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Abstracts

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PL-01

MECHANISM-BASED PKPD MODELING IN TRANSLATIONAL PHARMACOLOGY

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Mechanism-based PKPD models are based on principles from systems biology and contain specific expressions to characterize processes on the causal path between plasma concentration and response. This includes a) the target distribution, b) the target interaction/activation and c) the transduction and homestatic control mechanisms, which may be operative. The utilisation of these models relies on novel biomarkers characterising specific processes on the causal path in a quantitative manner. An essential feature of mechanism-based PKPD models is the strict distinction between “drug-specific” and “biological system-specific” pharmacodynamic parameters to describe in vivo drug effects. We have successfully developed mechanism-based PKPD models for drugs acting at various targets including A1 Adenosine, μ Opioid, 5-HT1A Serotonin and GABAA receptors. Our findings show that in general a drug's in vivo intrinsic efficacy can be accurately predicted on the basis of in vitro bioassays. Prediction of the in vivo potency on the other hand appears to be more difficult, presumably as result of complexities at the level of the target site distribution. Our results also show that equilibrium concentration-effect relationships can be readily scaled from pre-clinical animal models to humans. In contrast, the scaling of transduction and homeostatic feedback mechanisms appears to be more complex. It is concluded that mechanism-based PKPD models provide a scientific basis for the prediction of efficacy and safety of novel drugs in humans on the basis information from in vitro bioassays and/or in vivo animal studies.

PL-02

NEW ROLES FOR "OLD" RECEPTORS: UPDATE ON BETA3-ADRENERGIC SIGNALING IN CARDIOVASCULAR TISSUES

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Nitric oxide is a fundamental regulator of vessels and cardiac muscle function. It is produced by a family of enzymes, the nitric oxide synthases (NOS), all of which are represented in the diverse cell types composing the myocardial tissue. As such, they

participate in short-term regulation of contractility and perfusion, and long-term regulation of angiogenesis, myocardial remodeling and regeneration by cardiac stem cells. All of the above are desirable targets for pharmacologic regulation by drugs commonly used in cardiovascular diseases. Indeeed, recent understanding of the regulation of the cardiac NOS has unveiled pleiotropic effects of cardiovascular drugs that could be exploited for improved therapeutic efficacy. We will illustrate such "pleiotropism" for new generation beta-blockers for which we demonstrated the ability to promote NO-dependent beneficial effects in the vasculature and in the myocardium. Elucidation of the pharmacodynamic mechanisms underlying these effects, through activation of beta3-adrenergic receptors, open the way for the development new therapeutic approaches of cardiovascular diseases.

PL-03

RECIPROCAL INTERACTIONS BETWEEN WAKEFULNESS AND SLEEP

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Cognition is regulated across the 24-h sleep-wake cycle by circadian rhythmicity and sleep homeostasis. To better understand these mechanisms, we used functional neuroimaging (PET, fMRI, EEG/fMRI) both during wakefulness and sleep in normal human volunteers. In a first set of studies, we characterized the neural correlates of cognition during a normal sleep-wake cycle and during sleep-loss. The data show that dynamic changes in brain responses evolve across the sleep-wake and circadian cycles in a regionally-specific manner in such a way that the allocation of cortical resources through subcortical activation is constrained by sleep pressure and circadian phase. In a second set of studies, we show that non rapid eye movement (NREM) sleep is not a state of quiescence but is related with significant regional brain activity in synchrony with slow waves and spindles. A last set of studies aimed at showing that the regional brain activity is modified by previous experience, especially during NREM sleep following spatial learning. These studies provide some insights on the interaction between sleep and waking activity, and their influence on regional brain activity in humans.

PL-04

CURRENT INSIGHTS IN TO MECHANISMS OF INFLAMMATION IN A PROTOTYPE RHEUMATIC DISORDER, SPONDYLOARTHRITIS

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Over the past years, it has become clear that Tumor Necrosis Factor (TNF) is a key player in the pathogenesis of spondyloarthritis, a disease leading to joint inflame-mation of axial skeleton and peripheral joints, but the mechanisms by which this occurs are only partially known. Particularly, the cellular targets sufficient to mediate the articular and extra-articular manifestations (gut and skin inflammation) of spondyloarthritis remained to be defined, as well as the cellular constituents capable of modulating this TNF driven inflammation. Recently, we reported a peculiar role for mesenchymal cells in a mouse model of spondyloarthritis, characterized by enhanced TNF mRNA stability, resulting in Crohn´s like ileitis as well as peripheral arthritis. Hence, TNF-R1 expression on mesenchymal cells was sufficient to mediate combined gut and joint pathologies in this model of murine spondyloarthritis. However, it remained unclear whether regulatory T cell subsets could modulate this inflammation. More recently, we uncovered that a particular regulatory T cell lineage, invariant NKT (iNKT) cells, are natural regulators of TNF driven inflammation by modulating maturation and differentiation of antigen presenting cells in a pathway that is strictly dependent upon TNF. Altogether, these observations provide new insights in the regulatory as well as the effector mechanisms of spondyloarthritis.

PL-05

THE AMAZING MAMMALIAN BLOOD VESSEL: MORE SURPRISES FROM AN OLD FRIEND

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Over the past 10 years, research involving the mammalian blood vessel (my 'old friend') has involved major surprises. 1. The realisation that the internal elastic lamina is full of holes, through which endothelial cells send feet-like projections, has highlighted the diffusion pathways that are surely involved in the modulation of myocyte activity by endothelium-derived factors. 2. The roles of the Ca2+-sensitive K+ channels, SKCa and IKCa, present on the vascular endothelium is not totally clear but the demonstration of their localisation in different endothelial microdomains has been a fundamental advance. 3. The discovery that vascular endothelial cells possess a G protein-coupled receptor (the CaS) which senses extracellular Ca2+ was not expected. Even more surprising is the close coupling between the CaS and IKCa channels, with activation of the former triggering opening of IKCa without any involvement of SKCa. Speculatively, the function of the vascular CaS is to respond to increases in [Ca2+] in myoendothelial spaces (especially those associated with endothelial cell end-feet) following myocyte contraction. 4) In the brain, the role of astrocytes in neurovascular coupling has recently been clarified. Until now, it was believed that activation of only BKCa channels in astrocytic end-feet (encircling the cerebral arterioles) generated K+ clouds in the astro-myocyte spaces. Subsequent activation of myocyte inward rectifiers generated hyperpolarisation and vessel dilatation. It is now clear that not only BKCa but also IKCa channels (both on astrocytic end-feet) are involved in this neurovascular coupling phenomenon.

PL-06

MECHANISMS OF SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS

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Of all the faculties of mind, memory is the one that contributes most to our sense of self. For over a century it has been assumed that memory is stored as changes in the strength of connections between nerve cells in the vast neural networks of the brain. This process, known as synaptic plasticity, is widely studied in a brain region known as the hippocampus. In this talk I will describe some of the experiments that have led to our current understanding of the molecular machinery of memory. I will focus on how the brief activation of one class of L-glutamate receptor, the NMDA receptor, triggers long lasting changes in synaptic transmission mediated by a different class of L-glutamate receptor, the AMPA receptor, during the forms of synaptic plasticity known as long-term potentiation (LTP) and long-term depression (LTD). The role of various signaling molecules, such as Ca2+ and the protein kinase GSK-3β, will be described. The potential roles of aberrant synaptic plasticity in neurological disorders, such as Alzheimer's disease, will also be discussed.

PL-07

ALLERGEN SPECIFIC IMMUNOTHERAPY: LET'S MAKE THINGS BETTER

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Allergen specific immunotherapy (SIT) was first applied exactly a century ago by Noon and colleagues. Ever since, not much has changed in the original treatment protocol of subcutaneous injections with increasing doses of crude allergen extracts. This seems rather surprising since it is currently the only disease modifying treatment that offers long-term protection against allergic manifestations. Moreover, the efficacy of SIT is rather variable and appears to differ from patient to patient depending on the type of allergen, the type of allergic disease and on as yet unknown factors, including genetics. However, there is light at the end of the tunnel. Novel strategies are on their way to improve the burden of multiple subcutaneous injections by sublingual or intralymphatic administration, to improve its efficacy by using an adjuvant and to improve the standardization by using recombinant allergens. Despite its long history, the precise mechanism of action of SIT is still incompletely understood precluding rational improvement of SIT. To further dissect the mechanism(s) of action and to develop improved immunotherapeutic strategies, we have established a mouse

model of SIT. Using this mouse model we have determined that the immunoregulatory cytokine interleukin-10 (IL-10) plays a critical role in the beneficial effects of SIT in this mouse model. IL-10 can be produced by regulatory T-cells (Treg), which have been shown to increase in allergic patients after SIT. Novel strategies to increase the efficacy of SIT using the mouse model will be presented and discussed.

PO-08

CROSS-TALK BETWEEN 7TM-RECEPTORS FOR VASOACTIVE PEPTIDES

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Perivascular nerves and the endothelium contain potent vasoactive peptides such as calcitonin-gene related peptide (CGRP), neuropeptide Y (NPY) and endothelin-1 (ET-1). Because alterations of peripheral nervous function are observed in hypertension and upregulation of ET-1 can be part of the endothelial dysfunction that characterizes several diseases, these peptides might be valid pharmaco-therapeutic targets. In mesenteric resistance-sized arteries, endogenous CGRP released from sensory-motor nerves causes dilatations that are inhibited by endogenous NPY released from sympathetic nerves. This can be attributed to stimulatory and inhibitory effects of CGRP-receptors and NPY Y1-receptors, respectively, on the sarcolemmal adenylyl cyclase of the post-junctional arterial smooth muscle cells (ASMC; De Mey et al. JPET, 2008). The potent and long-lasting vasoconstrictor effects of ET-1 result from tight binding of the peptide to ASMC ETA-receptors. Antagonists and most vasodilators can not dissociate this binding and reversibly relax contractile effects initiated by ET-1. However, stimulation of ASMC CGRP-receptors by exogenous and endogenously released CGRP selectively promotes dissociation of ET-1/ETA-receptor complexes and terminates ET-1-induced vasoconstriction (Meens et al. PLoS ONE, 2010). This is observed in arteries isolated from several vascular beds and in vivo at the level of local vascular resistance and blood pressure. Because ECE and NEP are involved in the synthesis of ET-1 and in the degradation of CGRP, respectively, we tested the hypothesis that a dual NEP-/ECE-inhibitor (SOL-1) might have beneficial therapeutic effects. In young SHR rats, tissue contents of ET-1 and CGRP were increased and reduced, respectively, compared to age-matched WKY rats. Chronic treatment with SOL-1 normalized the tissue levels of the peptides and partly prevented the development of hypertension (Nelissen et al. this meeting). We conclude that crosstalk between vasoactive peptides might be a valid target for the treatment of multifactorial (cardiovascular) diseases.

PO-09

CLINICAL PHARMACOLOGY OF PURINES: FROM FOREARM TO HEART

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Preclinical research indicates that adenosine has important cardiovascular actions including inhibition of vascular inflammation, vascular calcification, atherosclerosis, prevention of ischemia-reperfusion injury and arrhythmias. These actions result from stimulation of specific adenosine receptors located on the cell membrane of various cells including endothelium, vascular smooth muscle cells, cardiomyocytes, autonomic nerve endings and inflammatory cells. Since disease, genetic background and co-medication could modulate the effects, formation and clearance of adenosine, investigations in humans in-vivo is clinically important. However, translation of this preclinical information to humans in-vivo is hampered by autonomic reflexes that occur in particular when adenosine is infused systemically. Furthermore, the rapid uptake of extracellular adenosine by the equilibrative nucleoside transporter into various cell types (including endothelium and erythrocytes) limits the access of intravenously infused adenosine to important target cells such as cardiomyocytes and vascular smooth muscle cells. Therefore, we have operationalized a human in-vivo method to study local actions of adenosine (both exogenous as well as endogenous) in the forearm vascular bed. In this overview, I will provide some examples of this research with particular attention to the effect of adenosine on consequences of ischemia-reperfusion and the effect of statins on the formation of extracellular adenosine. Finally, I will compare some of our forearm results with results from studies in isolated human atrial tissue and with results from clinical trials with clinically relevant cardiovascular endpoints to make the point that knowledge on the clinical pharmacology of adenosine in the forearm is also relevant for the heart.

PO-10

CONGENITAL NEPHROGENIC DIABETES INSIPIDUS: DO PHARMACO-CHAPERONES PROVIDE THE CURE?

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To maintain water homeostasis, vasopressin (AVP), released from the pituitary, binds its vasopressin type-2 receptor (V2R) in renal collecting duct cells, and induces translocation of Aquaporin-2 (AQP2) water channels from vesicles into the apical membrane, resulting in urine concentration. In congenital Nephrogenic Diabetes Insipidus (NDI), the kidney fails to concentrate urine in response to AVP, which is mostly due to mutations in the V2R gene. Missense V2R mutants in NDI are retained in the endoplasmic reticulum (ER), due to misfolding. As they are often functional, their ER retention is fundamental to the disease. V2R is thought to function only when expressed in the plasma membrane. To work toward treatment, we showed that cell permeable V2R and V1bR antagonists (CPAn) rescued the basolateral cell surface expression of ER-retained V2R mutants in NDI in polarized cells. In vivo studies with V1bR antagonists revealed a significant normalization of the daily urine

output and osmolality in patients, indicating that CPAn are promising pharmacological therapeutics to treat congenital NDI. Interestingly, the pharmaceutical industry developed non-peptide V2R agonists, meant to treat incontinence via oral administration. As these drugs thus have to pass the intestinal cell layer, we reasoned they must be cell membrane permeable. Indeed, treatment of cells with cell-permeable V2R agonist (CPAs), but not peptidic dDAVP, induced generation of cAMP from ER-retained V2R mutants in NDI and restored translocation of AQP2 to the apical membrane. Surprisingly, however, this effect was obtained without changing the intracellular localization or stability of the V2R mutants, which revealed that the V2R mutants can be activated inside the cell. As such, our data revealed a novel concept that GPCRs can be activated intracellularly and that CPAs are highly-promising novel therapeutics to treat diseases due to misfolded GPCRs in general, and NDI due to V2R mutations in particular.

PO-11

THE VOLTAGE SENSOR OF VOLTAGE-GATED K CHANNELS AS A TARGET FOR POTENTIAL ANTIEPILEPTIC AND ANTIARRHYTHMIC DRUGS

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Free polyunsaturated fatty acids (PUFAs) modulate the voltage dependence of voltage-gated ion channels. As an important consequence thereof, PUFAs can suppress epileptic seizures and cardiac arrhythmia. However, molecular details for the interaction between PUFA and ion channels are not well understood. In this study we have localized the PUFA-binding site in the voltage-gated Shaker K channel, by introducing positive charges on the channel surface which potentiated the PUFA effect. We furthermore found that PUFA mainly affects the final voltage-sensor movement, which is closely linked to channel opening, and that specific charges at the extracellular end of the voltage sensor are critical for the PUFA effect. Because different voltage-gated K channels have different charge profiles, this implies channel-specific PUFA effects. The identified site and the pharmacological mechanism will potentially be very useful in future drug design of small-molecule compounds specifically targeting neuronal and cardiac excitability.

O-01

G PROTEIN βγ-SUBUNITS IN VASORELAXING AND ANTI-ENDOTHELINERGIC EFFECTS OF CGRP

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Introduction: Calcitonin gene-related peptide (CGRP)–receptors can relax vascular smooth muscle cells (VSMC) via G-protein αs subunits (Gαs) and by promoting dissociation of endothelin-1 (ET-1) from ETA-receptors (Meens et al. 2010). The latter “terminator effect” is not mimicked by stimuli of Gαs. Thus, we evaluated involvement of G-protein βγ subunits (Gβγ) using i) myograph studies with isolated rat arteries, ii) radioligand-binding on membranes from CHO cells expressing human CGRP-receptors and iii) cAMP production assays on cultured rat VSMC. In several types of arteries, isoproterenol (ISO), forskolin and CGRP caused relaxation during K+-induced contractions. Relaxing effects of CGRP did not correlate with those of ISO or forskolin. In mesenteric arteries, contracted with K+ or ET-1, IBMX (PDE-inhibitor) increased sodium nitroprusside (SNP)- and ISO- but not CGRP-induced relaxations. While fluorescein (negative control) was without effect, gallein (Gβγ-inhibitor) increased receptor binding of [125I]-CGRP in the absence and presence of GTPγS. Gallein also significantly increased CGRP-induced cAMP production by VSMC but did not modify the sensitivity for CGRP. In isolated arteries, gallein and M119 (related Gβγ-inhibitor) selectively inhibited the relaxing and anti-endothelinergic effect of CGRP while not altering contractile reponses to K+ or ET-1 or relaxing reponses to ISO or SNP. Activated CGRP-receptors cause cyclic nucleotide-independent relaxation of VSMC and terminate arterial effects of ET-1 via Gβγ. Also, Gβγ may restrict agonist-binding to CGRP-receptors independently from Gα.

O-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 of perivascular adipose tissue in the regulation of vascular tone. The influence of moderate hypoxia on the effect 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. Hypoxia (bubbling with 95% N2, 5% CO2) 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 in the presence of indomethacin (10 µM) and nitro-L-arginine (0.1 mM). A minimal vasorelaxing effect was observed in both arteries without adipose tissue. Glibenclamide (30 µM) significantly diminished the hypoxic response in aorta, while apamin (1 µM) combined with charybdotoxin (0.1 µM) significantly reduced the hypoxic response in mesenteric arteries. 8-(p-sulfophenyl)theophylline (0.1 mM) did not influence the hypoxic response in both arteries. In contrast to aorta, removal of the endothelium significantly reduced the hypoxic relaxation in mesenteric arteries. Apamin (1 µM) combined with TRAM-34 (10 µM) partially reduced the hypoxic response in mesenteric arteries. Indomethacin (10 µM) and SQ-29548 (10 µM) significantly reduced the hypoxic vasoconstriction. From these results we conclude that in mice aorta hypoxia induces vasorelaxation in the presence of brown adipose tissue. This

relaxation is in part mediated by opening KATP channels and independent of adenosine receptors, suggesting the involvement of the "adipocyte-derived relaxing factor" (ADRF). In mice mesenteric arteries, hypoxia induces a biphasic response in the presence of white adipose tissue, suggesting the involvement of (a) vasoconstrictor(s) and dilator(s). The vasoconstrictor seems to be non-endothelial COX metabolites, whereas the vasodilator is at least in part the EDHF acting partially through opening KCa channels.

