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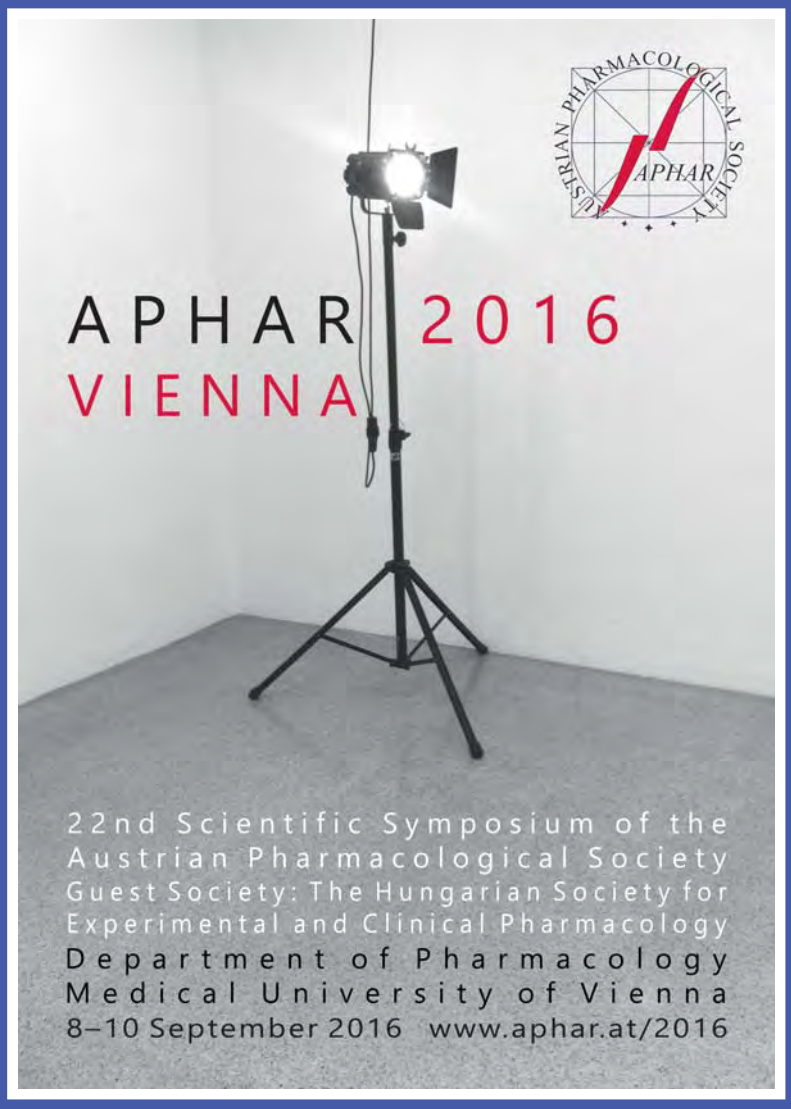

MEETING ABSTRACTS

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MEETING ABSTRACTS

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Cardiovascular Pharmacology and Endocrinology

A1.1

Formation of nitric oxide by aldehyde dehydrogenase-2 is essential for nitroglycerin-induced activation of soluble guanylate cyclase

