes in macrophages, which is a hallmark of autophagy. Local in vivo administration of imiquimod to rabbit atherosclerotic carotid arteries reduced the macrophage content in the plaques through autophagy, as shown by TEM, whereas the amount of SMCs was unaffected. In conclusion, imiquimod selectively decreased the macrophage load in rabbit atherosclerotic plaques. Therefore, TLR7 appears to be a promising pharmacological target for the stabilisation of atherosclerotic plaques.

P-01

VASORELAXANT ACTIVITY IN ISOLATED RAT AORTA OF EXTRACTS FROM PLANTS USED IN CONGOLESE TRADITIONAL MEDICINE

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Combretum laxiflorum Welw (Combretaceae), Combretum racemosum P. Beauv (Combretaceae) and Hymenocardia acida Tul (Euphorbiaceae) are traditionally used in RDCongo for the treatment of many diseases including cardiovascular disorders. To our best knowledge, there are no data on vascular relaxant effect of these medicinal plants. We assessed the vasorelaxant activity of extracts obtained from these plants in isolated rat thoracic aorta rings. Methanol extract of Combretum laxiflorum leaves (ClLv), Combretum racemosum leaves (CrLv) and bark root (CrBR) and Hymenocardia acida bark trunk (HaBTr) and bark root (HaBR) were prepared. Rings of aorta from male rats (Wistar), with or without intact endothelium, were mounted in tissue baths. After contraction with phenylephrine (Phe, 1µM) the plant extract was applied cumulatively (0.1-100µg/ml). In some experiments, prior to the addition of the extract, rings were pre-treated for 30 minutes with 100µM N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase (NOS) inhibitor, followed by the addition of 1µM Phe to obtain a similar contractile activity as in vessel rings not treated with L-NAME. All the tested extracts exhibited concentration-dependent vasorelaxations of Phe-induced contractions of intact aortic rings. HaBTr (5µg/ml), HaBR (10 µg/ml) and ClLv (10 µg/ml) methanol extracts potently relaxed aortic rings by respectively 100.4 ± 2.0 %, 93.9 ± 1.5 % and 85.1 ± 3.0 %. The maximum vasorelaxant responses of CrBR and CrLv methanol extracts were respectively 52.7 ± 4.9% and 48.7 ± 9.5% at a cumulative concentration of 100 µg/ml. The activity disappeared or was significantly attenuated by removal of functional endothelium or pre-treatment of the aortic tissues with L-NAME. After removal of the endothelium, the maximum relaxation was less than or equal to 10 % for all the extracts except for CrBR ($p>0.05$). Similar inhibitions were obtained with L-NAME incubation. These results support a significant role of endothelium/NO in these medicinal plants extracts-induced relaxations of rat aorta. The present study showed that the vasorelaxant effect of methanolic extracts from ClLv, CrLv, HaBTr and HaBR seems mainly mediated by the endothelial NO signaling in aortic tissues.

P-02

EFFECTS OF DIESEL EXHAUST MICROPARTICLES ON VASCULAR ENDOTHELIAL FUNCTION

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Epidemiological studies indicate that acute exposure to particulate matter is associated with significant adverse cardiovascular events. However, the pathophysiological mechanisms responsible for these effects are still unclear. We assessed the direct acute effects of diesel exhaust particles (DEP < 2.5µm) on vasomotor responses. We studied the endothelium-dependent relaxations to acetylcholine (ACh) in rat aortas incubated for 30 minutes with DEP, in presence or in absence of superoxide dismutase (SOD). Endothelium-independent relaxations to sodium nitroprusside (SNP) were also tested (on preparations without endothelium). Aortas were isolated from control rats and rats treated with rosuvastatin (10 mg/kg/d p.o.) for 5 weeks; this treatment down-regulates the p22phox subunit of the NAD(P)H oxidase pathway, considered to be the major source of ROS production in endothelial cells, without alteration in the eNOS pathway. Exposure to 100 µg/ml of DEP caused a decrease in maximal relaxations to ACh from 101.6 ± 2 % to 71.8 ± 3.8 % (% inhibition of phenylephrine-induced plateau, $p < 0.0001$). This effect was reversed in presence of SOD (200 UI), indicating that superoxide anion is responsible for the DEP-mediated endothelial dysfunction. Concentration-response curves (CRC) to SNP were slightly but significantly decreased by DEP suggesting a production of superoxide radicals by DEP themselves. In normocholesterolemic rats treated with rosuvastatin, similar results were observed, namely a significant impairment in the Ach-induced relaxations after DEP incubation. This reinforces the hypothesis that DEP themselves produce free radicals. In vivo treatment with apocynin (5 mg/kg/d p.o. for 5 days), an NAD(P)H oxidase inhibitor, also did not protect against this acute toxicity of DEP. The acute vascular toxicity of DEP is resistant to the antioxidant action of statin and apocynin and seems due to a direct generation of superoxide anion by DEP themselves.