O-03

INCREASED ARTERIAL STIFFNESS LEADS TO BRAIN INFARCTIONS AND SUDDEN DEATH IN APOLIPOPROTEIN E- DEFICIENT MICE

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Arterial stiffness has been associated with increased cardiovascular risk. We previously showed that arterial stiffness promotes both the progression of atherosclerosis and a more unstable plaque phenotype in apolipoprotein E (ApoE)-deficient mice with a mutation in the fibrillin-1 gene. In this study, we aimed to investigate whether these mice are suitable as an animal model for plaque rupture. Mice with a mutation (C1039G+/-) in the fibrillin-1 gene, leading to fragmentation of the elastic fibers, were crossbred with ApoE-deficient mice. At six weeks of age, female ApoE-/- Fibrillin-1 +/- and ApoE-/- Fibrillin-1 +/+ (control) mice were fed a western type diet for up to 52 weeks. Between 13 and 27 weeks (average 17 weeks) of a Western type diet, 75 % (12 out of 16) of ApoE-/- Fibrillin-1 +/- mice died suddenly, which was mostly preceded by head tilt and/or motor problems (loss of orientation and balance). In contrast, mortality was not seen in the control group (0 out of 15 mice at 52 weeks). Magnetic Resonance Imaging showed the presence of one or more brain infarctions in all ApoE-/- Fibrillin-1 +/- mice, whereas only one control mouse displayed a single brain infarct ($p < 0.001$). Brain lesions were characterised by the presence of foam cells and cholesterol clefts and stained positively for neutral lipids, smooth muscle cells and macrophages, indicative of cerebral embolization of plaque material after rupture. ApoE-/- Fibrillin-1 +/- mice seem to be a promising animal model for plaque rupture and stroke.

O-04

INTRA-ARTERIAL VEGF ANTIBODIES DO NOT ACUTELY AFFECT VASCULAR TONE IN HEALTHY VOLUNTEERS

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Angiogenesis inhibitors have remarkably improved treatment of patients with several types of cancer. One of the most reported side effects of angiogenesis inhibitors is hypertension. In patients treated with bevacizumab, a monoclonal antibody against vascular endothelial growth factor, hypertension had an overall incidence up to 32%. The increase in blood pressure occurs early in treatment. Understanding the pathogenesis of this side effect is essential for optimal treatment with this class of drugs. One of the main targets of angiogenesis-inhibitors is the vascular endothelial growth factor and its receptors. Animal and human studies show that VEGF induces vasodilatation and hypotension by stimulation of NO production. Moreover animal studies suggest that endogenous VEGF may play a role in maintaining normal vascular tone in blood vessels. Theoretically, inhibition of the VEGF-pathway in humans would decrease NO production causing vasoconstriction and thereby induce hypertension. Intra-arterial bevacizumab infusion causes acute local vasoconstriction. Methods: In 7 healthy male volunteers the acute vasomotor effect of bevacizumab was studied. During 15 minutes bevacizumab 144 µg/dl forearm volume/min was infused in the brachial artery of the non-dominant arm while assessing forearm blood flow with plethysmography. The dosage was calculated to reach a local concentration of 120 mg/l. resembling therapeutic concentrations. During bevacizumab infusion venous blood was collected from both arms to measure local and systemic concentrations of bevacizumab. Intra-arterial infusion of bevacuzimab did not significantly alter vascular tone expressed as vascular resistance or flow ratio. Intra-arterial bevacizumab was well tolerated. Intra-arterial Bevacizumab (144 µg/dl forearm volume/min for15 minutes) does not increase muscle vascular tone as a potential mechanism of hypertension.

O-05

ALTERED DIASTOLIC AND SYSTOLIC LEFT VENTRICULAR FUNCTION IN HORSES COMPLETING A LONG DISTANCE ENDURANCE RACE

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In horses, international endurance races are rides from 80 to 160 km performed in 6 to 12 h. This is comparable to effort performed by human athletes doing long duration strenuous exercise. Irreversible cardiac injury has been suspected after such exercise in some athletes. Little is known concerning the cardiac consequences of such races in horses. The objective of this study was to examine the effects of a long distance endurance race on the equine heart. Blood samples and echocardiography were performed before and within 45 minutes after completion of a race of 88 to 132 km in 13 horses. Systolic (s) and diastolic (d) left ventricular and aortic internal diameter (LVID and Ao, respectively) were measured from classical

echocardiographic views. Heart rate (HR), peak flow velocity, and flow velocity integral were measured from aortic pulsed-wave Doppler recordings. The left ventricular fractional shortening (FS) and ejection fraction (EF), the stroke volume (SV) and the cardiac output (CO) were calculated from those measurements. Blood samples were taken on plain tubes, centrifuged after clotting, and the serum was drawn and directly frozen at -20℃ until dosage of cardiac troponine I (CnTI) concentration using a commercially available Immunoassay System. The mean duration of the course was 7h28 ± 1h07 and the mean speed was 16.2 ± 1.3 km/h. After the race, LVIDd, LVIDs, Ao, EF, FS, FVI and SV were significantly lower and HR was significantly higher as compared with pre-race values. All horses had a CTnI under the detection limit (< 0.04 ng/ml) before the race. Eight of them had still a CTnI concentration under the detection limit after the race, and 5 of them had a detectable but physiological CTnI concentration after the race, with values ranging from 0.04 to 0.13 ng/ml. Those results suggest that in the studied horses, long duration endurance exercise was associated with a decrease in left ventricular preload and a myocardial fatigue, but was not associated with a significant myocardial injury.

O-06

TARGETING OF MYOFIBROBLASTS IN THE INFARCT AREA CAN PREVENT VENTRICULAR DILATATION AFTER MYOCARDIAL INFARCTION

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Progressive ventricular dilatation is a major complication of myocardial infarction (MI), resulting in heart failure. In a comparison of infarct healing in mice of different genetic backgrounds, we have established a correlation between myofibroblast numbers in the infarct area and preservation of left ventricular geometry. Myofibroblasts not only can produce extracellular matrix proteins, but also possess contractile properties similar to smooth muscle cells. These characteristics allow them to limit infarct expansion. However, our knowledge of the molecular control of myofibroblast differentiation is not complete. Myofibroblasts in the infarct area express Frizzled-1 and -2 (Fzd-1 and -2), receptors for Wnt proteins. We hypothesized that Fzd-1 and -2 can modulate the characteristics of these myofibroblasts. To test this, we blocked Fzd-1 and -2 with UM206 (6 microgram/kg.hr), a potent Fzd antagonist. UM206 administration significantly increased myofibroblast numbers, reduced adverse remodeling and completely prevented heart failure development. Subsequently we compared different treatment regimens for UM206 in mice subjected to MI and followed for 5 weeks: treatment for the first 2 weeks (wk1-2), the last three weeks (wk3-5) or the full 5 weeks (wk1-5). All regimens had a beneficial effect on EF (saline: 18±1%, wk1-2: 24±0,5%, wk3-5: 31±2%, wk1-5: 37±0,5%; $P<0.05$). The improvement of the EF was associated the myofibroblast numbers in the infarct area (saline: 4±0,2%, wk1-2: 6±0,4%, wk3-5: 11±0,9%, wk1-5: 13±1,4%; $P<0.05$). We conclude that blocking of Fzd-1 and -2 receptors with UM206 improves cardiac function after MI by increasing myofibroblast numbers in the infarct area, thereby reducing infarct expansion. Continuous UM206 therapy was found to be superior

over late or early therapy, but any UM206 treatment regimen was better than saline. The results show that pharmacotherapy specifically aimed at the myofibroblast can be a successful approach to prevent heart failure after MI.

O-07

MECHANISMS INVOLVED IN CORM-2 INDUCED VASORELAXATION

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Carbon monoxide (CO) plays an important physiological role in regulation of the vascular tone. CORM-2 is frequently used as a CO-donor to evaluate the physiological and pathophysiological properties of CO as well as the potential therapeutic applications of this diatomic molecule. The aim of this study was to examine the molecular mechanisms underlying the vasodilatory properties of CORM-2 as this has not yet been extensively explored. Isometric tension recordings were performed using different isolated blood vessels from both mice and rats. Responses to saturated CO solutions and CORM-2 were evaluated in the presence/absence of activators/inhibitors of different molecular pathways. The saturated CO solution was unable to relax mice isolated blood vessels, whereas it induced concentration-dependent relaxations in rat aortic ring segments. The response to CO was inhibited by both the soluble guanylyl cyclase inhibitor ODQ and potassium channel blocker TEA. CORM-2 relaxed both mice and rat isolated blood vessels in a concentration-dependent manner. The vasodilatory response was however only partially blocked by ODQ and TEA. Interestingly, 4-aminopyridine antagonised the CORM-2 induced vasodilatation whereas iberiotoxin had no influence on the response elicited by this CO-releasing molecule. It is concluded that the molecular mechanisms involved in CORM-2 induced vasodilatation differ from the mechanisms underlying CO induced vasorelaxation. CO induces vasorelaxation by activating sGC and/or calcium-activated potassium channels. In contrast, the vasodilatory properties of CORM-2 are only partially dependent upon sGC or potassium channel activation. Moreover, the CORM-2 induced vasodilatation seems to involve voltage-dependent potassium channels instead of calcium-activated potassium channels.

O-08

ENDOTHELIN-1 AND CALCITONIN GENE-RELATED PEPTIDE CONTRIBUTE TO THE DEVELOPING HYPERTENSION IN YOUNG SHR

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Binding of endothelin-1 (ET-1) to ETA-receptors promotes hypertension and can be terminated selectively by calcitonin gene-related peptide (CGRP). Endothelin converting enzyme (ECE) and neutral endopeptidase (NEP) are involved in ET-1 synthesis and in ET-1-and CGRP-degradation. Hypotheses: (1) ET-1 and CGRP contribute to the development of hypertension and (2) chronic ECE/NEP inhibition during this period lowers ET-1 levels and blood pressure (BP). The content of ET-1 and CGRP was determined by radioimmuno assay in the heart and kidney of male Spontaneously Hypertensive Rats (SHR) of 6, 8 and 12 weeks of age and age-matched Wistar Kyoto (WKY) rats. Also 4 week old male SHR and WKY rats were treated for 4 weeks with the novel ECE/NEP inhibitor SOL-1. At 8 weeks BP was determined intra-arterially. ET-1 content was significantly elevated in cardiac and renal tissue of 8 and 12 week old SHR compared to age-matched WKY. An age-dependent increase in cardiac CGRP content was observed only in WKY. In SHR, SOL-1 treatment significantly reduced BP and lowered cardiac and renal ET-1 content while CGRP content was significantly increased. The effect of chronic ECE/NEP inhibition on BP and on ET-1 and CGRP levels supports our hypothesis that ET-1 and CGRP are involved in the development of hypertension in SHR.

O-09

STUDY OF BASAL NITRIC OXIDE PRODUCTION BY ENDOTHELIAL NITRIC OXIDE SYNTHASE IN THE MOUSE AORTA

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Nitric oxide (NO) is an important anti-atherogenic factor and its production by endothelial NO synthase (eNOS) is disrupted early in plaque development. Constitutive (“basal”) NO production is believed to be of highest relevance in its protective effects. We noticed, however, that basal eNOS activity does not always correlate with agonist stimulated eNOS sensitivity, pointing to different regulation mechanisms. We investigated the effects of time and kinase inhibitors. C57Bl/6 mouse (4 months) thoracic aorta segments were suspended in organ baths. Basal eNOS activity was measured by its capability to suppress phenylephrine (1µM) contractions measured at 1h intervals, followed by dose-relaxation curves to acetylcholine. Responses in the presence of NOS inhibitors served as control. Afterwards, phosphorylation of eNOS at S1177 and T495, and Akt at S473 was investigated using Western blot. Phenylephrine contractions increased gradually in time (1.1±0.6, 4.4±1.1 and 7.9±0.8 mN, resp. at 1, 2 and 3h; n=4), suggesting decreased basal NO production, while acetylcholine sensitivity remained unaltered. Western blot showed a loss of Akt phosphorylation (-49±16%) after 2h without changes in eNOS phosphorylation. The phosphoinositide 3 kinase (PI3K) inhibitor wortmannin raised phenylephrine contractions (+57±7 %; n=4 at 1h), without changing sensitivity to acetylcholine or exogenous NO (DEANO), or smooth muscle function. This effect was abolished by preincubation with the tyrosine kinase inhibitor erbstatin. Finaly, wortmannin strongly inhibited Akt phosphorylation (-97±3%), but did not alter eNOS phosphorylation. These findings show that basal and agonist-stimulated eNOS activity are regulated by different mechanisms. The loss of basal

eNOS activity with time in the organ bath and wortmannin treatment and the concomitant decline in Akt phosphorylation point to involvement of the PI3K/Akt pathway and tyrosine phosphorylation, but not of eNOS phosphorylation of at S1177.

O-010

DIPYRIDAMOLE PROTECTS AGAINST ISCHEMIA AND REPERFUSION INJURY IN TWO DIFFERENT HUMAN MODELS

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Well-tolerated protection against ischemia-reperfusion (IR) could benefit many patients. We tested the hypothesis that dipyridamole, an adenosine uptake inhibitor, provides such protection. Experiment 1: 10 healthy men were treated for 7 days in a double-blind randomised cross-over design with dipyridamole (2x200mg) and placebo (wash-out > 3 weeks). At the end of each treatment, volunteers performed 10 minutes forearm ischemic exercise. At reperfusion, 450 MBq 99m-Tc-annexin A5 was administered to measure IR injury. Gamma scans of both hands were acquired at 1 and 4 hours of reperfusion. Annexin targeting was defined as % difference in counts/pixel between IR and control thenar muscle. In 6 CABG patients, atrial tissue was harvested during surgery. Ex-vivo, in two trabecles, contractile force recovery was measured after 90 minutes of simulated ischemia. In each patient, one trabecle was exposed to dipyridamole (1 mg/l) while the other served as control. Dipyridamole significantly reduced annexin A5 targeting in those volunteers who received placebo first and dipyridamole next (from 27±14% and 30±16% (1 and 4 hours of reperfusion) to 15±9% and 16±10%), as opposed to those who received dipyridamole first and placebo next (from 17±13% and 19±6% to 20±14% and 19±6%; $p=0.029$ for interaction between treatment order and treatment effect). Atrial contractile recovery after IR was higher in dipyridamole compared to controls (43 ±10.1% and 19 ±3.2% resp.; $p<0.05$). Dipyridamole treatment protects against IR in forearm skeletal muscle of healthy volunteers (considering the wash-out period, this effect appears to persist >4 weeks) as well as in cardiac tissue from patients with coronary artery disease.

O-11

CXCR2 ANTAGONISTS BLOCK THE N-AC-PGP-INDUCED NEUTROPHIL INFLUX IN THE AIRWAYS OF MICE, BUT NOT THE PRODUCTION KC

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Neutrophils are innate immune cells in chronic inflammatory diseases including chronic obstructive pulmonary disease (COPD) and can be attracted to the site of inflammation via the collagen breakdown product N-acetyl Proline-Glycine-Proline (N-Ac-PGP). To elucidate whether the CXCR2 receptor is involved in N-Ac-PGP-induced neutrophil migration and activation, studies using specific antagonists were performed in vivo. N-Ac-PGP and keratinocyte cell-derived chemokine (KC, CXCL1) were administered in C57Bl/6 mice via oropharyngeal aspiration. Intraperitoneal applications of CXCR2 antagonist SB225002 or SB332235 were administered 1 hour prior and 1 hour after oropharyngeal aspiration. Six hours after oropharyngeal aspiration mice were sacrificed. Neutrophil counts and CXCL1 levels were determined In bronchoalveolar lavage fluid, myleoperoxidase (MPO) levels were measured in lung tissue homogenates and an immunohistological staining for neutrophils was performed on lung tissue. N-Ac-PGP and CXCL1 induced a neutrophil influx in the bronchoalveolar lavage fluid and lung tissue, which was also reflected by increased MPO levels in lung tissue. The N-Ac-PGP- and CXCL1-induced neutrophil influx and the increased pulmonary tissue MPO levels were inhibited by the CXCR2 antagonists SB225002 and SB332235. Moreover, N-Ac-PGP administration enhanced the CXCL1 levels in bronchoalveolar lavage fluid, which could not be attenuated by both CXCR2 antagonists. It can be concluded that neutrophil migration induced by N-Ac-PGP is mediated via direct interaction with CXCR2 or indirectly via the release of CXCL1. The N-Ac-PGP-induced release of CXCL1 is independent of the CXCR2 receptor. Furthermore, N-Ac-PGP is more potent in inducing the neutrophil migration in the pulmonary tissue than into the bronchoalveolar lavage fluid.

O-12

INCREASED ARGINASE ACTIVITY CONTRIBUTES TO AIRWAY INFLAMMATION AND REMODELING IN A GUINEA PIG MODEL OF COPD

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Airway inflammation and remodeling are major features of COPD. In addition, pulmonary hypertension is a common comorbidity that is associated with a poor prognosis in COPD. Recent studies indicated that increased arginase activity contributes to allergen-induced airway inflammation, hyperresponsiveness and remodeling in a guinea pig model of allergic asthma. Although there is evidence that cigarette smoke and lipopolysaccharide (LPS), two risk factors of COPD, increase arginase expression, the role of arginase in the pathogenesis of COPD is currently unknown. This study aimed to investigate the role of arginase in pulmonary inflammation and remodeling using an animal model of COPD. Guinea pigs were instilled intranasally with either lipopolysaccharide (LPS) or saline twice weekly for 12 weeks

and were pretreated by inhalation with the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) or phosphate-buffered saline. Repeated LPS exposure increased lung arginase activity, resulting in increased L-ornithine/L-arginine and L-ornithine/L-citrulline ratio's. Both amino acid ratio's were reversed by ABH treatment. Repeated LPS exposure also induced increased IL-8 levels, neutrophils, goblet cells and hydroxyproline in the lung, which were all attenuated by ABH. Remarkably, LPS-induced increase in right ventricular mass, indicative of pulmonary hypertension, was fully abrogated by the arginase inhibitor. In conclusion, increased arginase activity contributes to pulmonary inflammation, airway remodeling and right ventricular hypertrophy in a guinea pig model of COPD, indicating that arginase inhibitors may have therapeutic potential in the treatment of this disease.

O-13

TLR 2/6 STIMULATION IN COMBINATION WITH INTESTINAL INFLAMMATION LEADS TO TH17 RESPONSES AGAINST ORALLY ADMINISTERED ANTIGENS

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Interest in T helper cells during the initiation and progression of inflammatory bowel disease (IBD) has increased as a result of the probable role of Th17 cells in IBD pathogenesis. The dextran sodium sulfate (DSS) model of colitis recruits many T lymphocytes to the inflamed colon, however, very little is known about the subtype (Th1, Th2, Th17 and Treg) and what factors steer their development. Research on gut antigen-primed T cells in DSS colitis is hampered by a lack of knowledge about the antigens presented in the gut. Thus, an oral tracker antigen (ovalbumin) was employed to follow gut antigen-primed T cells in mice under the influence of gastrointestinal Toll-like receptor (TLR) triggering during DSS-induced colitis. DSS-colitis was induced by administering DSS (1.5%) in the drink water over a period of 6 days. Ovalbumin and the TLR ligands were given orally during the DSS treatment and mice were sacrificed one week later. Adaptive immune responses were measured by examining T cell responses and numbers with flow cytometry before and after ex vivo stimulation with ovalbumin. Ovalbumin-specific CD4+ T cells were detected in the spleens and mesenteric lymph nodes of mice after the resolution of inflammation (14 days after the start of DSS administration). These responses were found in mice that were treated orally with bacteria or with ligands for the TLR2/6 heterodimer during colitis and not in mice that were treated orally with ligands for TLR1/2 and TLR4. Using antibodies specific for transcription factors, it was determined that the ovalbumin-specific CD4+ T cells were Th17 or Treg, expressing either RORγT or Foxp3 respectively. These results demonstrate that breaking tolerance against gut antigens requires a combination of local inflammatory signals to develop gut antigen-specific Th17 and Treg cells that may be found systemically after the resolution of inflammation. These insights will ultimately help elucidate how the gut environment and pathogen-associated molecular patterns steer the development of adaptive immune responses during the initiation of colitis and how pathogen-

recognition receptors can be used to manipulate the development or resolution of inflammation to treat gastrointestinal disease.