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Background: Aldehyde dehydrogenase-2 (ALDH2) catalyzes vascular bioactivation of the antianginal drug nitroglycerin (GTN), resulting in 3′,5′-cyclic guanosine monophosphate (cGMP)-mediated vasodilation through activation of soluble guanylate cyclase (sGC). We have previously shown that a minor reaction of ALDH2-catalyzed GTN bioconversion, accounting for about 5% of the main clearance-based turnover yielding inorganic nitrite, results in direct nitric oxide (NO) formation, and concluded that this minor pathway could provide the link between vascular GTN metabolism and activation of sGC. However, lack of detectable NO at therapeutically relevant GTN concentrations (<1 µM) in vascular tissue questioned the biological significance of NO formation by purified ALDH2. In the present study, we used a novel, highly sensitive genetically encoded fluorescent NO probe (C-geNOp) to visualize intracellular NO formation in cultured vascular smooth muscle cells (VSMC) expressing either wild-type ALDH2 or a mutant (C301S/C303S ALDH2) that reduces GTN to NO but lacks clearance-based GTN denitration activity.
Methods: Wild-type and C301S/C303S ALDH2 were expressed in murine ALDH2-deficient vascular smooth muscle and porcine aortic endothelial cells by adenoviral transfection, and protein expression was analyzed by western blot. For single-cell NO measurements, cells were co-infected with the fluorescent protein-based NO probe C-geNOp. ALDH2-catalyzed NO formation at low GTN concentrations (<1 µM) was measured by real-time cell imaging and compared with GTN-induced activation of sGC in VSMC lysates and cultured porcine aortic endothelial cells.
Results: Adenoviral transfection led to virtually identical protein expression levels of wild-type and mutated ALDH2. Addition of 1 µM GTN to VSMC expressing either wild-type or C301S/C303S ALDH2 resulted in a pronounced increase in intracellular NO, with maximal concentrations of 7 and 17 nM, respectively. Formation of GTN-derived NO correlated well with activation of purified sGC in VSMC lysates as well as cGMP accumulation in cultured porcine aortic endothelial cells that had been infected with wild-type or mutant ALDH2. Formation of NO and cGMP accumulation were inhibited by the ALDH inhibitors cloral hydrate and daidzin.
Discussion: The present study demonstrates that ALDH2-catalyzed NO formation is necessary and sufficient for GTN bioactivation in VSMC.
Acknowledgements: This work was supported by the Austrian Science Fund FWF, grant no. P24946.

A1.2

Effects of resveratrol supplementation in rats with diet-induced hypercholesterolemia

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Background: *Trans*-resveratrol (RSV) is a phytoalexin, class: stilbene, polyphenol compound from the non-flavonoids group, for which numerous *in vitro* and pre-clinical tests on animals have shown the ability to exhibit a wide range of potentially useful activities

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for human health, such as antioxidant, anti-inflammatory, cardio-protective, neuroprotective, anti-diabetic, and anti-cancer activity. There are several commercially available dietary supplements in Serbia, based on resveratrol, which are recommended to reduce the risk of cardiovascular, neurodegenerative and malignant diseases. However, like other available dietary supplements, they have not been sufficiently tested in terms of their quality and safety. Few previous studies on animals have showed that resveratrol has the ability to express cardioprotective effects via various mechanisms of action. The aim of this study was to examine the effect of a commercial supplement based on resveratrol on the lipid status of experimental animals in order to assess the hypolipidemic effect of the registered preparation.

Methods: Three groups containing 6 animals of male Wistar rats (250–300 g) were used for the study. Group I served as control and received only vehicle (saline) for 30 days. Group II was fed with a high-cholesterol diet (2% cholesterol and 0.5% cholic acid of a daily diet was dissolved in olive oil and given orally by probe) for 30 days. Group III was fed with a high-cholesterol diet and treated with the resveratrol-based dietary supplement (20 mg RSV/kg body weight) orally for 30 days.

Results: Cholesterol and cholic acid diet induced a significant increase in total cholesterol, triglyceride and serum LDL concentration in comparison to the control group ($p < 0.05$), while HDL levels has remained unchanged. Treatment with resveratrol-based dietary supplement (Group III) significantly decreased serum LDL and triglyceride levels, while it significantly increased the HDL levels in comparison to Group II.

Discussion: The present results show that a resveratrol-based dietary supplement possesses significant hypolipidemic effect in laboratory animals with cholesterol/cholic acid diet-induced hyper-lipoproteinemia which may explain protective effect of resveratrol against atherosclerosis and cardiovascular diseases.

Acknowledgements: This work was supported by the Provincial Secretariat for Science and Technological Development of the Autonomous Province of Vojvodina (project no. 114-451-698/2015-02).