P-03

RIGHT VENTRICULAR FAILURE IN PROLONGED OVERCIRCULATION-INDUCED PULMONARY HYPERTENSION: ROLE OF APOPTOSIS AND INFLAMMATION

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We previously reported on well preserved right ventriculo (RV)-arterial coupling in growing piglets with pulmonary arterial hypertension (PAH) induced by three-month aortapulmonary shunting. We hypothesized that a more prolonged period of shunting would induce more severe pulmonary hypertension and RV failure. Sixteen three-week old piglets were randomized to a modified Blalock-Taussig- or a SHAM-operation. Six months later, the animals underwent hemodynamic evaluation followed by pulmonary and myocardial tissue sampling for morphometry and patho-biological evaluation (by real-time quantitative polymerase chain reaction and Western Blotting). Six-month chronic systemic-to-pulmonary shunting induced similar pulmonary vascular changes than previously described in three-month shunted piglets, with pulmonary vascular resistance (PVR) increased from 2.8±0.3 to 6.4±1.0 mmHg.L-1.min.m-2 and increased arteriolar medial thickness compared to SHAM-operated piglets. However, cardiac output was decreased (5.4±0.6 vs 3.0±0.1 L/min), in relation to a RV-arterial uncoupling as shown by a ratio of end-systolic to arterial elastances (Ees/Ea) decreased from 1.4±0.1 to 0.7±0.1. At patho-biological level, pro-apoptotic Bax/Bcl2 mRNA ratio and caspase-3 activation were signifycantly increased in right ventricular tissue, with associated increased mRNA expressions in natriuretic peptide precursors A (NPPA) and B (NPPB). Proinflammatory cytokines, such as interleukin-1alpha and -1beta, were upregulated in the failing right ventricle, while expressions of antagonist (IL1RN) and receptor (IL1R) didn’t change. Prolonged aorta-pulmonary shunting in piglets does not further aggravate pulmonary hypertension, but induced a RV failure, which appears related to mechanical stress-induced myocardial inflammatory response probably responsible for the induction of apoptotic pathways and increased expression of hypertrophic markers.

P-04

ACTIVATION OF APOPTOTIC PATHWAYS IN EXPERIMENTAL ACUTE AFTERLOAD-INDUCED RIGHT VENTRICULAR FAILURE

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The pathobiology of persistent right ventricular (RV) failure observed after an acute increase in pulmonary artery pressure (Ppa) remains incompletely understood. We here tested the hypothesis that persistent RV dysfunction might be related to an activation of apoptotic pathways. Fourteen anesthetized dogs were randomised to a transient 90 minutes pulmonary artery constriction or to a SHAM operation, followed 30 minutes later by hemodynamic measurements including effective arterial elastance (Ea) to estimate RV afterload and endsystolic elastance (Ees) to estimate RV contractility, and sampling of myocardial tissue to assess apoptosis by real-time quantitative polymerase chain reaction, enzyme-linked immunosorbent assay and immunohistochemistry. Transient increase in Ppa persistently increased Ea from 0.75±0.08 to 1.37±0.18 mmHg/mL, and decreased Ees from 1.06±0.09 to 0.49±0.09 mmHg/mL, Ees/Ea from 1.44±0.06 to 0.34±0.03 and cardiac output from 3.78±0.16 to 1.46±0.10 L/min, indicating RV failure. As compared to the SHAM-operated group, and to left ventricular tissue in animals with persistent RV failure, there were decreased gene expressions of RV and septal Bcl-2, with no changes in the gene expressions of Bax and Bak, and an increase in the Bax/Bcl-2 ratio. RV and septal Bcl-XL, and RV Bcl-w gene expressions were decreased as compared to the SHAM-operated group. There were activations of RV Caspases-8 and -9, and of RV and septal Caspase-3. Diffuse RV and septal apoptosis was confirmed by TUNEL staining. There were also increased RV and septal protein expressions of tumor necrosis factor-alpha. Acute afterload-induced persistent RV failure appears to be related to an early activation of apoptotic pathways.