O-14

S1P1 RECEPTOR MODULATION IMPROVES SYSTEMIC VASCULAR DYS-FUNCTION AFTER CPB: A COMPARISON BETWEEN FTY720 AND SEW2871

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Cardiopulmonary bypass (CPB) is associated with a systemic inflammatory response syndrome and disturbances in endothelial function of systemic arteries. FTY720, a non-selective agonist for sphingosine 1-phosphate (S1P) receptors, evokes lymphopenia by sequestration of lymphocytes from circulation to secondary lymphoid organs. SEW2871 is a selective agonist of S1P1 receptors. We investigated whether FTY720 and SEW2871 improve vascular reactivity after CPB in the rat. Experiments were done in male Wistar rats (n=48). Anesthesia-induction consisted of isoflurane (2.5-3%), followed by fentanyl and midazolam during CPB. After catheterization of the left femoral and carotid artery and the right heart, normothermic extracorporeal circulation was instituted for 60 min. Following 1 day of recovery, constriction to phenylephrine (PE) and serotonin (SE) and relaxation to acetylcholine of small mesenteric artery segments was assessed in a wired myograph system. Relaxation was expressed as % of preconstriction and analyzed as the area under the concentration-response curve (AUC, arbitrary units). Contractile responses were inhibited after both CPB and SHAM experimental procedures. In mesenteric artery, FTY720 normalized SE- and PE-mediated vascular reactivity after CPB. FTY720 also increased total relaxation to acetylcholine as compared with untreated CPB groups (AUC: 256.8±43.9 and 168.2± 28.4; respectively). SEW2871 produced vascular effects comparable with FTY720. FTY720 and SEW2871 improve vascular function in mesenteric after CPB. This pharmacological effect was mediated mainly through S1P1 receptors and did not require lymphopenia. S1P1 receptor agonism may provide a promising therapeutic intervention to prevent CPB-related vascular dysfunction.

O-15

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

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Rupture-prone atherosclerotic plaques have a large necrotic core, numerous macrophages and a thin fibrous cap that consists of collagen-synthesizing smooth muscle cells (SMCs) and extracellular matrix. Plaques tend to rupture as a consequence of a weakened fibrous cap, particularly in the shoulder regions where most macrophages reside. Lesional macrophages produce matrix metalloproteinases that degrade the extracellular matrix, and induce SMC apoptosis which leads to a decrease in collagen-producing cells. Selective removal of macrophages from plaques by inducing macrophage-specific cell death may be a promising strategy to alter plaque composition, favoring plaque stability. Macrophages express Toll-like receptors (TLRs) to recognize pathogens, and eliminate intracellular pathogens by inducing autophagy. Since macrophages in human atherosclerotic plaques express TLR7, we investigated 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 characterized by an increased number of vesicular structures containing cytoplasmic debris, called autophagosomes, which is a hallmark of autophagy. The effects of imiquimod were abolished in TLR7-deficient macrophages, indicating that imiquimod induces autophagy via TLR7. 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 stabilizing atherosclerotic plaques.

O-16

LPS INDUCED TNF- A PRODUCTION BY BRONCHOAVEOLAR LAVAGE- AND LUNG CELLS IS INCREASED IN TLR 9 DEFICIENT MICE

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Toll-like receptors (TLRs) are proteins that recognize specific molecular patterns and have recently been implicated in the pathogenesis of chronic obstructive pulmonary diseases (COPD). Animal models that are used to study COPD involve for instance exposure to cigarette smoke (CS) or to lipopolysaccharide (LPS) which is known to stimulate TLR 4. Interestingly, CS induced cytokine production by macrophages is also mediated by TLR4 and TLR4 is implicated in CS induced pulmonary inflammation in a murine model of COPD (Karimi et al 2006, Maes et al 2006). Very recently we demonstrated that TLR 9 is involved in the CS-induced IL-8 production by human inflammatory cells (Mortaz et al, 2009). Now we want to confirm these data using TLR 9 KO mice. But before doing so, we wanted to exclude possible effects of LPS via TLR4 in these animals. Lungs of TLR 9-/- and wild type C57BL/6 (WT) mice were lavaged and bronchoalveolar lavage (BAL) fluid was examined for total cell

number and differential cell number using diff quick staining. Lung and spleen tissue was isolated and frozen for PCR analysis. Total cell cultures derived from BAL (2*10^5 cells/200 µl), lung (8*10^5 cells/200 µl) and spleen (2*10^5 cells/200 µl) were ex vivo stimulated with LPS (1000 ng/200µl). Tumor necrosis factor (TNF)-α was measured in supernatant using a matched antibody cytoset from Arcus biologicals. Statistical analysis was performed in Prism. Total BAL cells were 40% increased in TLR9-/- compared to WT mice. Differential cell count did not show PMN, eosinophil nor lymphocyte influx. The increase in cell number in TLR 9 -/- animals is caused by an increased number of bronchoalveolar macrophages. LPS increased the TNF-α production of BAL-, lung- and spleen cells of WT-mice. Surprisingly, the TNF-α levels were 42,6% increased in lung cell cultures from TLR 9 -/- animals compared to WT-mice ($P<0.05$). Similar trends were observed in BAL and spleen cell cultures. These data show that TLR 9 deficiency causes an enhanced response upon TLR 4 stimulation via LPS. This could be explained by an upregulation of TLR 4 expression which is currently being investigated.

O-17

IN INFLAMMATORY BOWEL DISEASE NEUTROPHILS HAVE INCREASED MIGRATORY ACTIVITY ASSOCIATED WITH ENHANCED CXCR1 AND CXCR2 EXPRESSION

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Neutrophils transmigrate from the blood into inflamed tissue via the interaction of interleukin 8 (CXCL8), produced in this tissue, with chemokine receptors, CXCR1 and CXCR2 that are expressed on the membranes of neutrophils. We investigated neutrophil migration and components of this pathway in patients with inflammatory bowel disease (IBD) and healthy controls. CXCL-8 induced chemotaxis of peripheral blood isolated neutrophils was studied in a transwell system. CXCL8, CXCR1 and CXCR2 expression and CXCL8 release were examined by qRT-PCR, ELISA and immunofluorescence in neutrophils and in intestinal tissue. IBD neutrophils (n=24) show a similar chemotactic index in response to CXCL8 when compared with healthy control neutrophils (n=6), although the basal migratory capacity is a 5-fold higher. The expression of both CXCR1 and CXCR2 is increased in IBD neutrophils, while the expression and release of CXCL8 is decreased. CXCL8 protein levels were lower in non-inflamed IBD tissue homogenates (n=38) and significantly increased in inflamed IBD tissue (n=50) when compared with healthy tissue homogenates of colorectal cancer patients (n=20). CXCL8 was mainly found in epithelial cells in inflamed IBD tissue while sporadically neutrophils expressing CXCL8 were found in IBD tissue. IBD neutrophils have different expression levels of components involved in CXCL8-CXCR1/2 chemotaxis. In addition to the enhanced CXCL8 levels observed in

inflamed IBD tissue, this might explain the enhanced migratory capacity of neutrophils in IBD.

O-18

CIGARETTE SMOKE INDUCES RELEASE OF CXCL8 FROM HUMAN BRONCHIAL EPITHELIAL CELLS VIA TLRS AND INDUCTION OF INFLAMMASOME

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Chronic Obstructive Pulmonary Diseases (COPD) such as bronchitis and lung emphysema are chronic airway diseases associated with inflammation and cigarette smoking. Airway epithelial cells are the first cells that will be exposed to cigarette smoke. In addition, epithelial cells are able to release the cytokines CXCL-8 and IL-1beta. These cytokines are involved in the acute and chronic character of inflammatory processes associated with COPD, respectively. The aim of this study was to investigate whether Toll Like Receptors (TLRs) in or on epithelial cells were involved in cigarette smoke-induced cytokine production. Here we demonstrate that cigarette smoke induces the release of CXCL-8, IL-1beta and IL-6 from human bronchial epithelial cells (HBE-14o cells). The cigarette smoke-induced CXCL-8 production was inhibited by an antibody against TLR4 and by inhibitory ODN without CpGODN motif suggesting the involvement of TLR4 and TLR9. In addition, exposure of HBE-14o cells to ligands specific for TLR4 (LPS) or TLR9 (CpGODN) resulted in the release of CXCL8 and IL1beta as well as IL-6. TLR4 and, interestingly, also TLR9 were present on the cell surface membrane of the HBE-14o cells. The surface expression of both receptors decreased after cigarette smoke exposure. To further investigate the molecular mechanism of the cigarette smoke-induced CXCL-8 production by human bronchial epithelial cells different inhibitors were used. It was concluded that purinergic P2X7 receptors and reactive oxygen species were involved. Interestingly, the inflammasome activator monosodium urate crystals (MSU) induced also the release of CXCL-8 and IL-1beta as well as IL-6, and the caspase-1 inhibitor, irreversible interleukin-1beta-converting enzyme (ICE) inhibitor Z-Val-Ala-Asp-dichlorobenzoate (Z-VADDCB), suppressed the cigarette smoke induced release of CXCL-8. In addition, cigarette smoke, CpGODN, LPS and MSU all increased the expression of caspase-1 and IL-1beta. In conclusion, our results demonstrate that cigarette smoke releases CXCL-8 from HBE-14o cells via TLR 4 and TLR9 and inflammasome activation.

O-19

ON THE ORIGIN OF THE PACEMAKER ACTIVITY OF MIDBRAIN DOPAMINERGIC NEURONS

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Midbrain dopaminergic (DA) neurons sustain important physiological functions such as control of motricity and signalling of positive error in reward prediction in the mesolimbic system. Under physiological conditions, DA neurons can switch between three distinct modes: tonic (pacemaker), irregular, and burst firing. The nature of the channels involved in the low frequency pacemaking of DA neurons is still highly discussed. Indeed, whereas many studies have shown that L-type calcium channels are critical for this spontaneous activity, others, including ours, have observed little effect of a blockade of these channels on this firing pattern. Therefore, the respective contribution of calcium and sodium channels in pacemaking remains unclear. However, it is commonly accepted that low-frequency spontaneous firing requires oscillations in the cytoplasmic free calcium concentration. In this paper, we use a mathematical analysis to extract the mechanisms underlying the spontaneous activity of dopaminergic neurons. For this purpose, we develop a basic model of a dopaminergic neuron, where we consider the minimal set of conductances that are able to reproduce the firing patterns exhibited by these cells.
We find that pacemaker firing in dopaminergic neurons is mainly sustained by the cooperation of sodium and L-type calcium channels, whereas variations of the intracellular calcium concentration play a major role in the rate of this spontaneous firing pattern. On the basis of this mechanism, we identify potential causes for the experimental discrepancies mentioned above, using the simple model of a dopaminergic neuron as well as the quantitative model. We observe that neurons only differing by less than 1% in their maximal sodium conductance might react oppositely to a blockade of L-type calcium channels. Experiments performed in rat brain slices confirm that L-type calcium and sodium channels cooperate to generate pacemaking in these neurons.

O-20

INVOLVEMENT OF THE CYSTINE/GLUTAMATE ANTIPORTER IN HIPPO-CAMPAL FUNCTIONING: IMPLICATIONS FOR SEIZURE SENSITIVITY

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System xc- exchanges intracellular glutamate for extracellular cystine, giving it a potential role in intracellular glutathione synthesis and regulating extracellular glutamate levels. We used mice lacking the xCT subunit of system xc- (xCT-/-) to investigate the possible involvement of this transporter in the susceptibility for limbic seizures. In a first set of experiments we could not detect alterations in hippocampal glutathione content, oxidative stress or brain atrophy in the xCT-/- mice. On the other

hand, extracellular hippocampal glutamate levels were significantly decreased. Consequently, xCT deletion elevated the threshold for limbic seizures. xCT-/- mice were less susceptible to the well known chemoconvulsants pilocarpine, kainic acid and NMDA. Furthermore, N-acetylcysteine, an activator of system xc-, was proconvulsant in the pilocarpine and 6 Hz model. This proconvulsant effect was absent in the xCT-/- mice. These findings sustain that system xc- is the major hippocampal source of extracellular glutamate and that impairing system xc- is clearly beneficial to decrease susceptibility for limbic seizures.

O-21

DIFFERENTIAL EFFECTS OF COCAINE ON DOPAMINE NEURON FIRING IN AWAKE AND ANAESTHETIZED RATS

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Cocaine is a natural alkaloid, which affects excitability of dopaminergic pathways, and may be able to "hijack" the endogenous reward system. The relationships between cocaine-induced behaviour and its action on the excitability of dopaminergic (and other) neurons are not clear. We investigated the effects of cocaine on the firing of midbrain dopaminergic (DA) neurons, both in anaesthetized (n = 7) and in freely moving (n = 13) Wistar rats, using pre-implanted multielectrode arrays and a recently developed telemetric recording system (Alpha-Omega, Israel). During the experiment, animals received intraperitoneal injections of saline or cocaine (10 mg/kg). To observe the effects of general anaesthesia on the activity of recorded units, 4 animals from the awake group were anaesthetized with chloral hydrate in the second part of the recording session. We also measured plasma and brain concentrations of cocaine and its main metabolite, benzoylecgonine, at the 10th or 30th minute after the cocaine injection. Average baseline activity of DA neurons consisted of irregular tonic firing interrupted by bursting periods. Injection of saline did not lead to any significant change in the firing of these neurons. After the injection of cocaine in awake rats (n = 52), we observed in a large population of DA neurons (n = 19, 37 %) a gradual increase in firing rate and bursting which was not time-locked to the locomotor activity of the animals. In other DA neurons, such increases were time-locked to the locomotor activity (n = 23, 44 % of the cells). In anaesthetized animals cocaine produced a general decrease of the firing rate and bursting of DA neurons (n = 27), sometimes preceded by a transient increase in both parameters. In particular, monotonous increases observed in awake animals were not observed in these conditions. Injection of chloral hydrate in awake rats induced an inhibitory effect in 7 out of 10 recorded presumably DA units. This can partly explain the difference between awake and anaesthetized groups. Measured brain cocaine and benzoylecgonine concentrations suggested that differences observed in electro-physiological experiments did not have a pharmacokinetic origin. Taken together, our results demonstrate that cocaine injection differentially affects the electrical activity of DA neurons in awake and anaesthetized rats. These observations show that electrophysiological recordings in awake animals allow to uncover changes in

neuronal excitability in the presence of an exogenous agent that are absent in anaesthetized animals.

O-22

NADPH OXIDASE AS A PHARMACOLOGICAL TARGET IN DISEASE-RELEVANT OXIDATIVE STRESS

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Oxidative stress is a key pathomechanism of cardiovascular diseases. However, attempts to exploit this with antioxidants have failed in clinical trials. Thus, alternative approaches are needed, i.e. to inhibit the relevant sources of reactive oxygen species (ROS). Here we aimed to establish the role of NADPH oxidases (NOX) as sources of ROS in hypertension and cerebral ischemia/reperfusion (I/R) injury. Aortas from aged spontaneously hypertensive rats (SHR) and WKY rats were used to determine ROS levels, NOX expression and localisation, as well as endothelial function. Ischemic strokes were induced by transient middle cerebral artery occlusion and photothrombosis in wildtype and NOX4 knockout mice of both sexes and different ages. NOX expression, infarct areas, oxidative stress markers, blood-brain barrier breakdown, apoptosis, neurological scores and survival were assessed. Aged SHR displayed endothelial dysfunction and increased vascular ROS levels compared to aged-matched WKY rats. Inhibition of NADPH oxidases with VAS2870 normalised aortic ROS levels and restored endothelial function in SHR aortas. The NADPH oxidase isoforms NOX1 and NOX2 were upregulated in SHR aortas with NOX1 displaying ectopic expression in the endothelium. In contrast, NOX4 levels were unchanged, and NOX4 knockout mice displayed normal blood pressures. A major pathophysiological role for NOX4 became evident in I/R induced tissue damage: NOX4 was upregulated after I/R in human and mouse brains. After acute and chronic ischemia, brains from NOX4 knockout mice were protected from oxidative stress, blood-brain barrier breakdown and neuronal apoptosis. VAS2870 application within a clinically relevant time frame, i.e. post-stroke, protected wild-type mice from stroke-induced I/R damage. NOX1 and NOX4 appear to represent promising targets to treat oxidative stress-associated hypertension and prevent I/R injury, respectively. Pharmacological inhibition of NADPH oxidases using specific compounds may pave new avenues for the treatment of hypertension and ischemic brain injury in humans.

O-23

INWARD RECTIFIER CURRENT INHIBITION BY PENTAMIDINE

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Cellular and ionic mechanisms of pentamidine-mediated acute and chronic inhibition of cardiac inward rectifier current were examined (IK1). Pentamidine is an antiprotozoal drug widely used in treatment of Leishmaniasis, Human African trypanosomiasis and opportunistic protozoal infections resulting in candidiasis and pneumonia. Pentamidine treatment may cause sudden cardiac death by provoking cardiac arrhythmias associated with QTc prolongation and U-wave alterations. We hypothesize that pentamidine reduces Kir2.1 carried IK1 current. Patch clamp measurements of IK1 on adult canine ventricular cardiomyocytes, Kir2.1-HEK293 cells and Kir2.x inside-out patches were performed. Kir2.1 protein expression was measured by western blotting. Pentamidine binding simulation was performed using a computer model. Pentamidine decreases IK1 in cardiomyocytes (10 µM) and Kir2.1-HEK293 (1 and 10 µM) after 24 h incubation. In Kir2.1-HEK293, Kir2.1 protein expression decreases as a function of pentamidine concentration (1-10 µM). The effect is not acute, but develops after chronic exposure to the agent (16-72 h). Inhibition of lysosomal, but not proteasomal, degradation partially rescues Kir2.1 protein expression levels. When applied from the cytoplasmic side, pentamidine block of IK1 is acute (IC50=0.17 µM), and depends on the negatively charged amino acids E224, D259 and E299 in the cytoplasmic pore region of Kir2.1, but is independent of the presence of polyamines or magnesium. Kir2.2 and Kir2.3 based IK1 is also sensitive to pentamidine blockade. Molecular computer modeling confirms stable binding of pentamidine in the cytoplasmic pore region with residues E224, D259 and E299. Pentamidine inhibits cardiac IK1 by a dual mechanism. There are a chronic (lowering Kir2.1 protein expression levels) and an acute component (direct Kir2.x ion channel block from the cytoplasmic side). Kir2.1 protein levels can be rescued by lysosomal inhibition.