A1.3

Assessment of regional blood–brain barrier integrity and cerebral efflux transporter function in patients with neuroepithelial tumors using [^{11}C]tariquidar PET

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Background: At the human blood–brain barrier (BBB) high levels of efflux transporters, such as P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2), are expressed, which restrict the brain distribution of many different drugs. The pharmacological treatment of brain tumors is often hampered by the impermeability of the BBB to anticancer drugs. It has been shown that the BBB may become disrupted in central necrotic parts of high-grade gliomas, leading to higher anticancer drug concentrations as compared to tumor-free brain tissue. However, little is known to which extent a BBB disruption exists in low-grade glioma. [^{11}C]Tariquidar is a small, drug-like radiotracer, which is subject to efflux transport by ABCB1 and ABCG2 at the human BBB. We used positron emission tomography (PET) with [^{11}C]tariquidar to study regional BBB integrity and cerebral efflux transporter function in patients with neuro-epithelial tumors of the central nervous system.

Methods: Seven patients diagnosed with a neuroepithelial tumor of the central nervous system (WHO I–III) and elected for neurosurgery underwent a 60-min dynamic [^{11}C]tariquidar brain PET scan and simultaneous arterial blood sampling. Four regions of interest (tumor, tumor rim, contralateral as well as ipsilateral tumor-free brain area) were delineated on MR-co-registered PET images. Logan graphical analysis was used to estimate the total distribution volume (V_T) as a parameter of [^{11}C]tariquidar distribution from blood to different brain regions.

Results: In all patients, brain distribution of [^{11}C]tariquidar was very low, which was consistent with ABCB1/ABCG2-mediated efflux transport at the BBB. There were no significant differences in [^{11}C]tariquidar V_T values between tumor tissue and tumor-free tissue and between the central part of the tumor and the tumor rim. In 4 patients, V_T values were lower in tumor tissue as compared to contralateral tumor-free brain tissue (−89 ± 78%, range: −22% to −200%). In 3 patients, V_T values were higher in tumor tissue as compared with contralateral tumor-free brain tissue (+27 ± 14%, range: +13% to +40%).

Discussion: We found no significant differences in distribution of [^{11}C]tariquidar to tumor tissue and tumor-free tissue, which argues against a major BBB disruption in these patients and suggests that anticancer drugs which are transported by ABCB1 and ABCG2, such as tyrosine kinase inhibitors, may not reach the tumor tissue in sufficiently high and therapeutically effective concentrations.

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A1.4

Screening for potential hazardous effects of an H_2S-donating anthracycline on the cardiovascular system

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http://www.intrinsicactivity.org/2016/4/S3/A1.4

Background: Conjugation of doxorubicin (DOX) with H_2S donors gave rise to novel anthracyclines, such as CC2790A, which failed to inhibit topoisomerase II and displayed a more potent cytotoxic effect and higher intracellular retention than the parent compound in DOX-resistant U-2 OS osteosarcoma cells [1]. The well-known cardio-vascular toxicity of anthracyclines, however, might limit their use.

Methods: Therefore, the aim of this study was to investigate CC2790A-induced effects on the mechanical activity of fresh and cultured rat aorta rings, on $Ca_V1.2$ channel current ($I_{Ca1.2}$) of aortic A7r5 cells as well as its cytotoxicity on A7r5, endothelial EA.hy926 cells, and H9c2 cardiomyocytes [1, 2]. DOX was used as reference compound.

Results: At concentrations of ≥1 µM, DOX partially increased phenylephrine-induced contraction in fresh endothelium-intact rings, while CC2790A was ineffective. Conversely, in endothelium-denuded

rings both drugs were ineffective. CC2790A and DOX did not affect the concentration–response curve to high KCl. In arteries cultured with both drugs for 7 days, CC2790A blocked both phenylephrine- and high-KCl-induced contractions at a concentration 10-fold higher than that of DOX. CC2790A, at the maximum concentration tested of 10 µM, exhibited a weak Ca^{2+}-antagonist property in single A7r5 cells. CC2790A and DOX exerted cytotoxic effects at concentrations >1 µM or >0.1 µM, respectively, in both EA.hy926 and A7r5 cells. DOX (0.01–1 µM), at variance with CC2790A (0.1–1 µM), induced cell-cycle arrest in G_0/G_1 phase and significantly increased the proportion of cells in the sub-G_0/G_1 phase. Furthermore, it caused apoptosis, as confirmed by phase-contrast microscopy (cell shrinkage, membrane blebbing, presence of apoptotic bodies and attachment loss), by phosphatidylserine externalization (annexin V/propidium iodide labelling) as well as DNA fragmentation (DAPI staining). CC2790A, retained within H9c2 cells like DOX, was significantly less toxic and produced lower amounts of intracellular reactive oxygen species than the lead.