P-05

BLEOMYCIN AEROSOLIZATION: THE BEST ROUTE OF ADMINISTRATION FOR PULMONARY FIBROSIS MODEL IMPROVEMENT?

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic disease characterized by the accumulation of fibrotic tissue in the lung. This accumulation results from a dysfunction in the healing process; but, the exact mechanisms that lead to this trouble are still poorly understood. Different experimental models of the disease exist, such as radiation damage, instillation of silica or asbestos and transgenic mice or gene transfer employing fibrogenic cytokines. However, the most frequently used model is the intratracheal (IT) instillation of bleomycin in rodent. In the present studies, we compared instillation and aerosolization of this fibrotic agent. 47 Wistar rats received 3IU of bleomycin intratracheally, either by instillation (22) or by spraying (25). Manipulated animals and shams were sacrificed at day 3, 7, 14, 21 and 56 and the presence and intensity of the fibrotic process were studied histologically. Therefore, slides were obtained from each pulmonary lobes and a trichrome blue staining was performed. Fibrosis was then quantified using a modified Ashcroft scale (grade 0 to 8). When fields from the most affected part of the each lobe were considered, the value obtained was on average 0.6±0.1 in shams, and 1.8±0.1, 1.9±0.4, 2.7±0.3, 2.8±0.5, 3.2±0.4 in the instillation group at day 3, 7, 14, 21 and 56 respectively. Corresponding values after aerosolization were 1.4±0.1, 1.9±0.2, 3.8±0.1, 4.1±0.2, 4.6±0.3. In contrast, when the mean value of randomly chosen fields in the six lobes were considered, the corresponding results were 0.6±0.1 in shams and 0.7±0.1, 0.3±0.1, 0.9±0.1, 0.6±0.1, 0.9±0.1 and 0.5±0.1, 0.5±0.1, 1.5±0.3, 1.8±0.4, 2.3±0.2 with instillation and spraying respectively (same times). Interobserver variability was also studied. In conclusions, both instillation and aerosolization of bleomycin induce the development of fibrosis as demonstrated by the same progression of the highest modified Ashcroft score. Aerosolization, however, allows a homogeneous distribution of lesions among the lungs as demonstrated by the persistence of a time effect when random sampling is considered for the analysis.

P-06

A NOVEL IMPLANTABLE VAGUS NERVE STIMULATION SYSTEM (ADNS-300) FOR COMBINED STIMULATION AND RECORDING OF THE VAGUS NERVE:PILOT TRIAL AT GHENT UNIVERSITY HOSPITAL

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Vagus nerve stimulation (VNS) is an established treatment for refractory epilepsy. The ADNS-300 is a new system for VNS that includes a rechargeable stimulus generator and an electrode for combined stimulation and recording. In this feasibility study, three patients were implanted with ADNS-300 for therapeutic VNS. In addition, Compound Action Potentials (CAPs) were recorded to evaluate activation of the vagus nerve in response to VNS. Three patients were implanted with a cuff-electrode around the left vagus nerve, that was connected to a rechargeable pulse generator under the left clavicula. Two weeks after surgery, therapeutic VNS (0.25 mA to 1.25 mA, 500µsec, 30 sec On, 10 min Off and 30 Hz) was initiated and stimulus-induced CAPs were recorded. The ADNS-300 system was successfully implanted in all three patients and patients were appropriately stimulated during six months of follow-up. A reduction in seizure frequency was demonstrated in two patients (43% and 40% in patient 1 and 3 respectively), while in patient 2 seizure frequency remained unchanged. CAPs could be recorded in patient 1 and 2, proving stimulation-induced activation of the vagus nerve. This feasibility study demonstrates that the ADNS-300 system can be used for combined therapeutic stimulation (in 3/3 patients) and recording of CAPs in response to VNS (in 2/3 patients) up to three weeks after surgery. Implantation in a larger number of patients will lead to a better understanding of the electrophysiology of the vagus nerve, which in turn could result in more adequate and individualized VNS parameter choice.