O-24

SQUATTING: A POSTURAL MANOEUVRE TO STUDY BAROREFLEX HOMEOSTASIS, AUTONOMIC NEUROPATHY AND DRUG-INDUCED ORTHOSTATIC HYPOTENSION

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The active postural “squat-stand” test can be used to assess baroreflex homeostasis by continuously monitoring changes in blood pressure (BP) and heart rate (HR) using a FinapresR device. The standing to squatting transition is accompanied by rises in BP and pulse pressure (PP) and by a reflex reduction in HR. Conversely, the squatting to standing transition imposes a major orthostatic stress, with a marked immediate drop in BP followed by both reflex tachycardia and vasoconstriction leading to a rapid return of BP and HR to baseline values. These ample mirror

changes in BP and HR, mimicking those observed with the classical pharmacological approach using vasopressor/vasodilating agents, allow the calculation of the so-called baroreflex gain (BRG), by plotting R-R intervals according to systolic BP levels during the squat-stand transition. In healthy subjects, BRG was reduced with aging (69 years : 2.04 vs 31 years : 3.63 msec.mm Hg-1; p=0.0006), was not affected by gender (women : 3.64 vs men : 3.42 msec.mm Hg-1; p=0.55) and was slightly dampened in overweight people (BMI 29 kg/m² : 2.85 vs BMI 22 kg/m² : 4.03 msec.mm Hg-1; p=0.03). In various pathological conditions, especially in diabetes with cardiovascular autonomic neuropathy (CAN), both the increase in BP and PP (greater pulsatile stress) during squatting and the BP reduction during standing (orthostatic hypotension) may be more important and sustained. Type 1 diabetic patients with CAN had lower GBR compared to aged-matched patients without CAN (1.39 vs 2.43 msec.mm Hg-1; p=0.0374) and more prolonged orthostatic hypotension. Several drugs (i.e. antihypertensive compounds, psychotropic agents) may also alter baroreflex homeostasis and aggravate orthostatic hypotension. GBR calculated during a squat-stand test was not affected by monotherapy with angiotensin converting enzyme inhibitors in diabetic patients, whereas it was significantly reduced by tricyclic antidepressants in nondiabetic individuals.

O-25

BIOAVAILABILITY OF ORAL MOXIFLOXACIN AFTER ROUX-EN-Y GASTRIC BYPASS SURGERY

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Roux-en-Y gastric bypass, whereby the stomach, the duodenum and the jejunum are circumvented, is the most commonly performed procedure for the treatment of morbid obesity. In healthy volunteers, moxifloxacin has an oral bioavailability of 86.2 ± 1.11 % (Stass & Kubitza, 1999). However, no data are available on the impact of gastric bypass on the oral bioavailability of the antibiotic moxifloxacin. To evaluate the effect of gastric bypass on the absolute oral bioavailability of moxifloxacin. This was a single-centre, open-label, randomized cross-over study in 12 healthy volunteers (8 female, age 25–57 yrs) who underwent gastric bypass surgery at least 6 months prior to inclusion in the trial, and had reached a stable body weight (81.4 ± 14.9 kg; BMI = 27.7 ± 4.3 kg/m²). Each subject received 2 single standard doses of 400mg moxifloxacin orally or intravenously (as a 1-hour infusion) administered on 2 occasions separated by a washout period of 1 week. Serial venous blood samples were drawn up to 72h after dosing, and the moxifloxacin plasma levels were measured by HPLC with fluorescence detection. After oral dosing, moxifloxacin plasma concentrations reached a maximum (Cmax) of 3.57 ± 1.25 µg/ml after 1.75h (0.75-4.00). After IV dosing, Cmax and tmax were 4.82 ± 1.86 µg/ml and 1.03h (0.75-

2.50), respectively. The mean areas under the plasma concentration time curve extrapolated to infinity (AUC∞) were 48.20 ± 14.91 h*µg/ml after oral dosing and 54.20 ± 15.56 h*µg/ml after IV dosing, resulting in an absolute oral bioavailability for moxifloxacin of 88.31 ± 1.06%. This study suggests that gastric bypass has no effect on the bioavailability of oral moxifloxacin.

O-26

MOLECULAR BASIS FOR DEPOLARIZATION DEPENDENT MODULATION OF CHANNEL GATE CLOSURE IN KV CHANNELS

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Kv channels are voltage-dependent potassium pores that shape the action potential duration and are critical for cellular excitability. Detection of membrane potential is done by a charged voltage sensor domain (VSD) whose reorientations generate a transient gating current (IQ). Prolonged depolarization of Shaker Kv channels pushes the VSD into the relaxed state, characterized by a slowing in IQOff. Kv channels also have two gates (in series) that seal off K+ permeation: the S6 bundle crossing (BC), directly tied to the VSD, and the selectivity filter linked to C-type inactivation. Direct comparison of K+-conduction in Shaker, reflecting the status of the BC gate, with IQ shows a strong correlation between both. As IQOff slowed down with prolonged depolarizations, BC gate closure displayed a similar 2-fold slowing when the duration of a +20mV pre-pulse was increased from 0.2 to 10 seconds. Introducing in Shaker the double mutation T449V+I470C that abolished C-type inactivation did not prevent this slowing process, indicating that the observed slowing was independent of the inactivation process. Simultaneous monitoring of the VSD movement (fluorescence recordings) and channel gate closure (ionic recordings) in the TMRM-labeled Shaker mutant M356C showed that the slowing in IQOff and BC gate closure occurs simultaneously. This indicates that the BC gate is strictly controlled by the movements of the VSD and most importantly that the BC gate remains open even when the VSD relaxes. Consequently, K+ conduction continues as long as C-type inactivation does not kick in and the SF gate closes. Therefore, we here propose a previously uncharacterized modulation mechanism for Kv channels in which the channel closure times are determined by the relaxation process of the VSD. Since such time course modulation of channel closure directly determines the refractory period time between action potentials, the VSD relaxation process may have an impact on regulating cell excitability.

O-27

SUSTAINED HYPERTENSION CAUSED BY TRANSIENT RENIN ANGIOTENSIN ALDOSTERONE SYSTEM STIMULATION IS PREVENTED BY AT1RECEPTOR BLOCKADE

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Transient stimulation (4-8 weeks of age) of the renin angiotensin aldosterone system (RAAS) permanently increases blood pressure (BP) in young transgenic rats with inducible hypertension. The chronic elevation in BP is associated with the induction of irreversible changes in renal structure and function. To identify the responsible RAAS components that mediate this phenotype, we tested if concomitant administration of an angiotensin II receptor blocker (ARB) during transient RAAS stimulation (TRS) could prevent the development of permanently elevated BP and end-organ damage. Studies were performed in Cyp1a1 Ren2 transgenic rats with inducible hypertension. These rats harbor a construct for the production of mouse renin which becomes activated when indole-3 carbinol (I3C) is added to the diet. Experimental rats received 0.3% I3C-treatment with either an ARB (losartan: 20mg/kg/day) or vehicle between 4-8 weeks of age via a subcutaneously implanted osmotic minipump. Cyp1a1 Ren2 rats without I3C-treatment, with or without losartan, served as controls. Intra-arterial BP was determined at 4, 8, 12 and 20 weeks of age. Additionally, renal vascular resistance RVR was determined. Data are presented as mean ± SEM. At 8 weeks of age TRS rats demonstrate fulminant hypertension (Mean Arterial Pressure (MAP) of 170±9 mmHg versus 126±2 mmHg). Treatment with losartan had no effect on BP in the control rats (123±3mmHg), whereas BP was completely restored to baseline values in the TRS rats (126±3mmHg). At 20 weeks of age, i.e. 12 weeks after TRS, I3C-treated rats without losartan still demonstrated elevated MAP (152±10 mmHg versus 123±2 mmHg), whereas BP of losartan treated TRS rats remained at control levels (119±2 mmHg). This study shows that TRS induced sustained hypertension can be completely prevented by an ARB suggesting that AT1R activation is the main contribution to the irreversible renal damage in young Cyp1a1 Ren2 transgenic rats.

O-28

REACTIVITY OF GLOMERULAR ARTERIOLES TO ANG II IN VIVO AND MYOGENIC CONSTRICTION IN VITRO PREDICT RENAL DAMAGE AFTER 5/6 NX

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Susceptibility to renal injury varies among individuals. Previously, it was shown that individuals with healthy baseline endothelial dilatory ability in isolated renal arterioles developed less renal damage after 5/6 Nx. Whether in vivo pre-existing vascular

integrity also predicts subsequent renal damage to 5/6 Nx is subject of the current study using intravital microscopy. In addition, we explored whether in vitro myogenic constriction of the extirpated kidney is also a predictor of renal damage after 5/6 Nx. Anaesthetized rats underwent intravital microscopy to envision glomerular afferent and efferent arterioles. After stabilization with a saline infusion, Ang II (30 ng/kg) was infused in the experimental group. Thereafter, renal damage was induced by 5/6 Nx and continued on saline infusion. During the intravital protocol, arterial blood pressure, heart rate and renal blood flow were measured. Images of glomeruli were recorded for measurements of changes in vascular diameter of the afferent and efferent glomerular arterioles. Myogenic constriction was investigated in vitro in small arteries isolated from the extirpated kidney at 5/6 Nx. After surgery, animals were followed for 12 weeks for measurement of blood pressure and proteinuria. Infusion of Ang II induced significant contraction of both afferent and efferent glomerular arterioles ($p<0.001$ compared to saline). Linear regression analysis between the change in afferent and efferent glomerular arteriolar diameter upon Ang II infusion and proteinuria 12 weeks after 5/6 Nx showed a significant correlation ($r = 0.73$; $p=0.01$ and $r = - 0.90$; $p=0.01$, respectively). Additionally, in vitro measured MC of small renal arteries correlated with proteinuria 12 weeks after 5/6 Nx ($r = - 0.71$, $p = 0.02$). Individual afferent and efferent responses to AII in the healthy rat is able to predict the severity of renal damage induced by 5/6 Nx. Further research underlying this baseline function can lead to novel preventive renoprotective therapies.

O-29

(PRO)RENIN RECEPTOR ((P)RR) BLOCKADE COUNTERACTS THE BENEFICIAL EFFECTS OF RENIN INHIBITION IN DIABETIC TRANSGENIC MREN2 RATS (TGR(MREN2)27)

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Elevated prorenin levels associate with microvascular complications in patients with diabetes mellitus. Prorenin may generate angiotensin I at tissue sites, possibly by binding to the (P)RR. We evaluated this possibility in diabetic TGR(mREN2)27 rats.
Rats made diabetic with streptozotocin were treated with vehicle, the renin inhibitor aliskiren with(out) the (P)RR antagonist HRP. Blood pressure and heart rate were monitored by telemetry. After sacrifice mesenteric arteries (MA) were used to evaluate vascular reactivity. relaxed MA of vehicle-treated rats. The NO synthase inhibitor L-NAME partially blocked the effect of ACh, whereas adding Tram34+apamin (inhibitors of intermediate/small conductance Ca2+-dependent K+ channels) on top of L-NAME reversed the relaxant ACh response into a contractile effect. Aliskiren did not alter the relaxant effect of ACh, nor the degree of blockade by L-NAME, but prevented the contractile response to ACh in the presence of L-NAME, Tram34+apamin. Yet, following co-treatment with HRP, the latter response returned, suggesting that HRP counteracts the aliskiren-induced downregulation of ACh-induced constriction, either by upregulating contractile muscarinic receptors and/or by enhancing the release of endothelium-derived contractile factor(s). Treatment did not

alter the NO-responsiveness of the vascular smooth muscle cells, evaluated with the NO donor SNAP. Endothelin-1 constricted MA identically with and without treatment. Yet, the ETA receptor antagonist BQ123 inhibited this effect in aliskiren+HRP-treated rats only, suggesting selective upregulation of ETA receptors by HRP. HRP upregulates ETA receptors and the contractile response to ACh, thereby counteracting the beneficial vascular effects of aliskiren. This occurs in a blood pressure-independent manner, and argues against detrimental effects of (P)RR-prorenin interaction.

O-30

ADDITIVE PROTECTIVE EFFECTS OF REMOTE AND LOCAL POST-CONDITIONING ON RENAL ISCHEMIA REPERFUSION INJURY

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Recently, we have shown that remote ischemic preconditioning using the hind limb is effective in reducing renal ischemia/reperfusion injury (IRI). However, preconditioning is not applicable in patients suffering from an unpredictable ischemic insult. In contrast, ischemic postconditioning (IPostC) is a strategy to reduce injury after an ischemic insult, by inducing short-term ischemia/reperfusion (I/R) shortly after reperfusion of the target organ. Similarly, short-term I/R of a remote organ (RIPostC) may also confer protection to a target organ after IRI. We investigated whether IPostC and RIPostC induce protection after renal IRI, and whether these strategies have an additive effect. In anesthetized rats, we induced 25' ischemia in the right kidney and nephrectomised the left kidney. Subsequently, rats underwent no postconditioning, IPostC, RIPostC, or both. The local IPostC stimulus consisted of 6 cycles of 8"/8" I/R. The RIPostC stimulus consisted of 3 cycles of 4'/4' I/R of the hind limb. After 48h of reperfusion, an IRI-induced rise in plasma creatinine, plasma urea and fractional sodium excretion (FENa) was observed in the non-postconditioned control group. RIPostC alone reduced the FENa from 8.4±6.2% to 3.8±2.1%. Remarkably, the combination of IPostC + RIPostC caused a 45% reduction in plasma creatinine, from 288.4±124.3 to 152.6±38.8 µmol/l. Similarly, plasma urea was reduced from 38.4±11.1 to 22.3±7.1 mmol/l and FENa was reduced by >60% to 3.1±1.5% in the RIPostC + IPostC group. In conclusion, we found that RIPostC using the hind limb as remote organ can ameliorate renal function. Moreover, the combination of remote and local postconditioning is more powerful in reducing renal damage. Our observations suggest that IPostC and RIPostC may work via different mechanisms, leading to an additive effect of these strategies. This makes postconditioning a promising tool to prevent renal damage in e.g. renal transplantation.

O-31

ROLES OF SGLT1, GLUT1 AND GLUT4 IN THE SECRETION OF GLUCOSE BY ACINAR PAROTID CELLS

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Salivary glucose concentration ranges between 25 to 100 µM in human subjects. A higher salivary glucose concentration prevails in diabetic patients than in non-diabetics. When an oral glucose tolerance test was conducted in normal subjects and diabetic patients, we observed a similar time course for salivary glucose and glycemia: the glucose concentration in unstimulated saliva progressively increased during the first 30 min and decreased thereafter. Salivary glucose is excreted by salivary glands. This study aims at investigating mechanisms for glucose secretion by salivary glands. In the literature, only the presence of SGLT1 has been so far shown at the baso-lateral membrane of parotid acinar cells in rats. We investigated the expression and localization of different glucose transporters in parotid gland from healthy rats and from streptozotocin-induced diabetic rats. Immunochemistry was used to localize SGLT1, GLUT1, GLUT2 and GLUT4. SGLT1, as well as GLUT1 and GLUT4, were observed in acinar cells of rat parotid glands. Their mRNA expression was confirmed by quantitative-PCR. GLUT4 mRNA level was significantly lower in the parotid cells of streptozotocin-induced diabetic as compared to normal rats. However, such a difference was not observed for SGLT1 and GLUT1 mRNA. By analogy with the kidney, we postulate the presence of SGLT1 in the baso-lateral membrane of acinar cells and the presence of GLUT4 and/or GLUT1 in the apical membrane. Further experiments are required to assess the validity of this proposal by co-localization immunohistochemistry.

O-32

EXPRESSION AND FUNCTION OF WNT-5A IN HUMAN AIRWAY SMOOTH MUSCLE OF ASTHMA PATIENTS

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Alterations in airway smooth muscle structure and function are key to the pathogenesis of asthma and may underlie increased airway narrowing and airways hyperresponsiveness. The mechanisms underlying these changes in smooth muscle phenotype and function are currently largely unknown. Here, we report a role for WNT-5A overexpression by airway smooth muscle from asthma patients and link this to the alterations in airway smooth muscle structure and function seen in this disease. Airway smooth muscle expressed a number of WNT ligands, of which WNT-5A was among the most abundant. mRNA and protein expression of WNT-5A was

increased in airway smooth muscle from asthma patients. Moreover, a single nucleotide polymorphism in the WNT-5A gene was found to associate with asthma susceptibility. WNT-5A expression in lung tissue and bronchoalveolar lavage fluid was inducible following in vivo allergen exposure. Further, in vitro exposure of cultured human airway smooth muscle cells to pro-inflammatory cytokines and growth factors including IL-1β, PDGF-AB and TGF-β1 induced mRNA and protein expression of WNT-5A. Autocrine WNT-5A production was found to regulate several functions of the airway smooth muscle, including cytokine production, cell proliferation, and extracellular matrix protein production. Collectively, we identify a novel role for WNT-5A in asthma, and indicate that WNT-5A overexpression by the airway smooth muscle may contribute to the alterations in airway smooth muscle structure and function that are characteristic of asthma.

O-33

COLLAGEN I-INDUCED AIRWAY SMOOTH MUSCLE PHENOTYPE SWITCHING REQUIRES SIGNALLING THROUGH FOCAL ADHESION KINASE

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Increased extracellular matrix (ECM) deposition and airway smooth muscle (ASM) mass are major contributors to airway remodeling in asthma. Increased deposition of the ECM protein collagen I is not only observed beneath the epithelium, but also surrounding the asthmatic ASM bundle. Recently, we have demonstrated that collagen I induces a proliferative, hypocontractile ASM phenotype. Little is known, however, on the signalling pathways involved. Using bovine tracheal smooth muscle (BTSM), we now investigated the role of focal adhesion kinase (FAK) and downstream signalling pathways in collagen I-induced phenotype modulation. Phosphorylation of FAK was increased during adhesion to uncoated and collagen I-coated plastic culture dishes, without differences between the matrices. No differences between cellular adhesion were found either. Inhibition of FAK activity, by overexpression of the FAK deletion mutants FAT (focal adhesion targeting domain) and FRNK (FAK-related non-kinase), attenuated adhesion. After attachment, FAK phosphorylation was time-dependently increased in cells cultured on collagen I, whereas no activation was found on the uncoated plastic matrix. In addition, collagen I time- and concentration-dependently increased BTSM cell proliferation, which was inhibited by FAT and FRNK. Collagen I-induced proliferation was also concentration-dependently inhibited by the pharmacological FAK Inhibitor PF-573,228 (FAK inhibitor II) at concentrations that were specific for FAK (IC50=65±16 nM). In addition, the induction of a hypocontractile phenotype by collagen I was inhibited by this compound. Specific pharmacological inhibitors of p38 MAPK (SB203580, 10 µM) and Src-kinase (PP2, 10 µM) fully inhibited collagen I-induced proliferation as well, whereas partial inhibition was observed by inhibition of PI3-kinase (LY294002, 10 µM) and MEK (U0126, 3 µM). Inhibition of cell proliferation by the inhibitors mentioned was associated with attenuation of the collagen I-induced hypocontractility. Collectively, the results indicate that induction of a proliferative,

hypocontractile ASM phenotype by collagen I is mediated by FAK and by p38 MAPK, MEK, PI3-kinase and Src-mediated signaling pathways downstream of FAK.