Discussion: In conclusion, CC2790A is a novel H_2S-donating anthracycline characterized by a more favourable toxicological profile and a better efficacy towards drug-resistant cells. In the context of earlier attempts to use H_2S-donating drugs in cancer therapy, CC2790A is worthy of further investigations in preclinical and clinical settings.

Acknowledgements: This work was supported by the Italian Ministry for Instruction, Universities and Research (Futuro in Ricerca 2012, RBFR12SOQ1 to S.S.).

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A1.5

In vitro assessement of tariquidar toxicity towards vasculature

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http://www.intrinsicactivity.org/2016/4/S3/A1.5

Background: The P-glycoprotein (P-gp) inhibitor tariquidar, intravenously administered, increases brain uptake of radiolabeled P-gp substrates, commonly used to detect functional alterations of blood–brain barrier pumps in PET imaging [1]. However, the doses that are required—up to 4-fold higher than those already used in clinical trials to reverse multidrug resistance—cause syncopal episodes and hypotension [2]. Therefore, we investigated the toxic hazard of these doses towards the vasculature.

Methods: The effects of tariquidar on A7r5 and EA.hy926 cell viability, on the mechanical activity of fresh and cultured rat aorta rings, as well as on A7r5 $Ca_V1.2$ channel current ($I_{Ca1.2}$) were analysed [3].

Results: In both A7r5 and EA.hy926 cells, tariquidar was generally devoid of cytotoxic effects up to a concentration of 1 µM. However, at 10 µM, it caused apoptosis already after 24 h treatment. In endothelium-denuded aorta rings, 10 µM tariquidar relaxed contractions induced by phenylephrine but not by high K^+. The contractile activity of rings cultured for 7 days was not affected by drug treatment. Finally, tariquidar did not modify $I_{Ca1.2}$ intensity and kinetics.

Discussion: Tariquidar exerts both cytotoxic and acute vascular effects at concentrations comparable to those employed in PET imaging. This may limit its use as diagnostic tool.

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A1.6

Vascular toxicity risk assessment of MC18 and MC70, novel potential diagnostic tools for *in vivo* PET studies

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Background: The P-glycoprotein (P-gp) inhibitor MC18 has recently been proposed as a valuable PET tracer to measure P-gp expression *in vivo* [1]. The aim of this study was to evaluate the toxic hazard towards the vasculature of MC18 along with the structurally related and more potent P-gp inhibitor MC70 [2].

Methods: Their effects on A7r5 and human endothelial EA.hy926 cell viability, on the mechanical activity of fresh and cultured rat aorta rings, as well as on $Ca_V1.2$ channel current ($I_{Ca1.2}$) of A7r5 cells were analysed [3].

Results: At concentrations >10 µM, MC18 and MC70 decreased cell viability causing evident morphological changes. In fresh rat aorta rings, both compounds antagonized phenylephrine-induced contractions in a concentration-dependent manner with IC_{50} values in the range 2.44–14.5 µM, whereas only MC18 caused a concentration-dependent decrease of the responses induced by 60 mM K^+ (K60). In rings cultured for 7 days in the presence of tested compounds, 10 µM MC70 significantly reduced, while 10 µM MC18 completely prevented the contractile response to both phenylephrine and K60. MC18 and MC70 inhibited $I_{Ca1.2}$ in a concentration-dependent manner with IC_{50} values of 16.81 and 32.13 µM, respectively. The effects of the two compounds on the induction of endothelial-mesenchymal transition in EA.hy926 cells are currently under investigation.