P-07

GLYCINE RECEPTOR ACTIVATION INFLUENCE EARLY CORTICAL DEVELOPMENT

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The glycine receptor (GlyR) is a member of the ligand-gated ion channel superfamily. The presence and function of glycine receptors in the adults it is well known, however, the presence of glycine receptor in the embryonic cortex has only been study after the embryonic day 19 (E19) (Flint et al., 1998) and there is no description about the physiological function of this receptor at early stages of cortical development. The development of the cortex during embryogenesis it is a complex process characterized by intense proliferation, cellular migration from the proliferative zones and differentiation that will end up with the generation cortical circuits. A wide range of factors are known to influence the different aspects of corticogenesis. Among those factors, neurotransmitters and ligand-gated ion channels have recently gained interest for being signals for central nervous system (CNS) development (Nguyen. et al., 2001; Ik-Tsen et al., 2007). In this study we have characterize the functional properties, by using the patch clamp technique, of GlyR mediated current during early stages of development (E13-E17) showing, for the first time, the presence of GlyR mediated currents in migrating neurons. The two main types of migrating neurons, the proyection neurons and interneurons express GlyR as soon as they start their migration to build the cortex. Along its migration the pharmacological properties of the receptor change as a possible effect of receptor maturation. The EC_{50} for glycine in migrating cells before passing the cortico stratial junction was 154 ± 41and after entering the cortex it changed to 69 ± 12 µM. All these GlyR mediated currents were blocked by the traditional blockers of GlyR, strychnine and picrotoxinin. With regard to the physiological role of GlyR in corticogenesis it was assessed the possible effects in migration and proliferation and it was found that glycine GlyR blockade actively modulate cell migration and possibly also cell proliferation.

P-08

HYPERTENSION AS A CO MORBIDITY FACTOR IN A STROKE MODEL FOR CONSCIOUS ANIMALS: EFFECTS ON INFARCT SIZE, NEUROLOGICAL DEFICIT AND ACTIVATION OF GLIAL CELLS

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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 has been shown to be neuroprotective in animal models of focal cerebral ischemia. For successful translation to clinical studies the Stroke Therapy Academic Industry Round Table (STAIR)-criteria are essential. Testing of drugs in conscious animals, the use of a co morbidity factor such as hypertension and a clinically relevant administration of the drug are three important criteria. We showed that subcutaneous administration of IGF-I in conscious rats with transient occlusion of the middle cerebral artery resulted in decreased infarct volumes. The purpose of our study is to develop and investigate a clinically relevant animal model for focal transient cerebral ischemia using conscious rats with a co morbidity factor. To this end, we tested the use of the Spontaneously Hypertensive Rat strain (SHR) in the endothelin-1 (Et-1) rat model. Using a stereotactic frame, 200 pmol Et-1 was applied in the vicinity of the middle cerebral artery of conscious controls and SHRs in order to induce a cerebral infarct. Motor/sensory functions were measured 1, 6 and 24 hours after the insult using the Neurological Deficit Score (NDS). Infarct size was assessed by cresylviolet staining. To investigate the inflammatory response, LPS was administered in the striatum of both control rats and SHR. A dose-range finding study was carried out applying 40 and 10 µg/4µl LPS. Rectal temperature was measured 1, 3 and 6 hours after the administration of 10 µg/4µl LPS. 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) 24 hours after Et-1 and LPS administration. One day after induction of focal cerebral ischemia, SHRs showed a significantly larger infarct volume (55.50 ± 4.24 mm^3, n= 6) compared to the controls (39.20 ± 2.71 mm^3, n=15). Accordingly, SHRs exhibited lower NDS at each time point, although these differences did not reach statistical significance. Despite the larger infarct size in SHR, microglial activation in response to the insult was reduced. Indeed, a reduced number of activated microglia ($ED1^+$) in the striatum (WKY: 398 ± 50, n= 4; SHR: 195 ± 16, n= 4) as well as in the cortex (WKY: 608 ± 93, n= 4; SHR: 224 ± 56, n= 4) was observed. In contrast, activation of astrocytes, as assessed by GFAP expression, was the same in both animal models. LPS-injection resulted in an equal activation of microglia and an increased activation of astrocytes in the SHRs when 10 µg/4µl was administered (WKY: 25.3 ± 1.6, n=4; SHR: 32.1 ± 1.1, n=3). SHRs also show a significant increase in rectal temperature at every time point (1h: WKY: -0.80 ± 0.40, n=4; SHR: 0.60 ± 0.20, n=4); (3h: WKY: -0.40 ± 0.18, n=4; SHR: 0.72 ± 0.048, n=4); (6h: WKY: 0.32 ± 0.31, n= 4; SHR: 1.47 ± 0.08, n=4). We conclude that the SHR can be used to induce a clinically relevant ischemic damage. Using this model, 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. With respect to the differences between the normal and hypertensive model for cerebral ischemia, it remains to be established whether reduced activation of microglia is responsible for the increased infarct size in the SHR.