O-34

ALLOSTERIC MODULATION OF ETA-RECEPTORS ON RAT RESISTANCE ARTERIES

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Endothelin-1 (ET-1) causes long-lasting vasoconstriction resulting from tight binding to ETA-receptors. This hinders effects of competitive antagonists. We hypothesized that ET-receptor function can be modulated by non-competitive, allosteric mechanisms. We studied isolated rat mesenteric arteries after exposure to capsaicin in the continuous presence of L-NAME and indomethacin. Endothelins caused contractions (potency ET-1 ≥ ET-2 >> ET-3). 4AlaET-1 (ETB-agonist) was ineffective. BQ788 (ETB-antagonist) did not modify ET-1-induced responses. Presence of BQ123 (ETA-antagonist) reduced the sensitivity to ETs to a markedly different extent (pA2 of preventive effect: ET-3 > ET-1 > ET-2). BQ123 also caused relaxation of contractions in the presence of ETs and of contractions persisting after exposure to ETs. These relaxing effects were agonist-dependent; smaller and larger than predicted by the preventive effect of BQ123 in the case of ET-1 and ET-2, respectively. ACT-062724 (ACT), a novel non-peptidergic inhibitor of the binding of 125I-ET-1 to human ETA-receptors was similarly investigated. Presence of ACT reduced the sensitivity to ET-1 and ET-2 to the same extent. ACT reversibly relaxed contractile responses to ET-1 and ET-2. For both agonists, relaxing effects of ACT were significantly larger than predicted by preventive effects. In conclusion, preventive and relaxing effects of putative ETA-receptor antagonists differ and differ between agonists. Differential effects of antagonists on receptor-affinity and -efficacy and agonist-dependence rank among the criteria of allosteric receptor modulation which thus seems to apply to ETA-receptors. Future efforts may focus on candidate drugs that work best on activated receptors.

PO-01

ENDOTHELIUM-DEPENDENT EFFECTS OF MEDICINAL PLANTS FROM DEMOCRATIC REPUBLIC OF CONGO ON ISOLATED RAT AORTA

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In our previous study, we showed that methanolic extracts from Combretum laxiflorum leaves (ClLv), Combretum racemosum leaves (CrLv) and bark root (CrBR) and Hymenocardia acida bark trunk (HaBTr) and bark root (HaBR) have an endothelium-dependent vasorelaxant effect on isolated rat thoracic aorta. These plants are used as antihypertensive agents in RDCongo. The present study was conducted to investigate the mechanism involved in this response and to assess the activity of fractions obtained from the more active extracts (ClLv and HaBR). To obtain the fractions, the methanolic extract was suspended in ethanol and mixed with polyamide powder (5g). A glass column was filled with this mixture and eluted in three steps: fraction 1: ethanol; fraction 2: ethanol-acetone-water (80:16:4); fraction 3: acetone-water (7:3). To explore the involvement of vasodilator prostanoids and soluble guanylyl cyclase/ cyclic guanosine 3,5-monophosphate (GC/cGMP) in the vasorelaxant activity of our congolese plant extracts and fractions, rings of rat aorta were pretreated with 10 µM indomethacin, a cyclooxygenase inhibitor or 10 µM ODQ, a guanylyl cyclase inhibitor. The plant extract or the fraction was applied cumulatively (0.1-50µg/ml) after contraction with 1 µM phenylephrine. The active fraction was also tested on rubbed aorta rings and in the presence of 100 µM L-NAME, a NO synthase inhibitor. The vasorelaxant activity of all the tested extracts was inhibited by ODQ. Indomethacin only inhibited the activity of CrLv and CrBR extracts. The responses induced by HaBR and ClLv was obtained in fraction 3 and reached respectively 100.3 ± 3.5% and 96 ± 2.8 % at 3µg/ml. This effect was significantly attenuated by endothelium removal and after pretreatment with L-NAME and ODQ but not with indomethacin. Methanolic extracts from ClLv, HaBTr and HaBR induce an endothelium-dependent vasorelaxation through the NO-cGMP pathway while CrLv and CrBR extracts also act via a vasodilator prostanoid. Fractions 3 containing procyanidins seem to be responsible for the activity of ClLv and HaBR extracts.

PO-02

CHARACTERIZATION OF THE VASOACTIVE PROPERTIES OF FINGOLIMOD AND DERIVATIVES

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Fingolimod (FTY720) is a new immunosuppressant drug for the treatment of multiple sclerosis that, after phosphorylation in vivo, targets several sphingosine-1-phosphate (S1P) receptors. Phase III clinical trials revealed that this drug modestly increases blood pressure by 3-5 mmHg. Here we investigated whether Fingolimod and several derivatives have vasoactive properties that can account for this increase in blood pressure. For this purpose we studied contractile or dilatory responses to FTY720, its phosphorylated bioactive form FTY720-P, and derivatives of FTY720 (such as VPC23153, VPC24191 and VPC23019) in isolated rat mesenteric arteries and aorta. FTY720-P, but not its parent compound FTY720, induces vasoconstriction in rat

mesenteric arteries most likely due to stimulation of S1P3 receptors in the smooth muscle. This constriction is partly counteracted by an endothelium-dependent vasodilation. In contrast, in rat aorta FTY720-P predominantly induces an endothelium-dependent vasodilation and no contractile properties were observed in this vessel type. Interestingly, closely related derivatives of Fingolimod all induce vasorelaxation in the two vascular beds, however, they do so by varying mechanisms. For instance, VPC24191 induces full, endothelium-independent relaxation in isolated mesenteric arteries, whereas in the aorta it induces a 50-60% endothelium-dependent relaxation. We conclude that FTY720-P has vasoactive properties that may favour vasoconstriction in resistance arteries, whereas closely related derivatives induce vasodilation in both mesenteric arteries and aorta. The divergent actions of FTY720 and its derivatives cannot be explained solely by receptor distribution and receptor selectivity of the compounds. This suggests that other mechanisms of actions partially account for the observed effects.

PO-03

TWO-DIMENSIONAL, M-MODE AND PULSED WAVE DOPPLER ECHOCARDIOGRAPHIC REFERENCE VALUES IN HEALTHY ADULT SAANEN GOATS

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Echocardiography has become a routine non invasive cardiac diagnostic tool in most species. Accurate measurement of cardiac dimensions requires reference values, which are poorly documented in goats. Goats are animals easy to handle with a body and heart size comparable to that of humans. This makes goats an attractive candidate for the development of animal models for human cardiology research. The aim of this study was to test the repeatability and to establish the reference values of bi-dimensional (2D-), time-motion (M-) mode and pulsed wave (PW) Doppler echocardiographic variables in adult goats. Six healthy female adult Saanen goats were investigated three times by the same observer at one day interval using a standardized 2D-, M-mode and PW Doppler echocardiographic protocol. Calculation of the coefficient of variation for each variable measured within day and depending on the day allowed to evaluate their degree of variability. A single echocardiographic examination was performed in 6 other goats by the same observer, and the obtained values were added to these obtained on the third day of the 6 first goats. Then the observed mean, the standard deviation and the range of these measurements were calculated to establish the reference values of echocardiographic parameters in unsedated adult healthy female Saanen goats. Statistical analysis revealed a good inter-day repeatability of the 2D- and M-mode echocardiographic cardiac measurements, but PW Doppler parameters presented moderate to high variability, as documented in other species. Echocardiographic reference values obtained in healthy adult Saanen goats were similar to those reported in healthy adult sheep and in healthy adult humans.

PO-04

ERM PROTEINS ARE INVOLVED IN VASCULAR SMOOTH MUSCLE CELL MIGRATION IN RESPONSE TO PDGF

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Ezrin, radixin and moesin possess a very similar structure with a C-terminal actin-binding domain and a N-terminal FERM interacting domain. They are known to modulate the cell cytoskeleton in several cell types, but their expression pattern is tissue specific. Their contribution to the regulation of vascular smooth muscle cells (VSMC) cytoskeleton is still unknown. The aim of this study was to investigate the expression of ERM proteins in VSMC and their role in cell migration evoked by PDGF, a growth factor involved in pathophysiological processes like angiogenesis or atherosclerosis. Primary cultured VSMC obtained from rat aorta were used. The expression of ERM proteins was determined by Western blot and was inhibited with specific siRNA. Control cells were transfected with a scramble siRNA. Cell migration was measured by the wound healing assay. The three proteins, ezrin, radixin and moesin, were detected in VSMC. Cell transfection with specific siRNAs led to an inhibition of 96 ± 2% , 99 ± 1% and 94 ± 2% of ezrin, radixin and moesin expression, respectively. In cells transfected with the three siRNA against ezrin, radixin and moesin simultaneously, total ERM proteins expression was inhibited by 98 ± 1%. In VSMC stimulated with PDGF, actin stress fibers were markedly reorganized, lamellipodia were formed and migration was increased. Simultaneous depletion of the three ERM proteins abolished the effects of PDGF on cell architecture and on migration, while depletion of ezrin, radixin or moesin only slightly modified actin cytoskeleton reorganization and did not affect migration in response to PDGF. These results indicate that ERM proteins function in a redundant manner and are involved in the migration of VSMC in response to PDGF.

PO-05

EFFECTS OF AGE ON ECHOCARDIOGRAPHIC MEASUREMENTS IN HEALTHY HORSES OF THE HALF-BLOOD BREED

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In any species, for echocardiography to allow distinguishing between normal subjects and subjects suffering from heart disease, it is essential to dispose of reliable specific reference values. In human medicine, the effect of several physiological factors such as body size, ethnicity, gender, ageing, growth, training, or pregnancy on

echocardiographic reference values have been demonstrated. In horses, the effect of training, body weight (BW), sex and breed on echocardiographic parameters has been described, but the effect of growth and ageing was poorly documented in this species. The aim of this study was to describe the relationship between age and echocardiographic measurements in horses. Echocardiography was performed in 59 healthy half-blood horses, 24 females and 35 males, weighing 78 to 662 kg and aged from 10 days to 35 years-old. Standard bidimensional and time-motion mode echocardiography was performed in each horse, which allowed the measurement of the right ventricular internal diameter in diastole, the left ventricular internal diameter in systole and diastole, the interventricular septum and left ventricular free wall thickness in systole and diastole, and the aortic, pulmonary and left atrium internal diameter in diastole. The correlation between the echocardiographic measurements and the age was studied using a simple linear and a logarithmic regression test. All echocardiographic parameters showed a moderate to strong significant ($p < 0.05$) correlation with age, and the coefficient of determination obtained using the logarithmic regression test was always higher (R2 0.35 to 0.80) than the one obtained using the linear regression test (R2 0.25 to 0.49). In the equine species, especially in the early growing period (up to 3 years of age), echocardiographic reference values should be established in each breed using logarithmic regression equations as a function of age or BW (the BW showing a strong correlation with age in a given breed). This could increase the diagnostic value of echocardiography in equine cardiology.

PO-06

REVERSAL OF ACEPROMAZINE-INDUCED HEMODYNAMIC ALTERATIONS BY NOREPINEPHRINE IN STANDING HORSES

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Acepromazine (ACP) is a phenothiazine commonly used to sedate horses. Additionally, ACP exerts strong anti-inflammatory effects, which might have a therapeutic potential in horses suffering from systemic inflammatory response syndrome (SIRS). However, the ACP-induced vasodilation precludes its use in horses with SIRS-related cardiovascular compromise. The objective of this study was to test if the hemodynamic effects of the administration of 0,1 mg/kg of ACP could be counteracted by an intravenous infusion of norepinephrine (NOR) at 1 ug/kg/min in healthy horses. In 5 healthy adult horses, a 15 minutes NOR IV infusion was administered 45 minutes after an injection of 0,1 mg/kg of ACP IV. The systolic arterial blood pressure (SAP) was non-invasively measured by Doppler sphingometry at the tail. Hemodynamics of the median artery of the left forelimb were studied using Doppler ultrasonography, through calculation of the vessel's surface (SURF), diameter (DIAM), circumference (CIRC), and peak systolic velocity (PSV), end diastolic velocity (EDV), mean velocity (MV), volumetric flow (VF) and resistivity index (RI) of the flow. Both SAP and Doppler parameters were determined at regular

intervals during the entire study. ACP induced a hypotension and a vasodilatation, that were evidenced by a significant rise of the SURF, DIAM, CIRC, PSV, EDV, MV and VF and reduction of the SAP and RI. During NOR infusion, all these ACP-induced hemodynamic changes were reversed. These findings suggest that a continuous IV NOR infusion at 1 ug/kg/min is able to revert ACP-induced hypotension and vasodilation in healthy adult horses.

PO-07

EFFECT OF RHO-KINASE-INHIBITOR H-1152 ON VASCULAR CONTRACTILITY EARLY AFTER MYOCARDIAL INFARCTION

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The involvement of Rho-kinase (ROCK) in VEGF-driven endothelial migration and angiogenesis has been implied, and current studies address ROCK-inhibition as a potential therapeutic target after myocardial infarction (MI). However, ROCK-inhibitors also interfere with contractile processes and potentially blunt the early compensatory phase after MI. Hence, neurohumoral activation of CNS and RAAS in the early phase after MI is thought to help maintain cardiac output and organ perfusion. The present study investigated the effect of H-1152 on vascular contractility shortly after MI. Normal Wistar rats were subjected either to sham-surgery, MI-surgery or MI-surgery plus treatment with the ROCK-inhibitor H-1152 started immediately after surgery. One week following surgery rats were sacrificed, the aorta removed and studied in vitro for contractile responses to phenylephrine (PE) and angiotensin II (AngII). Contractile responses both to PE and AngII were profoundly increased in untreated MI, as compared to untreated sham rats. Presence of the eNOS-inhibitor L-NMMA increased and fully abolished the differences in PE-responses, thus suggesting reduced basal release of NO to inhibit contractility in MI-rats. Although presence of L-NMMA also increased responses to AngII in sham and MI, maximal contraction remained increased in the latter. Interestingly, pre-incubation with AG 1478 – an EGF-receptor inhibitor – reduced contractile responses to AngII in MI, but not sham. The latter finding is suggestive for increased involvement of AT1-EGR receptor transactivation following MI. Finally, PE- and AngII-induced responses did not differ between MI rats treated or without H-1152. Increased contractility to PE and AngII following MI involved reduced basal release of NO and possibly increased AT1-EGF-receptor transactivation. Treatment with the ROCK-inhibitor H-1152 did not adversely affect these adaptations in contractility following MI.

PO-08

CONSTRUCTING CELL LINE AND EXPERIMENT SPECIFIC GENE REGULATORY NETWORKS BY COMBINING GENERAL AND SPECIFIC MICRO ARRAY DATA

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Based on public and own microarray data sets and by integrating additional available interaction data, a bioinformatics pipeline was established for the fast and convenient extraction of cell line- and experiment-specific candidate networks. The new automated workflow allows obtaining such networks and identifying genes which play significant roles in signalling pathways of interest. Two subsequent filtering steps are thus applied: 1) cell type-specific array data available from public databases is used for co-expression analysis (assuming that co-expressed genes are also co-regulated) to identify cell type-specific clusters and general interactions; 2) additional own stimulation data of specific expression profiles helps to further filter the co-expression network. Only genes with an absolute fold change over a certain threshold were included in the candidate networks. To avoid the exclusion of important central nodes of the network, IPA Knowledge Base (Ingenuity) was used to ensure significance and completeness of the networks. The candidate networks can then be subjected to mathematical modelling and further analysis, e.g. using Probabilistic Boolean Modelling. The pipeline was applied to atherosclerosis related microarray data from the human umbilical vein endothelial cell line (HUVEC) which was chosen to mimic endothelial behaviour during the process of atherosclerosis development. Cells were stimulated during 1, 4 and 24 hours with the cytokine TNF-alpha and/or the micronutrient 1alpha,25(OH)2 vitamin D3, looking for interactions between stress-sensing nuclear factors and nutrient-sensing nuclear receptors which could be part of an imbalanced molecular mechanism towards atherosclerosis. The process was applied to HUVEC and THP-1, a cell model to mimic the behaviour of monocytes in atherosclerosis development, resulting in co-expression networks with 1519 and 2554 nodes, respectively. For HUVEC, this network was already filtered to a final core network of 84 genes.

PO-09

THE EFFECTS OF METHYLSUFONYLMETHANE (MSM) ON INFLAMMATORY MARKERS IN MONOCROTALINE-INDUCED PULMONARY HYPERTENSION

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MSM is naturally accruing organic sulphur that is known as a potent anti-oxidant/anti-inflammatory compound. The aim of this study was to investigate the effect of MSM on hemodynamics functions in rats with monocrotaline (MCT)-induced pulmonary

arterial hypertension (PAH). Wistar rats were randomly assigned to 38-days pretreatment or 28-days treatment. MSM was administered to rats at 100, 200, and 400 mg/kg/day doses either 10 days before or after a single dose of 60 mg/kg, IP, MCT. Rats were anesthetized with pentobarbital and heart tissue samples were obtained to evaluate changes in the antioxidative system and inflammatory genes expression levels including endotheline-1 (ET-1), transforming growth factor-β1 (TGF-β1), angiotensinogen. Analyses of gene expression by RT-PCR found a significantly reduced ET-1, TGF-β1 and angiotensinogen levels at efficient dose in MCT-induced pulmonary arterial hypertensive rats. There was also a similar, non-significant trend for the treatment protocol. Our present results suggest that long term administration of the MSM attenuates MCT-induced PAH related inflammation in rats. Should similar effects of MSM be found on markers of oxidative stress, this may have promise in the treatment and prevention of PAH.

PO-10

TIME-DEPENDENT EXPRESSION OF CELL SURFACE ANTIGENS DURING THE DIFFERENTIATION OF HL-60 AND PLB-985 MYELOID CELLS INTO MATURE GRANULOCYTES

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Neutrophil surface molecules function in part as biological sensors. Surface antigens undergo several changes during myeloid differentiation to accommodate cell functions. Here we describe time-dependent changes in antigen expression at the surface of HL-60 myeloid cells when differentiation is induced into granulocytes by dimethylsulphoxide (DMSO), all-trans retinoic acid (ATRA), as well as PLB-985 myeloid cells induced to differentiate into neutrophils by a mixture of N,N-Dimethylformamide (DMF) and Nutridoma-SP. Terminally differentiated cells were compared to circulating neutrophils in terms of cell surface antigen expression by flow cytometry analysis using a panel of pertinently purported 35 antibodies. The changes in myeloid cell surface antigens induced by DMSO, ATRA or DMF/Nutridoma-SP paralleled the expression pattern of these molecules in normal granulopoeisis with the exception of 7 antigens up-regulated and 9 down-regulated, indicating that the maturation was most probably not achieved and thus partially defective. All these differentiation inducers failed to induce the expression of neutrophil specific markers CD16 and CD66b. Differentiated PLB-985 cells appeared closer to neutrophils, in term of maturation and CD expression than the DMSO- or ATRA-differentiated HL-60. Finally, single cell analysis of the expression dynamics of several differentiation markers (CD11b, CD14, CD35, CD71) revealed a bistable switch and not a graded change of expression when measured as a cell population average. These results demonstrate kinetic changes in cell surface antigen expression during the transformation of a proliferating leukaemic cell into a potentially mature and terminally differentiated cell, which might be of substantial importance for analyzing the gene expression pathways that govern granulopoiesis.