Discussion: These findings demonstrate that MC18-induced vascular effects take place at concentrations that are at least three orders of magnitude higher than those allowing in vivo measurement of P-gp expression (≤10 nM). Thus, MC18, and possibly MC70, can be considered promising PET tools for *in vivo* P-gp quantification.

Acknowledgements: This work was supported by the Italian Ministry for Instruction, Universities and Research (Futuro in Ricerca 2012, RBFR12SOQ1 to S.S.).

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A1.7

Fluctuating estradiol levels in female controls correlate with white matter microstructure

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Background: Diffusion tensor imaging (DTI) provides an excellent method for the assessment of white matter microstructural organization in the human brain by measuring the diffusion of water molecules in the three-dimensional space [1]. A frequently used parameter is fractional anisotropy (FA) which indicates the degree of diffusion anisotropy. Large studies investigating sex differences in WM microstructure demonstrated FA differences between sexes with lower FA values in multiple regions in women compared to men [2]. Although specific biological underpinnings of these differences remain to be determined, sex-steroid hormones, such as estradiol, may play a crucial role. To test this hypothesis, changes in hormone levels over time were investigated in a healthy female population with normal menstrual cycle and correlated with FA value changes.

Methods: Thirteen healthy female controls (FC) (mean age ± SD: 25.0 ± 6.2) were included and measured at two time points with an interval of 4 months. Participants underwent MRI scans on a 3T TIM Trio Scanner (Siemens Medical, Germany). DTI acquisition was performed with an isotropic resolution of 1.6 mm^3 acquiring diffusion-weighted images in 30 directions with a *b* value of 800 s/mm^2. Calculation of fractional anisotropy (FA) maps was done in FSL (www.fmrib.ox.ac.uk/fsl/) after eddy current correction. Correlational analysis between changes of FA values and estradiol levels between the two time points was performed with tract-based spatial statistics to evaluate specific influences on DTI metrics.

Results: We observed a negative correlation between changes in estradiol levels and FA values in several white matter tracts. Increases in estradiol were associated with decreases in FA values and *vice versa*. Significant clusters were found in splenium of corpus callosum, left anterior, posterior and retrolenticular part of internal capsule, left superior and posterior corona radiata, left external capsule, left fornix / stria terminalis, left superior longitudinal fasciculus, left precentral and superior parietal blades. All reported correlations were $p < 0.05$ FWE-corrected.

Discussion: Our results indicate that fluctuating hormonal levels in a population of healthy female controls affect white matter micro-structure metrics. More specifically, a negative correlation between estradiol and FA values has been observed in several white matter tracts. As FA values are thought to reflect the constitution of axon caliber, myelination and fiber organization in white matter pathways [3, 4] our results suggest that even fluctuating levels of estradiol in the adult human brain affect those biological domains. Our results are in line with recent animal research showing that hormones contribute essentially to the regulation of axon biology [5].

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A1.8

Biological activities of the traditional Chinese medicine formula Fang Feng Tong Sheng San

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Background: Fang Feng Tong Sheng San (FFTS) is a well respected Chinese herbal medicine formulated over 800 years ago. In traditional Chinese medicine (TCM) it dispels 'pathogenic wind' (heat) from the body surface and purges away the 'dampness heat' from the interior. For modernization and internationalization of FFTS biological activities have to be determined.

Methods: In the present study, the formula was tested *in vitro* for antidiabetic, antioxidant and antimicrobial activities. Four FFTS preparations were selected from different Chinese manufacturers containing 14 powdered component herbs and three salts. The pills were crushed, extracted with methanol applying ultrasonic treatment. The supernatant was dried and used for the bioassays. As α-glycosidase is a key enzyme in carbohydrate digestion the inhibitory effect of the formula against this enzyme was tested [1]. The samples were compared to acarbose, which, as an inhibitor of α-glycosidase, can be used in the treatment of diabetes type 2. The evaluation of the antioxidant activity was performed by the DPPH radical scavenging method [2]. The standard disc diffusion assay [3]

was applied for testing the antimicrobial effect against gram-positive and gram-negative bacteria as well as against yeast.