P-09

MATERNAL INFLAMMATION AFFECTS EMBRYONIC MICROGLIA

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Infection during pregnancy can lead to maternal inflammation. Several studies have suggested that maternal inflammation increases the risk on neuropsychiatric disorders, like autism, in the offspring. The cause of autism remains unknown. Vargas et al. demonstrated the presence of an active neuroinflammatory process in the brains of autistic patients, with marked microglial cell activation. Microglia colonize the central nervous system early in embryonic development, at the moment that neuronal migration to the cortical plate is peaking and neuronal differentiation and synaptogenesis are underway. By their production of growth factors, it has been suggested that microglia can influence axonal growth and synaptogenesis. The aim of this study is to determine the localization, activation stages and migration routes of the microglia present in the embryonic murine neocortex, in healthy embryos and embryos subjected to maternal inflammation. In the control group, as expected, the cell density and number of microglial ramifications increase as the embryo ages. This increase in cell density is also present in the inflammation group, however compared to the control it is more pronounced. Based on the expression levels of CD68 and CD11b, the microglial cells of the embryos subjected to maternal inflammation show a higher activation profile. The orientation of the protruding ramifications of microglia present in the parenchyma suggests that the cells migrate along radial glial fibers to reach their final position. Confocal images confirm contact between both cell types.

P-10

MEDICATION HISTORY RECONCILIATION BY A PHARMACY STUDENT IN PATIENTS ADMITTED TO THE EMERGENCY DEPARTMENT

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Background and Objective: Medication discrepancies (MD) are common at transition periods such as hospital admission, transfer to another care unit and discharge. These discrepancies can lead to medication errors or other drug related problems, and have the potential to cause harm to the patient. The aim of this study was to investigate the incidence and the type of MD that affect patients who were admitted to the emergency department (ED). *Design:* Observational, prospective cohort study. At hospital admission, the medication history was structurally documented by a senior year pharmacy student. This list was compared to the physician-acquired medication history and investigated for discrepancies in acute and chronic medications. When patients were transferred from the ED to another ward, the focus was on discrepancies in chronic drugs. *Setting:* The urgent care facility of the ED of a Belgian university hospital (UZ Brussel). *Main outcome measures:* The percentage of patients with unintended MD and incidence & type of MD. *Results:* The study group consisted of 86 patients of which 68 took acute medication and 56 took chronic medication; 38 patients took both acute and chronic medication. For the acute medication group, the pharmacist (median 2, range 0-5) found more home medications per patient than documented by the physician (median 1, range 0-5; p<0,001) and 81% of patients had MD with a median of 1 MD per patient (range 0-7). MD were present with 87% of acute drugs (n=121). For acute medication the most common MD was omission of an acute drug (49% of MD). For the chronic medication group, the pharmacist also found more chronic home medications per patient (median 4, range 0-15) than documented by the physician (median 3, range 0-13; p=0,001) and 98% of patients had MD with a median of 3 MD per patient (range 0-15). One or more MD were present with 72% of chronic drugs (n=244). The most common MD for chronic medication was absence of documenting the frequency of administration (43% of MD). Of the 86 initial patients, 29 were transferred to another care unit; 24 of them took chronic medication. Of the 24 patients, 14 (58%) had one or more MD in their prescribed chronic medication. In total, 35 MD were detected with a median of 1.5 MD per patient (range 0–4). MD were present with 27% of the chronic drugs (n=129). The most common MD was omission of chronic medication (74%). *Conclusions:* The high number of MD emphasizes the importance of accurate and complete medication histories acquired by structural interviews at the time of ED admission. These medication histories should also include acute drugs because they, as well as chronic drugs may have triggered the ED visit. At the time of transfer to another care unit, the prescribed medication should be reconciled with respect to the original home medication.