PO-11

TRANSCRIPTIONAL MODULATION OF PATHOGEN RECOGNITION RECEPTORS AND T-CELL ASSOCIATED CYTOKINES DURING COLITIS IN MICE

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The two major forms of inflammatory bowel disease (IBD) are Crohn's disease and ulcerative colitis, which are characterized by Th1/Th17- or Th2-cell-mediated inflammation, respectively. The mechanisms underlying these diseases remain unclear, however, studies from germ-free animals reveal an important role of the intestinal microflora within these diseases. Since pathogen recognition receptors (PRR), like Toll like receptors (TLRs) and nucleotide-binding oligomerization domains (NOD)-like receptors (NLRs), can induce immune responses against intestinal microflora, PRRs may contribute to the development of IBD. Colitis was induced by adding 1.25% dextran sodium sulfate (DSS) into the drinking water of mice. On day 7, mice were sacrificed; the colons were isolated and divided into four equal sections. The mRNA expression level of PRRs, cytokines, and T cell subset-associated master transcription factors has been determined by using quantitative PCR. Both NLRs and TLRs were up-regulated in colonic regions with intense inflammation, except TLR1 and TLR5. Furthermore, the Th1/Th17 associated transcription factors for cytokines were up-regulated in inflamed colons. Th1-associated master transcription factor was also increased. The Th2 associated transcription factor did show an increased mRNA expression, but the expression level of associated cytokines did not change. In adition, the regulatory T cell- and Th17 associated transcription factors did not show an up-regulated mRNA expression in inflamed colons, however, their associated cytokines were up-regulated. This study provides an overview of mRNA expression of PRRs along the colon in both healthy mice and mice suffering from DSS-induced colitis. An analysis of T cell subset-associated transcription factors and their associated cytokines suggests Th1/Th17 polarization within the DSS-induced colitis. These data will help us to further understand the role of PRRs during the intestinal inflammation and the involvement of TLRs in the mechanism of action of probiotics in IBD.

PO-12

CHEMOTACTIC COLLEGEN BREAKDOWN PRODUCT, PROLINE-GLYCINE-PROLINE, IS INVOLVED IN INFLAMMATORY BOWEL DISEASES: FUTURE TARGET?

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The migration of inflammatory cells to the sites of inflammation relies on the coordinated action of chemokines receptors (CXCRs) present on the inflammatory cells and chemotactic proteins produced at the site of inflammation. CXCR1 and CXCR2 are the CXCRs present on neutrophils and recognize CXCL8/IL-8 and are responsible for neutrophil infiltration at sites of inflammation. Recently, a collagen-breakdown product, Proline-Glycine-Proline (PGP) was shown to induce the infiltration of neutrophils via CXCR1 and CXCR2 in several lung diseases. PGP is formed from collagen by a combinational action of matrix metalloproteinase-9(MMP-9) and prolyl endopeptidase(PE), enzymes often found at sites of inflammation. To this end, we investigated the expression of both proteases in inflammatory bowel diseases (IBD). PE, large amount of MMP-9, as well as PGP were found in inflamed bowel tissue. Both MMP9 and PE are expressed by neutrophils, which provides a self-generating accumulation loop for the production of PGP and homing of neutrophils. Similar tot the human disease, expression of both proteases and PGP itself were elevated in the dextran sodium sulfate (DSS) mouse model of IBD. To examine if PGP could be used as a treatment target both a complementary peptide, L-arginine-threonine-arginine (RTR) which has been shown to bind to PGP sequences, and an anti-PGP antibody were injected into mice with colitis. Both treatments lead to reductions in severity of the DSS-colitis in these mice, confirming the importance of the collagen breakdown products in IBD, and opens new venues for the treatment of IBD in humans.

PO-13

CIGARETTE SMOKE INDUCES RELEASE OF TGF-BETA IN AIRWAYS AND MODULATES TRYPTASE EXPRESSION AND MAST CELL MATURATION

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The possible involvement of mast cells in the pathogenesis of lung emphysema is not well described. In the current study, the effect of cigarette smoke on mast cells was investigated in vitro and in vivo. We studied the distribution of mast cells during development of lung emphysema in an animal model for cigarette smoke-induced emphysema. We show that cigarette smoke induced the expression of tryptase and suppressed surface expression of Fc-epsilon-RI and c-kit, and increased the number of tryptase-positive mast cells in the airways. Cigarette smoke induced in vitro TGF-beta production by mast cells. Besides, TGF-beta was increased in BALF of smoke-exposed mice. Neutralization of TGF-beta suppresses induction of tryptase induced by cigarette smoke. To investigate the crucial importance of TGF-beta in the

induction of tryptase expression in mast cell cultures by cigarette smoke extracts, we have performed similar experiments in the presence of a TGF-beta-neutralizing antibody. In cell activation experiments positive and negative controls were included. In FACS experiments isotype controls were included to control for a specific binding. TGF-beta expression induced by cigarette smoke increases tryptase expression in mast cells and affects mast cell development, which could be of functional importance in the pathogenesis of lung emphysema.

PO-14

NORADRENALINE, BUT NOT SEROTONINE, IS REDUCED IN HIPPOCAMPUS AND PREFRONTAL CORTEX AFTER A BILATERAL 6-HYDROXYDOPAMINE LESION

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Approximately 45 % of patients suffering from Parkinson's disease (PD) are affected by depressive disorders. However, the pathophysiology of PD-associated depression remains largely unknown. In the present study, we determined the monoamine content of the hippocampus (HIP) and the prefrontal cortex (PFC), structures related to depression, and of the striatum in the bilateral 6-hydroxydopamine (6-OHDA) rat model. Rats were lesioned bilaterally in the substantia nigra pars compacta or the striatum. One or two weeks after the lesion, the levels of dopamine (DA), noradrenaline (NAD) and serotonine (5-HT) were determined by LC in homogenates of the various brain regions. Striatal DA levels decreased in striatally and nigrally lesioned rats by respectively 50% and 90% compared to control rats. The DA levels in the HIP and PFC showed a trend to reduce in both protocols one week after the lesion and restored to baseline levels two weeks post-lesioning. There was no significant effect of the two lesion protocols on 5-HT levels in all regions studied, only a brief decrease in the HIP 1 week after the nigral lesion was observed. Finally, these lesions resulted in significantly decreased hippocampal and PFC NAD levels up to approximately 50 % compared to controls. Nigrostriatal degeneration alters the NAD content of the HIP and PFC but has no important effect on 5-HT levels. No clear effects on DA levels were observed in the HIP and PFC in both lesion protocols. Striatal and nigral lesions resulted in similar effects on monoamine content in depression-related brain structures. These findings could contribute to a better understanding of depression in PD.

PO-15

ANTIDEPRESSANT-LIKE EFFECTS OF OXYTOCIN IN MICE SEEM DEPENDENT ON METABOLISATION BY INSULIN-REGULATED AMINOPEPTIDASE

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Oxytocin (OT) is a neuromodulator with antidepressant (AD)-like effects. In vitro, it is rapidly cleaved by insulin-regulated aminopeptidase (IRAP). OT metabolites are known to exert strong central activities that are different from the parent molecule. Our goal is to investigate in vivo whether IRAP deletion can modify the AD-like effects of OT and whether ageing interferes with AD-like effects of OT. A dose-response curve was performed in male and female C57Bl/6 mice (3 and 14 months) injected subcutaneously with saline (10 ml/kg) or oxytocin (0.05 mg/kg- 0.5 mg/kg). One hour after injection, mice were placed in an open field (OF) for 10 minutes to study locomotor behaviour and they were subjected to a 5 minutes forced swim during which immobility was timed. Young (3-6 months) and middle-aged (12-18 months) male and female IRAP wild-type (WT) and knock-out (KO) mice were also subjected to OF and FST, after injection with the doses OT that showed AD-like effects in C57Bl/6 mice. Treatment of young male C57Bl/6 mice with 0.15 mg/kg and 0.25 mg/kg OT resulted in a decreased immobility time. The effect of 0.25 mg/kg OT was reproduced in young male IRAP WT mice, but not in IRAP KO mice. OT had no effect in either young female C57Bl/6 mice or in young female IRAP WT and KO mice. However, we found an AD-like effect of 0.15 mg/kg OT in middle-aged female C57Bl/6 mice and IRAP WT mice, that was absent in middle-aged female IRAP KO mice. OT did not influence locomotor behaviour in mice, as shown with the OF. The observed AD-like effects of OT in young male and middle-aged female IRAP WT mice were absent in age-matched IRAP KO mice, suggesting that IRAP metabolizes OT in vivo. We suggest that these metabolites rather than the intact neuropeptide mediate the AD-like activity. In addition, AD-like effects of OT are influenced by ageing. This could be explained by a difference in circulating oestrogen and OT levels, however, further work is required to confirm this hypothesis.

PO-16

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.

PO-17

MORPHOLOGICAL ALTERATIONS IN THE CEREBELLAR GRANLE CELL LAYER OF MICE LACKING CALRETININ

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Calcium binding proteins, such as calretinin, are abundantly expressed in distinctive patterns in the central nervous system, but their physiological functions remain poorly understood. Calretinin is expressed in cerebellar granule cells and calretinin deficient mice suffer from alterations in motor coordination. Using confocal microscopy, we demonstrate that calretinin deficient mice exhibit a significantly decreased density of granule cells at the level of the cerebellar cortex. Moreover, it has been shown that migration of granule cells is tighly associated with intracellular calcium fluctuations. Therefore, we hypothesize that the perturbation of the calcium dynamics in calretinin deficient mice may be the cause of the observed morphological alterations. To test this assumption, we are currently developping two strategies.First, using confocal microscopy and cerebellar microexplant cultures, we are studying calcium transients occuring during granule cell migration in wild type and calretinin knock-out mice. On the other hand, we are developping a dedicated computational model for [Ca2+]i transients. This model will shed light on the possible mechanism responsible for the modulation by calretinin of calcium oscillations during granule cell migration.

PO-18

L-LTP CAN BE INDUCED BY A SINGLE TRAIN OF STIMULATION IN PRESENCE OF PROTEIN-SYNTHESIS INHIBITORS

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Encoding of memories relies on modifications in the strength of the synapses connecting the different cells within a neuronal network. The selective increases in synaptic weight are thought to be biologically implemented by long-term potentiation (LTP). Classically, in area CA1 of hippocampal slices a single train at 100-Hz (1s) triggers a short-lasting LTP, which lasts 1-2 h and is independent of new protein synthesis, whereas multiple trains induce a long-lasting LTP, which lasts several

hours and can be blocked by protein synthesis inhibitors. However, it has been repeatedly shown that the threshold and the features of these LTP depend on the history of the neurons, a phenomenon known as metaplasticity. We already demonstrated that slices recovery conditions modified the characteristics of LTP (Capron et al. 2006). By maintaining the slices in submersion during recovery, it was possible to induce a long-lasting LTP with a single train. Further investigations demonstrated that long-lasting LTP (more than 10 hours) can be induced by a very short stimulation (15 pulses) and that increasing the number of pulses increased just the level of potentiation. Moreover, this kind of LTP became nearly independent of new protein synthesis. In this case, synaptic tagging could not be observed anymore. The only way to impair this long-lasting potentiation was to increase the frequency of basal stimulation: stimulating the slices every 10 sec prevent the stabilization of LTP induced by one or 4 trains. But in this case again, synaptic tagging could not be observed. So, in vitro, de novo protein-synthesis dependence of LTP and synaptic tagging can only be observed in very specific conditions depending on the set-up used, the experimenter, the conditions of slices recovery, the composition of ACSF….This dramatically increases the difficulty for comparing the different results obtained in different labs and prevents rapid progress in the understanding of this phenomena.

PO-19

5-HT4 RECEPTOR ACTIVATION DOES NOT FACILITATE ACETYLCHOLINE OUTFLOW IN HIPPOCAMPAL BRAIN SLICES

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5-HT4 receptor (5-HT4R) activation increases memory and learning. As the loss of cholinergic markers is one of the major neurochemical deficits in Alzheimer's disease (AD), an increase in acetylcholine (ACh) release by 5-HT4R activation may be of therapeutic benefit to alleviate symptoms in AD. The facilitation of ACh release from hippocampal brain slices by 5-HT4R activation has been described by Siniscalchi et al. (1999). We used this method to evaluate the influence of novel selective 5-HT4R agonists on hippocampal ACh release. The hippocampus was dissected from rat brain. Hippocampal slices were cut at 350 µm, loaded with [^{3}H]-choline, and transferred to 200 µl chambers (perfused with carbogen-saturated Krebs' solution containing hemicholinium-3 (2.10-6 M); flow rate of 1.2 ml/min). After 1 hour of perfusion, 3 min samples of the effluent were collected and assayed for tritium content (by scintillation counting) as a measure of [^{3}H]-ACh . Electrical field stimulation (biphasic pulses, 1 ms duration, 50 mA, 2 Hz, for 2 min) was applied at 15 and 45 min after beginning the collection (S1, S2); drugs were added 15 min before S2. In accordance with literature, atropine (10-6 M) increased and the adenosine receptor agonist CADO (10-5 M) decreased the tritium release by 36% and 55%, respectively ($p<0.001$ vs. control). This corresponds with the blockade and stimulation of presynaptic inhibitory control on ACh release through muscarinic and adenosine receptors respectively. However, neither BIMU-8 (2.10-6 M), the 5-HT4R

agonist that was reported by Siniscalchi to increase tritium efflux by 43%, nor the highly selective 5-HT4R agonist prucalopride (10-6 M) increased tritium outflow. In the present study, selective 5-HT4R agonists do not facilitate ACh release from hippocampal brain slices. This might be related to the model system (ex vivo) as the facilitatory effect of 5-HT4R activation on hippocampal ACh release was shown in vivo (Matsumoto, 2001, JPET).

PO-20

ELECTROPHYSIOLOGICAL PROPERTIES AS PHENOTYPIC ACTIVATION MARKERS OF MICROGLIA DURING AUTOIMMUNE ENCEPHALOMYELITIS

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Microglia is considered as the resident macrophages of the central nervous system. It is generally accepted that microglia shifts between different activated phenotypes: depending on the stimulus, microglia can execute pro-inflammatory or anti-inflammatory actions in terms of e.g. nitric oxide or cytokine production. Moreover, a continuum of activation stages exists between these two extremes. Controlling the activation stage of microglia is believed to be a good therapeutic strategy in the treatment of diseases with a neuro-inflammatory context. In this study the microglial activation phenotypes were investigated using an electrophysiological approach. Differential expression of ion channels (voltage-gated and inward rectifier potassium channels), transporters and receptors is known to occur during strong activation of immune cells. Yet, a correlation of the expression profile of these proteins with the various activation stages is lacking. The electrophysiological phenotyping of microglia is performed on ex vivo brain slices of adult CX3CR1+/eGFP mice subjected to autoimmune encephalomyelitits (EAE). In these mice the microglia/macrophages are eGFP-labeled and EAE lesion in the brain slices are recognized by an increased density of these cells. In addition, the different activation stages are hypothesized to play a role in the balance between pro- and anti-inflammatory events during the disease progression of EAE and therefore microglial phenotypes are studied at different time points of the disease: before the onset, during the onset, at the peak an in the chronic phase of the disease. Microglia present in the active EAE lesions of brain slices of EAE animals in the peak and chronic stage show increased functional expression of delayed rectifier potassium channels. Preliminary results show no differential expression of ligand-gated ion channels (glycine, glutamate, serotonin acetylcholine).

PO-21

MOUSE EMBRYONIC STEM CELL-DERIVED PYRAMIDAL NEURONS ARE ABLE TO SURVIVE IN A KAINIC ACID CORTICAL LESION AND IN A SCLEROTIC HIPPOCAMPUS

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Like other neurodegenerative disorders, epilepsy is characterized by neuronal loss. Temporal lobe epilepsy is the most common form of difficult to treat epilepsy and is frequently associated with hippocampal sclerosis, a lesion characterized by reactive gliosis and loss of pyramidal- and interneurons in specific hippocampal cell layers. Replacement of lost cells seems to be a vital step for functional repair of the brain. A promising technique for replacement of lost cells is neurotransplantation. This technique tries to repair damaged neuronal networks or to deliver anti-epileptic substances by means of cell transplantation. In this study we evaluate if predifferentiated pyramidal neurons, derived from mouse embryonic stem cells, are able to survive in a kainic acid (KA) cortical lesion and in the sclerotic hippocampus of the intrahippocampal KA status epilepticus mouse model. In vitro generated tau-Green Fluorescent Protein (GFP) mouse embryonic stem cells were differentiated to precursors of pyramidal neurons and transplanted in a cortical and hippocampal lesion induced by focal injection of KA three days before grafting. In a first experiment, a cortical lesion was induced in mice by focal injection of 200 ng KA in 50 nl saline. Three different cell numbers were transplanted in the lesion [1,000 (n=4); 5,000 (n=6) and 25,000 cells (n=6) in 0.5 µl medium]. In a second experiment an intrahippocampal lesion was induced by focal injection of 100 (n=5) or 200 ng KA (n=4), dissolved in 50 nl saline, and 700 cells in 0.5 µl medium were grafted in the sclerotic hippocampus. Four weeks after transplantation mice were transcardially perfused to evaluate the presence of GFP-positive neuronal projections from grafted cells. In the first experiment, a dense network of projections was found in KA lesioned cortex, in 5 out of 6 mice grafted with 25,000 cells, in 4 out of 6 mice grafted with 5,000 cells and in 1 out of 4 mice grafted with 1,000 cells. A high number of axonal projections, running down along the lower layers of the cortex and along the external capsule, were seen. These projections were strikingly similar to the projections of the endogeneous cortical neurons. In the second experiment, a dense network of GFP positive neuronal projections was found in the sclerotic hippocampus in 3 out of 4 mice, injected with 200 ng KA, and in 2 out of 5 mice, injected with 100 ng KA. The projection pattern of grafted neurons was very similar in the successfully grafted mice. GFP positive neuronal fibers were confined to the ipsilateral and contralateral hippocampus and found in all layers of the hippocampal formation. These promising results show that mouse embryonic stem cells, in vitro predifferentiated to pyramidal neurons, are able to survive in a KA cortical lesion and in a sclerotic hippocampus. Further research will be done to determine the functional integration of the grafted neuronal precursors.

LACOSAMIDE ATTENUATES CORTICAL EXCITABILITY IN THE RAT CORTICAL STIMULATION MODEL

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Lacosamide is a novel antiepileptic drug which is currently used as an add-on treatment for partial seizures with or without secondary generalisation. Its anti-seizure effect is believed to result from a selective enhancement of the slow inactivation of voltage-gated sodium channels. Lacosamide is effective in the maximal electroshock model, the kindling model and the pentylenetetrazol model. The effect of lacosamide on cortical excitability has not been studied in vivo. Our aim in this study was to evaluate the effect of lacosamide on motor cortex excitability in the cortical stimulation model (CSM). In the CSM, a ramp-shaped pulse train with increasing intensity is delivered through epidural electrodes placed over the motor cortex. The threshold intensity for eliciting forelimb clonus is determined through behavioural observation, and is used as a measure for cortical excitability. Several known antiepileptic drugs including diazepam and carbamazepine increase threshold intensity in this model. Benefits of the CSM are that the activity of a drug can be accurately, rapidly and repeatedly determined in the same animal with a short interval between measurements. The CSM is useful for dose-finding and pharmacokinetic studies. Male Wistar rats (145-220g) were surgically implanted with epidural stimulation electrodes positioned over the motor cortex (AP: -1 mm; ML: +/-3 mm). Following surgery, animals were allowed to recover for one week. Subsequently, all animals were stimulated twice daily for 10 days (ramp pulse 0-1 mA, 50 Hz, pulse width 2 ms) to obtain a stable threshold intensity to elicit forelimb clonus. A first group of 12 stabilized animals underwent intraperitoneal (i.p.) administration of 0, 2.5, 5, 10 and 20 mg/kg lacosamide in a random order on 5 consecutive days. Threshold intensity was determined 1 h before and 30 min, 2 h and 5 h after every injection. To determine whether tolerance to lacosamide occurs after repeated administration, a second group of 12 animals underwent i.p. administration of 20 mg/kg lacosamide once daily on 5 consecutive days. During the stabilization period (stimulation days 1-10), threshold intensity to forelimb clonus decreased from 568 +/- 73 µA to 394 +/- 91 µA (n=25). Lacosamide increased the threshold intensity to forelimb clonus in a dose-dependent manner: 2.5, 5, 10 and 20 mg/kg lacosamide increased threshold intensity by 32 +/- 25 µA, 60 +/- 41 µA, 111 +/- 34 µA and 166 +/- 49 µA respectively. Threshold intensity reached a maximum 30 min after injection of lacosamide and returned to baseline 2 h after injection. Administration of 20 mg/kg lacosamide on 5 consecutive days resulted in a partial attenuation of its effect on cortical excitability: threshold intensity increased by 137 +/- 43 µA after the first injection, but only by 102 +/- 33 µA after the fifth. Lacosamide, administered i.p. at doses ranging from 2.5 to 20 mg/kg, decreases cortical excitability in the CSM in a dose-dependent manner. The effect of lacosamide on cortical excitability was maximal 30 min after injection of the drug, and lasted for 2 h. This suggests that lacosamide's half-life may be shorter in rodents than in humans (13 h). Repeated administration of lacosamide on consecutive days resulted in a slight, non-significant trend towards attenuation of its effect on cortical excitability.