Results: An average of 51.55 ± 0.90% inhibition of α-glycosidase was achieved with the four different FFTS preparations in comparison to acarbose (89.20 ± 4.91%). Antioxidant activity showed an average IC_{50} of 0.337 ± 0.002 mg/ml and was compared to rutin, which had an IC_{50} of 0.012 ± 0.011 mg/ml. No antimicrobial activity was detected against bacteria and yeast.

Discussion: Traditional Chinese medicines contain a large number of herbs and other components that, in different combinations, often produce unique biological effects. FFTS showed pronounced anti-diabetic and antioxidant properties. No antimicrobial activity could be determined. This study clearly establishes FFTS as a valuable source of natural antioxidants. It might also be regarded as an interesting alternative candidate for the treatment of diabetes type 2.

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Immunopharmacology and Infection

A2.1

Detection of ascaridole activation in *Leishmania*

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Background: Leishmaniasis is an infectious disease caused by protozoal parasites, which are transmitted by sandflies, and is leading to skin lesions, destruction of mucous membranes or even death. The disease is an increasing problem not only in Asia and Latin America but also in southern regions of Europe. Currently about 12 million people worldwide are infected. Since standard first-line treatment with antimonials often causes serious side effects and also resistances against established therapies are rising there is a big need for new drugs. A promising compound is the endoperoxidic monoterpene ascaridole (Asc), a major component of the essential oil of *Chenopodium ambrosioides*. In previous studies, the effectiveness of Asc in a leishmaniasis mouse model against infections of these parasites was demonstrated [1]. From biomimetic experiments it was predicted that Asc requires activation. However, how this activation takes place in *Leishmania* is completely unknown. Therefore, the molecular activation of Asc in *Leishmania* requires further elucidation.

Methods: Activation of Asc was studied by electron spin resonance (ESR) spectroscopy in combination with spin trapping using 2-methyl-2-nitrosopropane (MNP) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). Experiments were carried out in cell-free buffers and *Leishmania tarentolae* promastigote (LtP) cell suspensions. In addition, decomposition of Asc was studied by gas chromatography and flame ionization detection (GC/FID). The viability of LtP was assessed by a resazurin assay.

Results: Activation of Asc by Fe^{2+} in a cell-free system resulted in ESR spectra consisting of a triplet of duplets (a_N = 16.8 G, a_H = 1.8 G) using MNP to trap the radicals formed. In the cellular system (LtP) the less cytotoxic DMPO was used for spin trapping, resulting in a six-line ESR signal (a_N = 16.1 G, a_H = 24.6 G) without addition of iron. These coupling constants suggest the formation of carbon-centered radicals from Asc and are compatible with *iso*-propyl radicals as primary intermediates. Additional experiments in LtP in the presence of iron chelators and thiols revealed a modulation of the Asc activation. GC/FID measurements showed that Asc decomposition strongly depends on the buffer/cell suspension system. Furthermore, viability of LtP was much more influenced by Asc than by a non-peroxide analogue with similar structure.

Discussion: In summary, experimental data show that Asc requires activation by univalent reduction to exert its pharmacological function as antileishmanial agent. *Iso*-propyl radicals were identified as primary drug intermediates in LtP. The activation is influenced by cellular iron and the thiol status. Further studies will elucidate more details about the mechanism of Asc activation to improve the effectivity and safety of Asc application as antileishmanial agent.

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A2.2

Fighting antibiotic resistance: the concept of mutant selection window and mutant prevention concentration

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Background: Appropriate antibiotic dosing is an important factor in preventing the emergence and proliferation of antibiotic-resistant strains. The mutant selection window (MSW) concept offers knowledge of the relationship between antibacterial pharmacodynamics and resistance development which is crucial for optimizing the use of existing antibacterial agents. It is based on a novel pharmacodynamic measure of antibiotic potency—the mutant prevention concentration (MPC). This review aimed to explore these concepts with regard to their clinical applicability.

Methods: A literature search (–2015) using the keywords 'mutant prevention concentration' or 'mutant selection window', and 'antibiotic' or 'antibiotics' or 'antibacterial' of the PubMed database.