P-11

BLOCK OF SK CHANNELS BY THE SIGMA AGONIST 1,3-DI-O-TOLYL-GUANIDINE: EVIDENCE FOR A NOVEL SITE OF ACTION FOR SK BLOCKERS

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Among ion channels involved in the control of neuronal activity, small conductance calcium-activated potassium channels (SK) represent an interesting therapeutic target. Indeed, they underlie medium duration afterhyperpolarizations (mAHPs) in many types of neurons, thus inhibiting cell excitability. Three subtypes of SK subunits, SK1, SK2 and SK3, have been cloned and are expressed differentially within the central nervous system (CNS). Blocking SK channels might be beneficial in the treatment of several CNS disorders such as depression (SK3), Parkinson's disease (SK3) and cognitive disorders (SK2). So far, the prototypical blocker of SK channels is apamin, an octadecapeptide from bee venom. We have recently shown that apamin blocks SK channels by binding to a site distinct from that used by classical pore blockers such as tetraethylammonium (TEA) (Lamy et al. J. Biol. Chem. 2010, 285, 27067-77). We have also demonstrated that the nonpeptide blocker N-methyl-laudanosine (NML) (Scuvée-Moreau et al. J. Pharmacol. Exp. Ther. 2002, 302, 1176-83) competes for the binding site of the toxin. Further, our research team has recently shown that the sigma agonist 1,3-di-o-tolyl-guanidine (DTG) directly blocks SK currents in a voltage-independent manner (Lamy et al. Eur. J. Pharmacol. 2010, 641, 23-8). We have combined patch clamp experiments on cell lines with molecular modelling and mutagenesis, to try to identify the site where DTG blocks. DTG was found to be equipotent on wild-type (WT) and apamin-insensitive (e.g. SK2H337N) channels. Moreover, mutated channels with increased sensitivity to TEA (SK3V520F: mean IC_{50} of TEA: 0.34 mM versus 11 mM for WT channels) were blocked by DTG with the same potency as WT channels. Thus, DTG does not seem to share the site of either apamin or TEA. Modelling data were in agreement with this possibility because of the identification of various potential binding sites. Although preliminary, these results suggest the existence of yet another binding site in the outer pore region of SK channels.

P-12

INCREASE IN HIPPOCAMPAL URIC ACID AFTER KAINIC ACID INDUCED SEIZURES IS SUPPRESSED BY ALLOPURINOL

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Recent studies have shown that changes in uric acid concentration play a role in different neurologic diseases. Given these findings, the purpose of this study is to measure hippocampal uric acid concentration in a mouse model for temporal lobe seizures and to evaluate the effect of allopurinol on hippocampal uric acid levels during seizures. Wild type C57BL/6 mice (n=14, 20 g) were stereotactically implanted with a bipolar electrode and a microdialysis guide cannula in the left dorsal hippocampus. Microdialysis and EEG were performed before and after kainic acid (KA) administration. Microdialysis samples were analysed with high performance liquid chromatography (HPLC) to determine the uric acid concentration in 7 mice that received allopurinol 100 mg/kg 30 minutes before KA administration and 7 others that received a placebo. EEG recordings were screened for number and duration of seizures. In the control group a marked increase in hippocampal uric acid concentration was found with a peak increase between one and two hours after KA infusion. In the experimental group on the other hand, allopurinol significantly suppressed KA-induced increase in hippocampal uric acid levels. Dialysis samples collected 50 and 100 minutes after KA infusion demonstrated significantly lower uric acid levels in the experimental group compared to the control group (sample 50': $p = 0.023$, sample 100': $p = 0.005$ Mann-Withney U test). Our results clearly show an increase in uric acid after KA induced status epilepticus which can be blocked for at least 6 hours by a single injection of allopurinol 30 minutes prior to KA infusion. In our experiment, there wasn't a significant difference in the number or duration of seizures between the control group and the experimental group during the status.