PO-23

ANTICONVULSANT ACTIONS OF CORTISTATIN-14 ARE ABOLISHED BY SELECTIVE SST2 AND SST3 ANTAGONISM

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Anticonvulsant actions have already widely been proven for somatostatin-14. Cortistatin-14 (CST-14) was shown in one report to protect against kainate-induced seizures (Braun et al. Brain Res.1998;803(1-2):54-60). Although these neuropeptides are products of different genes, they are structurally related. This explains the interaction of CST-14 with the SST receptors (sst1-sst5). We here used in vivo micro-dialysis and telemetry-based electrocorticography (ECoG) in rats and administered CST-14 (0.1µM–1µM–10µM) in the presence and absence of highly selective antagonists for sst2 and sst3 receptor subtypes via intrahippocampal administration. Seizures were evoked by intrahippocampal pilocapine perfusion (10mM,40min) and seizure severity was assessed using a previously validated behavioural scoring system. Moreover, behavioural seizure severity assessment was verified with ECoG in at least 2 rats of each test group treated with CST-14. ECoG recordings of the rats treated with 0.1µM CST-14 showed clear epileptic discharges following pilocarpine perfusion. Indeed, the test group treated with 0.1µM CST-14 was not protected against pilocarpine-induced seizure severity. In none of the rats epileptic discharges were recorded during treatment with 1µM and 10µM CST-14. Hence, intrahippocampal administration of 1µM and 10µM CST-14 was anticonvulsant against pilocarpine-induced seizures. Furthermore, we showed that the CST-14 (1µM)-mediated anticonvulsant actions were reversed in the presence of a selective sst2 receptor antagonist Cyanamid154806 (0.1µM) or a selective sst3 receptor antagonist SST3-ODN-8 (0.1µM). Intrahippocampal perfusion of the selective sst2 or the selective sst3 receptor antagonists alone did not affect the pilocarpine-induced seizure severity per se. In conclusion, our results show that CST-14 is able to prevent seizures in a focal pilocarpine rat model and that selective sst2 or selective sst3 receptor antagonism is able to abolish these anticonvulsant actions.

PO-24

LONG TERM HIPPOCAMPAL DEEP BRAIN STIMULATION EFFICIENTLY REDUCES SEIZURES IN THE KAINIC ACID MODEL

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Epilepsy is a chronic neurological disorder, affecting up to 50 million people world-wide. Temporal lobe epilepsy remains one of the most difficult to treat forms of

epilepsy, as one third of all patients remain refractory to anti-epileptic drugs. Temporal lobe epilepsy is usually caused by a precipitating event, such as febrile seizures, head trauma, status epilepticus, etc. This event is followed by a latent period, during which several molecular and cellular changes occur, and eventually lead to the formation of an epileptic network, that gives rise to a condition with spontaneously recurring seizures. Recent studies have shown that hippocampal Deep Brain Stimulation (DBS) can efficiently suppress these spontaneous seizures. Despite these promising results, the precise mechanism of action of DBS remains undetermined. In this animal experimental study, we evaluated the seizure suppressive effect of deep brain stimulation at different stages after the initial event. Rats (n=32) were implanted with a bipolar DBS electrode in the right hippocampus and a bipolar EEG recording electrode in both hippocampi. After recovery from surgery, all rats were subjected to a status epilepticus (SE), that was elicited through intraperitoneal injections of kainic acid (KA). Immediately following SE, one group (n=16) was subjected to DBS (Poisson Distributed Stimulation, 130Hz, 100µs pulse width) during 70 days; the other group received sham stimulation. Continuous EEG was recorded throughout the entire experiment, to evaluate the latency to the first seizure after SE, and evaluate seizure frequency during the first 20 days, and during the last 15 days of the experiment. The mean latency for the first seizure to occur after the start of the SE was the same in control (9 ± 3 days) and DBS treated rats (9 ± 8 days). During the first 20 days after SE, seizure frequency was not different in control and treatment group (1 Sz/day). During the last 15 days of the experiment, there is a significant difference in seizure frequency in the control group (11 ± 1 Sz/day) vs the treatment group (3 ± 1 Sz/day). In kainic acid treated rats, hippocampal deep brain stimulation does not prevent seizures from occurring in an early phase after SE. Hippocampal deep brain stimulation does affect seizures occurring in a later stage when rats exhibit spontaneously recurring seizures. These results suggest that hippocampal deep brain stimulation using a specific set of stimulation parameters is effective after a longer period of stimulation in this model at a time when the epileptic network is fully developed.

PO-25

DETERMINATION AND EVOLUTION OF OPTIMAL CURRENT VALUES FOR ACUTE VAGUS NERVE STIMULATION IN DOGS

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Vagus nerve stimulation (VNS) is a well-established treatment modality for patients with refractory seizures. The optimal output current is individually determined by evaluating patient tolerance or reaching seizure control. VNS is currently also investigated as a potential treatment for epilepsy in dogs. In one study the optimal output current in dogs with refractory epilepsy was determined to be the highest

current that did not elicit coughing. Ten Beagle dogs were implanted with a Cyberonics® pulse generator and helical electrodes around the left vagosympathetic trunk. One dog developed a left-sided Horner syndrome and was excluded. Starting 4 weeks after implantation, the optimal output current was assessed on a weekly basis for 6 weeks by stimulating the vagal nerve during 2 minutes with intermittent pulse trains (500 µs, 30 Hz, 7s on, 0.3 min off). The initial stimulation intensity was set at 0.125mA and this output current was gradually increased in steps of 0.125mA until coughing was observed (coughing threshold). The optimal current was defined as 0.125mA below the coughing threshold. During the ramping-up procedure laryngeal vibrations could be palpated 0-0.5mA beneath the coughing threshold. Overall, the optimal output current was stable over time (7 dogs). At 4 weeks after implantation the optimal output current ranged between 0.125 and 1.5mA (mean±SD=0.722±0.384mA). At 10 weeks after implantation the optimal current ranged between 0.375 mA and 1 mA (mean±SD=0.611±0.202 mA). In 3 out of 7 dogs a small decrease of 0.125 mA between week 5 and 6 after the implantation was found. In 1 dog the current decreased from 1.5 mA to 0.625 mA and in 1 dog there was an increase from 0.125 mA to 0.375 mA. There is a wide range in optimal current values for acute VNS in dogs. Week 6 after implantation seems to be an ideal moment to assess optimal current values. Moreover, laryngeal vibrations can be palpated in dogs during VNS.

PO-26

NANO LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE MONITORING OF NEUROPEPTIDES POSSIBLY INVOLVED IN LIMBIC EPILEPSY

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Neuropeptides are an important class of neuronal signalling molecules. In order to further characterise the peptides involved in the physiopathology of epilepsy, it is essential to monitor their concentration in the brain. Quantification of neuropeptides in dialysates is challenging due to their low extracellular concentrations, their low microdialysis efficiencies and the tendency of peptides to stick to surfaces. Therefore the use of very sensitive nano LC-MS/MS methods is required. In this study the feasibility of quantifying eight neuropeptides, possibly involved in epilepsy, is investigated, namely bradykinin, dynorphin A (1-13), galanin, ghrelin, neuromedin B, neuromedin N, neuropeptide Y and neurotensin. Samples were concentrated on a C18 precolumn (5 mm x 300 µm id, 5 µm) and back-flushed onto a nano C18 column (15 cm x 75 µm id) at 300 nl/min. Gradient elution was performed. The nano LC system was hyphenated to the nanosource of a Quattro Premier triple quadrupole MS using Picotip nanospray emitters (10 µm id). Detection was performed in ESI+ mode and quantification was executed in SRM mode. First, the compound-specific MS/MS parameters of the peptides are optimised. Second, all peptides were injected onto the nano LC-MS/MS system using a generic gradient. Optimisation of the LC method is necessary for ghrelin and dynorphin A (1-13) to decrease the peak width.

Galanin and neuropeptide Y could not be measured at low concentrations. The limit of detection for bradykinin, neurotensin, neuromedin N and neuromedin B was found to be 7.0; 2.6; 1.5 and 23 pM respectively. Third, a screening was performed in basal hippocampal dialysates. Neuromedin N, neurotensin and ghrelin were detected. These preliminary results show that, after optimisation and validation, the quantification of the selected neuropeptides with nano LC-MS/MS is feasible.

PO-27

THE PHARMACODYNAMIC PROFILE OF ARIPIPRAZOLE IS NOT ALTERED IN A MODEL OF DOPAMINERGIC HYPERSENSITIVITY

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The partial agonist profile of novel antipsychotics such as aripiprazole has hardly been demonstrated in biochemical assays on animal tissues. As it is established that responses induced by dopamine D2 receptor agonists are increased in models of dopaminergic sensitization, this paradigm was used in order to facilitate the detection of the partial agonist properties of aripiprazole. At variance with all other partial and full agonists tested, the partial agonist properties of aripiprazole were not revealed in [35S]GTPγS binding assays on striatal membranes from haloperidol treated rats. Hence, aripiprazole behaved as an antagonist, efficiently inhibiting the functional response to dopamine. Similarly, in behavioural assays, aripiprazole dose-dependently inhibited the stereotypies elicited by apomorphine. However, at variance with haloperidol, repeated administrations of aripiprazole (3 weeks) at the doses of 10 and 30mg/kg did not induce any up-regulation or hyperfunctionality of the dopamine D2 receptors in the striatum. These data highlight the putative involvement of other pharmacological targets for aripiprazole that would support in the prevention of secondary effects commonly associated with the blockade of striatal dopamine D2 receptors. Hence, in additional experiments, aripiprazole was found to efficiently promote [35S]GTPγS binding in hippocampal membranes through activation of 5-HT1A receptors. Further experiments investigating the second-messenger cascades should be performed so as to establish the functional properties of aripiprazole and understand the mechanism underlying the prevention of dopamine receptor regulation in spite of the observed antagonism.

PO-28

MODULATION BY LONG-CHAIN POLYUNSATURATED Ω3 AND Ω6 FATTY ACIDS OF THE METABOLIC SYNDROME IN RATS EXPOSED TO A FRUCTOSE-RICH DIET

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The present study concerns the metabolic and hormonal effects of dietary ω3 and w6 fatty acids in rats exposed to a fructose-rich diet. Four groups of 6 rats each were exposed from the 8th week after birth and for the ensuing 8 weeks to diets containing either 64% (w/w) starch and 5% sunflower oil, or 64% fructose and either 5% sunflower oil, 3.4% sunflower oil and 1.6% salmon oil, or 3.4% sunflower oil and 1.6% safflower oil. The time course for changes in food intake, body weight, plasma D-glucose and insulin concentrations was monitored throughout the experiment. At day 50, an intraperitoneal glucose tolerance test was performed after overnight starvation. At sacrifice, other variables, e.g. the percentage of glycated hemoglobin and plasma concentration of D-fructose and albumin, were also measured. Last pancreatic islets were isolated from each group of rats and incubated in vitro for measurement in insulin secretion and content upon exposure to several distinct nutrients secretagogues, as well as D-[U-14C] glucose oxidation and D-[5-3H]glucose utilization. Several variables, including the insulinogenic index and HOMA index for insulin resistance, were found to differ significantly in the ω3-depleted rats exposed to the starch- or fructose- containing diet. In the latter case, significant effects of partial substitution of sunflower oil by either salmon or safflower oil were also documented.

PO-29

COPD MANAGEMENT IN PRIMARY CARE: AN OBSERVATIONAL COMMUNITY PHARMACY-BASED STUDY

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To date, observational studies on COPD management in primary care are scarce. This study aimed to provide a detailed description of (1) drug therapy, (2) drug adherence, (3) inhalation technique, and (4) health status of COPD patients recruited via community pharmacies. Cross-sectional, observational study in 93 pharmacies (Belgium). Participants (n=555) completed a questionnaire to collect demographic information, smoking history, influenza vaccination, type of COPD medication and side effects. Adherence to controller COPD medication was 1 year-retrospectively analysed through prescription refill rates. Inhalation technique was scored using a checklist. Health status was evaluated with the SGRQ, CCQ and MRC dyspnoea scale. Mean age of the COPD patients was 68.6 yr, 73.7% were men and 37.2% were current smokers. Influenza vaccination status was significantly lower in patients aged <65 yr (65.9%) than in patients aged 65 years or older (86.0%) ($p<0.001$). Inhaled corticosteroid + long-acting β2-agonist combinations were the most

frequently used COPD medications (75.4%). About 48% of patients was underadherent (<80% adherence), 47% was adherent (80-120% adherence) and 5% was overadherent (>120% adherence). Predictors for underadherence with maintenance treatment were age, SGRQ and n° of drug s. Twenty-one % made major inhalation technique errors with the rescue medication, which were all errors in hand-breathing coordination with pMDI's. This observational study on COPD management highlights 4 main aspects which could be improved: (1) drug adherence, (2) inhalation technique with pMDI's, (3) influenza vaccination in COPD patients <65 yr and (4) smoking cessation.

PO-30

DRUG-RELATED PROBLEMS AMONG HOME-DWELLING OLDER PATIENTS WITH CHRONIC DISEASES: IDENTIFICATION OF COMMUNITY PHARMACIST ROLES

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Drug-related problems are common among elderly. The current study aimed to provide an overall picture of medication management by home-dwelling elderly with chronic diseases, by examining: (1) drug utilization, (2) drug adherence, (3) drug knowledge and (4) practical drug management capacity. Such integrated view should allow us to identify potential problems and to indicate target areas for community pharmacist intervention. Cross-sectional, observational study in 86 community pharmacies (Belgium). The patients' current chronic medication regimen was taken from the pharmacy database. Drug adherence was determined by prescription refill rate, pill count and self-report. Drug knowledge and practical drug management capacity were assessed by questionnaire. The study population (n=338) used a median of 5 chronic drugs per patient. Half of our sample chronically used psychotropic medication, mainly benzodiazepines. In 100 patients (29.6%) at least one drug-drug interaction of potential clinical significance was observed. The overall mean adherence per patient was very high: 104.8% according to prescription refill rate and 98.1% according to pill count. Most patients (95.9%) self-reported to take their medicines according to the GP's instructions, although nearly one fifth (n = 82) admitted forgetting medicine intake occasionally. Moreover, 16.9% (n = 57) admitted having stopped a medication on their own initiative. Seventy-six % (n=258) of patients had an acceptable knowledge of the indication for at least 75% of their medication. The participants reported several practical drug taking problems: difficulties with vision (32.0%), blister opening (12.1%), tablet swallowing (14.8%), tablet splitting (29.7% [represents % of patients who have to split tablets]) and distinction between different drug packages (23.4%). These data should allow us to design a rational community pharmacist intervention for this vulnerable patient group.

PO-31

THE (PRO)RENIN RECEPTOR IS REQUIRED FOR VACUOLAR H+-ATPASE REGULATION IN COLLECTING DUCT CELLS

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Prorenin was long considered merely an inactive precursor of renin until the recent discovery of a specific (pro)renin receptor, (P)RR. The (P)RR non-proteolytically activates prorenin, resulting in Angiotensin I (Ang I) formation. The (P)RR, however, can also act as a bona fide signaling receptor, as binding of (pro)renin to the (P)RR can activate signaling pathways, independent of the generation of Ang I. The C-terminal domain of the (P)RR was previously identified as an accessory protein of vacuolar H+-ATPase (V-ATPase), and in collecting duct cells V-ATPase activity is required for (pro)renin induced ERK1/2 phosphorylation. Recent studies have also identified (pro)renin-independent functions for the (P)RR-V-ATPase axis in V-ATPase stability and Wnt signaling. However, so far the exact role of the (P)RR in the regulation of V-ATPase activity remains unknown. In this study, we knocked down the (P)RR in Mardin Darby canine kidney cells (MDCK.C11), that represent the intercalated cells of the collecting duct, using siRNA. We subsequently measured basal and vasopressin-induced V-ATPase activity in cells loaded with the pH-sensitive dye BCECF after an NH4Cl induced acid load. Transfection of MDCK.C11 cells with siRNA against (P)RR decreased (P)RR protein levels by 80%. Basal V-ATPase activity was similar in cells treated with siRNA targeting (P)RR (0.02905 ± 0.003934 pH units/min, n=23) and cells treated with control siRNA (0.03062 ± 0.003153 pH units/min, n=20). In the presence of 100 nM vasopressin, cells treated with control siRNA showed a significant ($p<0.01$) increase in V-ATPase activity (0.03565 ± 0.004543 pH units/min, n=24), whereas V-ATPase activity was not increased in cells treated with siRNA targeting (P)RR (0.02988 ± 0.004199 pH units/min, n=27). Western blotting showed decreased expression of the VOa2, but not the VOd2 subunit of the V-ATPase. Our results suggest that in collecting duct cells, the (P)RR is required for V-ATPase stability and vasopressin-induced V-ATPase activity, and indicate an important function for the (P)RR in V-ATPase regulation.

PO-32

NEW BENZOPYRAN DERIVATIVES ACTIVATING THE INSULIN-SECRETING CELLS KATP CHANNELS

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ATP-sensitive potassium (Katp) channels have been identified in many excitable cell types, into which they play a wide variety of physiological roles. Such ionic channels have been depicted in pancreatic B-cells and have been shown to be tightly involved in the insulin secretory process. In vascular smooth muscle cells, Katp channels participate in the control of muscle tone. The potential and recognized therapeutic indications for potassium channel openers (PCOs) include the treatment of arterial hypertension, angina pectoris, androgenic alopecia... PCOs have also been proposed for the prevention and/or management of type I, type II diabetes, obesity, nesidioblastosis and insulinomas. In order to be used as therapeutic agents and to avoid side effects, PCOs need to express a potent activity associated with high tissue selectivity. By exploring a series of 4,6-disubstituted R/S-3,4-dihydro-2,2-dimethyl-2H-1-benzopyrans structurally related to (±)-cromakalim, we have recently identified compounds relatively potent and selective for the pancreatic Katp channel. Such compounds were bearing a bulky tert-butyloxycarbonylamino group at the 6-position as well as, at the 4-position, a phenylthiourea moiety substituted on the phenyl ring by a meta or a para-electron- withdrawing group such as Cl or CN. In the light of such data, the present work aimed at exploring the influence of other groups at the 6-position, keeping the same substitutions at the 4-position. Preliminary results indicated that the new molecules inhibited the insulin secretory rate and affected the vascular smooth muscle contractile activity. Radioisotopic experiments further revealed that the mechanism of action was related to the activation of Katp channels.