Results: The search yielded 450 results and, after checking the titles, 181 abstracts; 84 articles were assessed in full text. The concept of MSW was first reported in 1999 in relation to fluoroquinolones. The basic concept is simple: within a susceptible bacterial population, a fraction of cells is not affected when exposed to an antimicrobial agent, and multiplication of this resistant subpopulation occurs in a range of concentrations (MSW) between the minimum inhibitory

concentration (MIC) of the susceptible cells, and the mutant prevention concentration (MPC). MPC represents a concentration of antibiotic that prevents the development of first-step resistant mutants—the MIC of the least drug-susceptible mutant sub-population. Multiple studies that monitored the increase in MIC after bacterial exposure to different concentrations of antibiotics confirmed that resistant mutants are selectively enriched when the antibiotic concentrations remain within the MSW. Besides on fluoroquinolones, this hypothesis has been tested in other classes of antibacterial agents such as polimixines, macrolides, aminoglycosides and beta-lactams *in vitro*. A limited number of studies tested these principles *in vivo*.

Discussion: Traditional dosing regimens often provide antibacterial concentrations within the MSW, allowing selective amplification of resistant mutants. Depending on the host defence mechanism, this leads to killing of susceptible bacteria and to a successful clinical response, but the possibility does exist that less susceptible microorganisms are selected. The MSW has been widely confirmed *in vitro*, but not all data generated *in vitro* agree with the results attained *in vivo*. The mutant selection window concept is definitely relevant for fluoroquinolones based on *in vitro* and *in vivo* experiments, but further research is necessary to determine the applicability of MSW *in vivo* for other antibacterial groups. For fluoroquinolones, MSW determined *in vitro* can be a reliable tool for guiding the optimization of antimicrobial treatment regimens for suppression of the selection of antimicrobial resistance, and clinical implementation of a selection window dosing strategy is feasible.

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A2.3

Vascular and metabolic effects of anti-TNFα biologics in rheumatoid arthritis

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Background: Cardiovascular (CV) morbidity and mortality are increased in patients with rheumatoid arthritis (RA). Biologics may influence vascular function and lipids in RA, however, most studies have been short-term and less information has become available on etanercept (ETN) and certolizumab pegol (CZP). We wished to determine the effects of these TNFα blockers on common carotid intima–media thickness (ccIMT), brachial artery flow-mediated, endothelium-dependent vasodilatation (FMD) and the arterial stiffness marker pulse wave velocity (PWV) in context with laboratory assessments in RA patients after 12 months of biological therapy.

Methods: Twenty-six patients (22 female, 4 male) were studied. They received either ETN or CZP for 12 months. Brachial and carotid ultrasonography was performed to determine FMD, ccIMT and PWV, respectively. We also assessed immunological, inflammatory and metabolic laboratory markers.

Results: At baseline, mean ccIMT was 0.56 mm (normal range: 0.4–0.9 mm), mean FMD was 6.5% (normal: >10%), and the mean PWV was 8.4 m/s (normal range: 4–20 m/s). At baseline, ccIMT correlated with disease duration ($r = 0.446$, $p = 0.015$), while FMD and PWV did not. ccIMT ($r = 0.393$, $p = 0.023$) and PWV ($r = 0.511$, $p = 0.005$) also correlated with age at RA onset. PWV correlated with serum triglyceride levels. After 12 months of anti-TNFα treatment, DAS28 ($p < 0.001$), CRP ($p = 0.004$), FMD ($p = 0.04$) and PWV ($p = 0.035$) significantly improved.

Discussion: In patients with RA, FMD, a marker of endothel dysfunction, and PWV, a marker of arterial stiffness, significantly improved after 12 months of anti-TNFα treatment with ETN or CZP, whereas ccIMT may require more time to improve.

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A2.4

Pharmacokinetics of anidulafungin in ascites and pleural effusion

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Background: Echinocandins are recommended for treatment of invasive candidiasis in critically ill patients. Anidulafungin displays particularly favourable pharmacokinetics, because its elimination is independent from hepatic and renal function. Data on anidulafungin target-site penetration and kinetics is sparse so far. Therefore, we determined anidulafungin pharmacokinetics in ascites and pleural effusion of critically ill patients. We addressed the question whether effective anidulafungin concentrations can be achieved by standard doses in these compartments.