P-13

EBP50 MODULATES MIGRATION AND CYTOKINESIS IN VASCULAR SMOOTH MUSCLE CELLS

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EBP50, also known as NHERF1, is a scaffold protein that possesses two PDZ interacting domains and an ERM binding domain. In a previous study, we characterized the interaction of EBP50 with several proteins of the cell cytoskeleton in intact arteries, after agonist stimulation. The aim of this work was to further characterize the role of EBP50 in the modulation of cell cytoskeleton in primary vascular smooth muscle cells.
Immunostaining of EBP50 in these cells revealed a localization along thick filaments that crosses the entire cell. Transfection of two different siRNAs against EBP50 depleted its expression by 99±0.5% and 97±0.5% after 96 hours of culture. Cells transfected with a scramble siRNA exhibited thick actin bundles, large and numerous focal adhesions and microtubules that spread uniformly in the cell from the perinuclear region. On the other hand, cells depleted for EBP50 lack actin bundles, show lamellipodiae and trailing tails, had fewer and smaller focal adhesions and microtubules mainly spread into lamellae projections. Cells transfected with a scramble siRNA were able to migrate constitutively. EBP50-knocked down cells exhibited faster migratory properties. Moreover, 19±0.5 and 21±1% of binucleated cells were observed in cells transfected with first and second siRNAs against EBP50, respectively, while only 5±1% of binucleated cells were observed among cells transfected with a scramble siRNA. This observation has been confirmed by propidium iodure staining and FACS analysis of DNA content: 6±0.5% and 16±2% of the cells were in G2/M phase, respectively in control cells and in cells transfected with EBP50 specific siRNA, indicating an alteration in the cytokinesis.
These results indicate that EBP50 is a key player in cellular migration and cytokinesis. Further studies would be performed to investigate how EBP50 modulates these cellular processes.

P-14

REGULATION OF AGONIST-EVOKED CALCIUM ENTRY BY RHO KINASE IN AORTA AND CULTURED AORTIC SMOOTH MUSCLE CELLS

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We have demonstrated that, besides his role in the calcium-sensitization of the contractile process in smooth muscle, Rho-dependent kinase (ROK) is involved in noradrenaline-activated calcium entry in rat arteries (Ghisdal,et al. 2003). The objective of the present study was to investigate the involvement of the ROK pathway in the calcium signal in vascular smooth muscle cells, by comparing the effect of the ROK inhibitor, Y-27632, in whole artery and in cultured vascular smooth muscle cells. Endothelium denuded rat aorta rings and primary cultured rat aortic smooth muscle cells were used. Cytosolic calcium concentration was measured in fura-2-loaded arteries or cells. Vasopressin was first applied in Ca2+-free solution to release intracellular Ca2+ and Ca2+ entry was activated by the addition of Ca2+ in the bath solution. ROK expression was measured by western blot analysis and immunofluorescence, and its activity was determined by measuring its downstream targets, phospho-ERM (pERM). Protein kinase C (PKC) involvement on pERM was also investigated by measuring the effect of the PKC inhibitor, Gö-6983. In isolated rat aorta, the ROK inhibitor Y-27632 completely inhibited vasopressin-evoked contraction, depressed calcium entry signal by 52 ± 6 % (n=5) and inhibited ERM phosphorylation. On the contrary, in cultured aortic smooth muscle cells, Y-27632 did affect neither the calcium entry signal evoked by vasopressin nor ERM phosphorylation, although ROK expression was confirmed. On the other hand, ERM phosphorylation was inhibited by the PKC inhibitor Gö-6983 in cultured cells but not in isolated aorta. These results suggest that the mechanism of agonist-evoked Ca2+ entry is different in the whole artery compared to cultured cells, which probably lack an essential effector in the ROK pathway and might involve the PKC pathway.

P-15

EXPRESSION OF GLUT-2 AND THE ELECTROGENIC NA^+-HCO_3^--COTRANSPORTER NBCE1 IN HUMAN ENDOCRINE PANCREAS

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Species-related differences in the expression of selected proteins in pancreatic islet cells cannot be ignored. For instance, GLUT-2 was reported to be expressed at very low levels and not to be functionally essential in human ß cells. Likewise, whilst the expression of cystic fibrosis transmembrane conductance regulatory (CFTR) protein was documented in rat pancreatic islets by RT-PCR, Western blotting and imunocytochemistry, no conclusive evidence for the expression of CFTR in human islets was so far obtained. Such considerations led us to investigate in human pancreatic islets the expression of GLUT-2 and that of the electrogenic Na^+- HCO_3^--cotransporter (NBCe1), recently documented by RT-PCR, Western blotting and immunocytochemistry in both rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells. GLUT-2 immunocytochemistry was compared in rat and human pancreatic sections. NBCe1 expression was investigated by RT-PCR in human islets and immunocytochemistry in human pancreatic sections. Immunostaining of GLUT-2 was comparable in human and normal rat islets. Expression of NBCe1 in islet cells was documented by RT-PCR in isolated human islets, and confirmed by immunocytochemistry of human pancreatic sections. The present findings argue, on one hand, against the view recently formulated that GLUT-2 expression is functionally not essential in human ß cells and, hence, too low to allow D-mannoheptulose entry into such cells. The fact already documented more than 40 years ago that the latter heptose inhibits insulin secretion in humans and the finding reported 10 years ago that tritiated D-mannoheptulose is indeed efficiently taken up by isolated human islets also argue against such a view. The observation that NBCe1 is well expressed in human islets warrants, on the other hand, further investigations on the participation of this cotransporter in the fluxes of bicarbonate anions in islet cells.