PO-33

DISTRIBUTION OF MECHANICAL ADVANTAGE AND RESPIRATORY ACTIVITY IN THE LEVATOR COSTAE MUSCLE

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In intercostal muscles, inspiratory EMG activity predominates in the upper part of the chest and decreases caudally. This activity gradient parallels the distribution of mechanical advantage, so that the most effective part is the most activated. The levator costae muscle inserts between each rib and the vertebra immediately rostral. In the cat, its activity was progressively stronger in the muscles located in the more caudal thoracic segments. This paradoxical distribution prompted us to study the mechanical advantage, muscle fibre type, EMG activity and metabolic activity of these muscles in the rabbit. In 7 anesthetized animals, the mechanical advantage was evaluated by measuring the fractional change in muscle length during passive inflation. The muscle shortened in each interspace and the mean shortening was -4.7±0.5, 4.0±0.4, -3.3±0.5, -0.8±0.6, 1.4±0.5 %LFRC in the interspaces 2, 3, 5, 6, 7 and 8 respectively. Animals were then sacrificed and the rostro-caudal distribution of type I muscle fibre was evaluated immunohistologically. The proportion of type I fibre peaked at the top (interspace 2: 44±2%) and decreased caudally (interspace 4:

34±1%; 6: 28±3%; 8: 24±2%). In another group of animals, the raw EMG activity was recorded at rest and during resistive loading. The time lag between the air flow reversal and the onset of activity was measured. At rest and with resistive loading, EMG activity appeared earlier in the 2nd interspace than in the mid-thoracic segments and was delayed in the 7th and 8th interspaces. Muscles were freeze-clamped and the intensity of metabolic activity was then quantified using a 31P NMR evaluation. The ratio of inorganic phosphorus to phosphocreatine was highest in the 2nd interspace. We concluded that in levator costae the mechanical advantage and respiratory activity predominate in the upper interspaces, as in intercostal muscles. This neuromechanical matching is reflected in the muscle fibre type composition.

PO-34

SIMULTANEOUS 384-WELL AUTOMATED ELECTROPHYSIOLOGY RECORDING OF LIGAND- AND VOLTAGE-GATED CHANNELS WITH IONWORKS® BARRACUDA™

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Over the last decade the advent of higher throughput screening technologies such as automated patch clamp has alleviated a major bottleneck in early stage drug discovery for ion channels. We present data from the IonWorks® Barracuda™.
The system allows simultaneous and continuous measurement of ionic currents at 384 separate recording sites. The system is equipped with 384 individual patch-clamp amplifiers together with a 384-channel fluidic pipettor. Currents are measured measured in the perforated patch clamp configuration using a single hole (SH) or an array of 64 holes in each well, Population Patch Clamp™ (PPC) technology. Data presented include LGIC recordings of acid sensing ion channels (ASIC1a), as well as VGIC recordings of hERG channels. The ASIC1a and human hERG channels stably transfected in CHO cells were generously provided by ChanTest Corporation (Cleveland, Ohio).ASIC1a current responses were elicited by the application of pH 5.0 buffer. Concentration-dependence of ASIC1a channel responses were inhibition by benzamil and amiloride determined in two different PPC experiments and gave IC50 values of 2.857e-006 M (n=384), and 1.041e-005 M (n=384), respectively. Concentration-dependence of hERG channel inhibition was determined for 3 different compounds, cisapride, terfenadine and quinidine, in three different PPC experiments. These gave IC50 values of 7.972e-008 M (n=384), 4.070e-007 M (n= 384) and 1.924e-006 M (n= 384) respectively. Additional data will be presented from Ligand-Gated (LGIC) and 1 nACh,αVoltage-Gated (VGIC) ion channels, including, Nav1.5, Kv1.3, α-1 nACh and α-7 nACH, and GABA channels. With a throughput of over 10,000 data points per day the IonWorks® Barracuda™ is a high-throughput automated electrophysiology system suitable for the screening ligand-gated and voltage-gated ion channel targets.

PO-35

SUCCESSIVE CHANGES IN HYALURONAN (HA) SYNTHESIS AND FRAGMENTATION IN THE RAT POST-ISCHEMIC KIDNEY

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Since the time-course of hyaluronan (HA) accumulation and fragmentation in inflammatory tissues remains unknown, our study was performed to analyse the expression of the main hyaluronidases and HA synthases, as well as HA fragmentation, after ischemia/reperfusion injury (IR) in the rat kidney. The expression of HYAL1, HYAL2, HAS1, HAS2 and HAS3 was evaluated by real-time PCR in the outer [OSOM] and inner stripes [ISOM] of the renal outer medulla up to Day 14 after IR injury. We also assessed HA fragmentation by membrane filtration followed by pseudo-ELISA method. HAS1 was up-regulated more than 50- and 35-fold in OSOM and ISOM respectively, at 12 h post-IR, returning to baseline faster in ISOM than in OSOM. HAS2 mRNA increased only after a 2-day delay and remained elevated until Day 14 post-IR (mRNA for HAS3 was not detected). Both HYAL1 and HYAL2 were strongly but transiently (12-48 h) repressed. The progressive HA accumulation in the post-ischemic kidney, detected by immunohistochemistry (Declèves et al., 2006), was confirmed by the peudo-ELISA method. Indeed, the amount of HA in control kidneys averaged 29+/-9 and 370+/-28 ng/mg in OSOM and ISOM, and was significantly enhanced to 1201+/-119 and 1524+/-132 ng/mg at Day 14 post-IR, respectively. However, the amount of low molecular weight HA (i.e., HA fragments) decreased by 40% at 24 h post-IR in ISOM. At later stages post-IR, the amount of HA fragments increased in both zones (x80 in OSOM and x6 in ISOM versus baseline, at Day 14). In summary, within 24 h post-IR, there is a massive activation of HAS1 combined with a repression of hyaluronidases, leading to a pool of predominantly high MW HA. Significant amounts of HA fragments appear later (Day 7-14), when HAS2 is induced and HYAL1 and HYAL2 levels are back to baseline. This modulation of HA breakdown after IR injury further suggest that this process might be involved in post-ischemic events such as inflammation and/or regeneration.

PO-36

REMOTE ISCHEMIC PRECONDITIONING: A LEG UP ON RENAL IRI

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Remote ischemic preconditioning (RIPreC) is a strategy to protect a target organ against ischemia/reperfusion injury (IRI) by inducing short-term ischemia/reperfusion (I/R) in a remote organ. We investigated if a remote ischemic stimulus in the hind

limb can protect the kidney against IRI, and whether this protection is adenosine dependent. Anesthetized rats underwent either no RIPreC, unilateral (one limb) or bilateral RIPreC (both limbs). The preconditioning stimulus was continuous (12'/12' I/R), or fractionated (3 times 4'/4' I/R). After the last reperfusion period, we induced 25' ischemia in the right kidney and nephrectomised the left kidney. After 48h of reperfusion, RIPreC ameliorated renal function (plasma creatinine and urea, Ccr and FENa) and reduced renal damage (histology) and kidney injury molecule-1 (KIM-1) transcription ($p<0.05$ or $p<0.01$ for all parameters, depending on the RIPreC protocol). Bilateral RIPreC appeared to be more effective than unilateral RIPreC. Administration of the adenosine receptor antagonist 8-SPT did not abolish the beneficial effects of either continuous or fractionated RIPreC on renal function. In conclusion, we found that RIPreC using the hind limb as remote organ can reduce renal IRI, making this a promising, safe, cheap and non-invasive method to prevent renal damage in e.g. transplantation and aortic surgery. Both fractionated and continuous RIPreC appeared to reduce renal damage via an adenosine-independent mechanism. Preliminary results indicate that opiates, noradrenaline, and/or cannabinoids may play a role in this RIPreC model.

PO-37

THE PRESENCE OF SGLT1 AND GLUT4 IN DUCTAL CELLS OF RAT AND HUMAN SALIVARY GLANDS

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We have documented that the human salivary glucose comes mainly from salivary glands (parotid, sub-maxillary and sublingual). The primary saliva is isotonic and becomes hypotonic during its transport to the oral cavity because of the reabsorption of sodium by ductal cells. Therefore, this study aims, by analogy to enterocytes, to assess whether if the glucose secreted by acinar cells was reabsorbed by ductal cells. Quantitative polymerase chain reaction was used to measure mRNA expression of GLUT4 in different salivary glands. Total RNA from salivary glands was extracted and subjected to mRNA quantification using specific primers for SGLT1 and Glut4 and SYBR Green on a LightCycler 480. Anti-GLUT4 antibody (Millipore 07-1404, 1/500) and anti-SGLT1 antibody (Millipore 07-1417, 1/500) were applied on the fixed salivary glands sections. Staining included avidin-biotin-peroxidase (Vector Labs, Belgium) and diaminobenzidine (Dako, Belgium), with hematoxylin counterstaining. The presence of SGLT1 was detected in rat parotid and sub-maxillary ductal cells by immunehistochemistry. SGLT1 is also present in human parotid ductal cells. Moreover, we have discovered the presence of GLUT4 in ductal cells from human parotid, and both rat parotid and sub-maxillary glands. The presence of both SGLT1 and GLUT4 was also confirmed by qRT-PCR in theses salivary glands. In conclusion this study documents the presence of SGLT1 and GLUT4 in ductal cells of human parotid and rat parotid and sub-maxillary glands. We postulate that their presence in ductal cells of salivary glands may allow the

reabsorption of glucose secreted by acinar cells of salivary glands. Further experiments are required to assess the validity of this proposal by co-localization immunohistochemistry.

PO-38

MODULATION OF MICROGLIA-ASTROCYTE INTERACTION THROUGH LXR ACTIVATION

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In neurodegenerative brain pathologies, such as Alzheimer's and Parkinson's disease, microglia are frequently activated by a local and chronic inflammation. Pro-inflammatory compounds secreted by activated microglia are responsible for astrocyte activation. These astrocytes also produce pro-inflammatory molecules leading to an inflammatory vicious circle in the brain. This toxic environment produced by both cell types seems to be one of the causes of neuronal death in patient's brain.Liver X receptor (LXR) is a ligand-activated nuclear receptor playing a role in cholesterol homeostasis control but also regulating inflammatory responses in many cell types.Is activated LXR able to modulate the dialogue taking place between microglia and astrocytes during inflammation? Can LXR modulate the activation state of these two cell types in order to break this inflammatory vicious circle and, by consequence, protect neurons from death? To study these cellular interactions, astrocytes and microglia co-cultures were performed. These cells were treated by pro-inflammatory molecules and a LXR agonist. The pro-inflammatory gene/protein expression profiles of these cells were analysed by Real-Time PCR and ELISA assay, respectively. Our results show that activated LXR reduces microglia activation, but has no direct effect on astrocyte activation. Moreover, in microglia, LXR activation regulates the mRNA expression of cytokines and chemokines involved in cell-cell signalling. In co-culture experiments, we also show that LXR-treated microglia are able to down-regulate astrocytic activation. Activated LXR appears to be indirectly able to modulate the phenotype of astrocytes through its action on microglia. This work emphasizes the role of activated LXR in the cellular communication between microglia and astrocytes. Thus, LXR activation could reduce brain inflammation and consequently protect from neuronal death.

PO-39

CHARACTERIZATION OF A NEW SIGNALING PATHWAY INVOLVED IN THE NEUTROPHIL NADPH OXIDASE REGULATION VIA S100A8/A9 TRANS-LOCATION

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The neutrophil NADPH oxidase (NOX2), through the production of reactive oxygen species, is a key enzyme for host defense against invading pathogens. Although, it is established that the translocation of S100A8/S100A9, two Ca2+-binding proteins, is involved in NOX2 regulation, mechanisms underlining such a process remains elusive. Neutrophil-like HL-60 cells and human neutrophil were subjected to pharmacological inhibitors or transfected by specific siRNA. NOX2 activity and intracellular Ca2+ variations were quantified by spectrofluorimetry using Amplex red and Fura-2/AM for H2O2 and Ca2+ measurements. S100A8/A9 translocation was monitored by immunofluorescent labeling with Mac387 antibody. p38 MAPK activity was performed using a p38 MAPK activity test. Finally, protein-protein interactions were characterized by GST pull-down. Our data show that sphingosine kinases (SphK) are involved in the S100A8/A9 recruitment to the plasma membrane. Depletion of internal Ca2+ stores is required to mediate SphK activity dependent-S100A8/A9 translocation. Further, SphK knock-down resulted in a decrease of p38 MAPK activity. In addition, we observed that inhibition of S100A8/A9 translocation by SphK knock-down is associated to a decrease of NOX2 activation. S100A8/A9 interacts with cytosolic subunits of NOX2 (p67phox, p47phox, Rac1 and Rac2). These interactions are not inhibited by intracellular Ca2+ chelation. Ca2+ store depletion-induced SphK activity regulates NOX2 activity through the p38 MAPK dependent-translocation of S100A8/A9. Moreover, S100A8/A9 interacts with NOX2 cytosolic factors in a Ca2+ independent manner suggesting that S100A8/A9 regulates NOX2 assembly to the plasma membrane.

PO-40

STRESS- AND NUTRITION-SENSING TRANSCRIPTION FACTORS INTER-ACTIONS IN ATHEROSCLEROSIS: INVOLVEMENT OF CCL2, 4 AND 5

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An imbalance of functional interactions between nutrient-sensing nuclear receptors (e.g. Vitamin D receptor, VDR) and stress-sensing transcription factors (e.g. NF-kappaB, NFkB) induced by lifetime exposure to micronutrients and cytokines may be a central molecular process towards atherosclerosis. We wanted to highlight potential interactions between VDR and NFkB signalling in vascular endothelial cells, the first cell population implicated in this disease. Microarray analyses were carried out on total RNA extracted from EA.hy926 (human umbilical vein endothelial cells line) after stimulation with TNFalpha (TNF) and/or 1-alpha 25(OH)2 vitamin D3 (VD) up to 24h. Transformation/normalisation of raw data (Bioconductor software) delineated a variety of genes regulated by TNF or by VD alone, or by TNF and VD in a synergistic or antagonistic way. We focused on the genes regulated by TNF and VD in an opposite way and identified a group of genes potentially implicated in macrophage chemo-attraction (Chemokine (C-C motif) ligand 2, 4 and 5 (CCL2, 4 and 5)): they were up-regulated by TNF and these up-regulations are reduced by VD. Microarray data were further verified and confirmed by real time RT-PCR for CCL2, 4 and 5. Also, the effect of VD was abolished when VDR expression was switched off by

siRNA. The same results were obtained when cycloheximide was added to the TNF and VD treatment. The implication of VDR seems however to be indirect and to involve a de novo synthesis of protein(s). IPA Knowledge Base (Ingenuity) helped to identify proteins which could be part of this regulation. Among other proteins, we showed for the first time that GILZ (Glucocorticoid-induced leucine zipper; TSC22D3), hypothesised in the literature to decrease the production of CCL5, was up-regulated by VD. GILZ could therefore be responsible for a potential protective effect of VD on inflammation-induced expression of CCL2, 4 and 5.

PO-41

PHARMACOLOGICAL CHARACTERIZATION OF THE ZEBRAFISH VERSUS THE HUMAN SPHINGOSINE-1-PHOSPHATE TYPE 1 RECEPTOR

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The sphingosine-1-phosphate type 1 (S1P1) receptor is a lipid receptor which belongs to the family of G protein-coupled receptors and predominantly couples to and activates Gi/o proteins. Genetic deletion and pharmacological intervention studies revealed a crucial role for this receptor in the vascular and immune system. Probably because of its important role in these systems S1P1 receptor expression has been conserved from non-mammalian vertebrates including the zebrafish to mammalian species. Although the zebrafish S1P1 receptor has already been identified in 2000 it has not been extensively characterized yet. In this study we characterized the zebrafish S1P1 receptor using various pharmacological tools that recently have become available and compared the signalling properties directly to those of the human S1P1 receptor. N-terminally HisG-tagged zebrafish and human S1P1 receptors were stably expressed in CHO-FlpIn cells and signal transduction was measured by determining the effect of various selective and non-selective S1P1 ligands on the forskolin-induced cAMP accumulation. Alignment of the zebrafish and human S1P1 sequence reveals a relatively low overall amino acid homology of about 70%. However, the signalling properties between the zebrafish and human S1P1 receptor seem to be well conserved. As found for the human receptor the zebrafish S1P1 receptor also mediates the S1P-induced inhibition of adenylyl cyclase which is pertussis toxin-dependent indicating the involvement of Gi/o proteins. Overall, the potency profiles of the S1P1 selective (SEW2871, CYM5442) and unselective (S1P, FTY720-P, dihydroS1P) ligands was comparable at both receptor species although the potency at the human receptor generally was a bit higher. The S1P1 antagonist W146 also behaved as a competitive antagonist at the zebrafish S1P1 receptor.
In conclusion, despite a relatively low amino acid homology between the zebrafish and human S1P1 receptor the signalling properties between these two species are well conserved.

PO42

HYPOTONICITY-INDUCED INSULIN RELEASE IN BRIN BD11 CELLS IS MEDIATED BY HYDROGEN PEROXIDE

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NAD(P)H oxidase (NOX)-derived H2O2 was recently proposed to act, in several cells, as the signal mediating the activation of volume-regulated anion channels (VRAC) under a variety of physiological conditions. The present study aims at investigating whether a similar situation prevails in insulin-secreting BRIN-BD11 cells. Exogenous H2O2 stimulated insulin secretion with a threshold concentration close to 38 µM and a maximal response at about 100 µM. The inhibitor of VRAC, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) inhibited the secretory response to exogenous H2O2. In patch clamp experiments, exogenous H2O2 was observed to stimulate NPPB-sensitive anion channel activity, which induced cell membrane depolarization. Exposure of the BRIN-BD11 cells to a hypotonic medium caused a detectable increase in intracellular level of reactive oxygen species (ROS) that was abolished by diphenylene iodinium chloride (DPI), a general NOX inhibitor. NOX inhibitors such as DPI and plumbagin nearly totally inhibited insulin release provoked by exposure of the BRIN-BD11 cells to a hypotonic medium. Preincubation with two other drugs also abolished hypotonicity-induced insulin release and reduced basal insulin output: 1) N-acetyl cysteine (NAC), a glutathione precursor that serves as general antioxidant and 2) betulinic acid a compound that almost totally abolished NOX4 expression. As NPPB, each of these inhibitors (DPI, plumbagin, preincubation with NAC or betulinic acid) strongly reduced the volume regulatory decrease observed following a hypotonic shock, providing an independent proof that VRAC activation is mediated by H2O2. Taken together, these data suggest that NOX-derived H2O2 plays a key role in the insulin secretory response of BRIN-BD11 cells to extracellular hypotonicity.

Meeting information

First Benelux Congress on Physiology and Pharmacology – Liège (Belgium)
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