P-16

LACK OF TRPC1 CHANNELS IMPAIRS SKELETAL MUSCLE REGENERATION

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We have previously showed in vitro that Ca^{2+} entry through TRPC1 ion channels regulates myoblasts migration and differentiation by activating calpain, a Ca^{2+}-dependent protease which cleaves myristoylated alanine-rich C-kinase substrate (MARCKS) protein to allow myoblasts migration. To explore, in vivo, whether the absence of TRPC1 channel impairs skeletal muscle regeneration, we used cardiotoxin injections to induce muscle injury in adult $TRPC1^{+/+}$ and $TRPC1^{-/-}$ mice. Interestingly, we observed that regenerated $TRPC1^{-/-}$ muscles had a smaller fibre size and a decreased specific force respectively after 10 and 14 days of regeneration. We also observed an increase of central nuclei at day 14 of regeneration in $TRPC1^{-/-}$ whereas, at this stage, in $TRPC1^{+/+}$ muscles, nuclei were essentially situated in the periphery of the fibres. These observations indicate a delay in muscle regeneration in $TRPC1^{-/-}$ mice in comparison with their controls. To understand the molecular mechanisms which sustain this delayed regeneration in $TRPC1^{-/-}$ mice, we investigated myogenic transcription factors implicated in the control of myogenesis. In comparison with $TRPC1^{-/-}$, $TRPC1^{+/+}$ muscles showed an earlier increase of the mRNA level and of the protein expression of MyoD and Myf5. Interestingly, developmental Myosin Heavy Chain (MHCd), a well known downstream target of MyoD during muscle regeneration, was also expressed earlier in $TRPC1^{+/+}$ than in $TRPC^{-/-}$ muscles. Finally, we also observed a more important and earlier phosphorylation of both Akt and P70S6k in $TRPC1^{+/+}$ muscles than in $TRPC1^{-/-}$ muscles, suggesting, as previously reported, an involvement of Akt / mTOR / P70S6K pathway in the control of protein synthesis, muscles fibres size and muscle regeneration in vivo. Altogether, our results demonstrate the importance of TRPC1 channels in skeletal muscles development both in vitro and in vivo and identify Akt / mTOR / P70S6K as the main pathway affected in $TRPC1^{-/-}$ during muscle regeneration in vivo.

P-17

MOLECULAR COMPOSITION OF THE DELAYED RECTIFIER K-CURRENT IN SINGLE ADULT MOUSE 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 adult mouse dorsal root ganglia (DRG) neurons. Using extracellular Stromatoxin (ScTx) and TEA, we isolated the delayed rectifier, which is carried by both homotetrameric Kv2 and heterotetrameric Kv2/silent Kv channel complexes. In order to identify the exact molecular composition we correlated the mRNA content with the recorded currents. The mRNA is extracted with the patch clamp pipette and amplified by an RNA amplification prior to quantitative real time PCR.

P-18

DYNAMIC CONTROL OF NEURONAL FIRING THRESHOLD BY CALCIUM BUFFERING : A NEW ROLE FOR CALCIUM BINDING PROTEINS

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We have investigated the detailed regulation of neuronal firing threshold by the cytosolic calcium buffering capacity using a combination of mathematical modeling and patch clamp recording in acute slice. Theoretical results show that, at similar free calcium concentration, increased calcium buffer concentration lowers the firing threshold of cerebellar granule cells. We show that this effect is a direct consequence of the major slowdown of calcium dynamics. Patch clamp recordings on cerebellar granule cells loaded with a high concentration of the fast calcium buffer BAPTA (15 mM) reveal alterations in the excitability threshold as compared to cells loaded with 0.15 mM BAPTA. In high calcium buffering conditions, granule cells exhibit a significative lower firing threshold. These results suggest that cytosolic calcium buffering capacity can tightly modulate neuronal firing threshold and therefore that calcium-binding proteins may play a critical role in the information processing in the central nervous system.