Methods: Samples of ascites and of pleural effusion were taken during routine paracentesis or via ascites or pleural drain applied for therapeutic purpose. When sampling was performed via drainage seven samples were drawn within the dosage interval for assessment of anidulafungin kinetics. Anidulafungin was measured by high-performance liquid chromatography (HPLC) and UV detection after sample preparation by protein precipitation with acetonitrile. Gradient elution was done with ammonium acetate and acetonitrile at a flowrate of 1.0 ml/min. Anidulafungin was detected at 306 nm. Quantification was validated according to the European Medicine Agency (EMA) guidelines. The lower limit of quantification was 0.05 mg/l.

Results: Seven critically ill patients suffering from septic shock and multi-organ dysfunction syndrome were enrolled. Anidulafungin kinetics was determined in ascites of four patients and in pleural effusion of two patients. A single concentration was measured in one sample obtained from paracentesis. Ascites concentrations were lower than plasma levels (peak level [C_{max}] 0.34–0.98 *vs.* 3.82–7.70 mg/l) and displayed a slower rise and decline than in plasma (t_{max} [time to C_{max}] 4–12 h *vs.* 1 h). The penetration ratio expressed by the ratio between the area under the concentration–time curve (AUC) in ascites and the AUC in plasma was 0.07–0.37. C_{max} values of anidulafungin in pleural effusion were 1.02 and 2.02 mg/l.

Discussion: Anidulafungin was detectable in all samples. Ascites and pleural effusion concentrations exceeded the minimal inhibitory concentrations (MICs) reported for numerous *Candida* strains. But less susceptible isolates have also been described. Antifungal activity

of anidulafungin in ascites and pleural effusion has to be assessed by further studies.

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A2.5

The role of D-type prostanoid receptor 1 in eosinophil apoptosis

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Background: Allergic asthma is a progressive inflammatory disease with growing incidence in western countries. Although many aspects of the disease development and symptom manifestation are well understood, more research is required to illuminate how the allergic process, for instance, is initiated and how it can be prevented. Eosinophil granulocytes are members of the innate immune system and strongly involved in the pathophysiology observed in subjects suffering from allergic disease. Their recruitment and activation during asthmatic responses is facilitated by mediators secreted by mast cells and other cells. One of these mediators is prostaglandin (PG) D_2, a lipid compound that is found in high concentrations in inflamed tissues. Eosinophils express two distinct G protein-coupled receptors for PGD_2 on their cellular surface, DP (D-type prostanoid receptor 1) and CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). Both receptors bind PGD_2 with a similar affinity. Although it is known that PGD_2 induces several morphological and functional changes through DP (intracellular cAMP increase) and CRTH2 (calcium increase, shape change, chemotaxis), its role in eosinophil survival has not been explained in detail, although previous published data link DP signalling to eosinophil survival.

Methods: Functional assays to measure the activity of effector caspases 3 and 7 were performed and protein expression was examined through means of western blotting, flow cytometry and fluorescence microscopy.

Results: We observed that the DP-specific agonist BW245C, but not the PGD_2- or CRTH2-specific agonist DK-PGD_2 could significantly decrease caspase 3/7 activation in isolated human eosinophils. The PGD_2 metabolite 15-deoxy-Δ12,14-prostaglandin J_2 (15d-PGJ_2) increased caspase 3/7 activity in a PPAR-γ-independent manner. Flow-cytometry data suggested an upregulation of the anti-apoptotic protein Bcl-xL after 18 hours, independently of receptor activation, while preliminary fluorescence microscopy data suggested that CRTH2 regulates Bcl-xL expression. This indicated that not only DP but also CRTH2 might be involved in anti-apoptotic signalling.

Discussion: In conclusion, we have shown that DP acts as an anti-apoptotic receptor by blocking caspase 3 and 7 activity. This process is mediated by Bcl-xL. The metabolite 15d-PGJ_2 increases caspase activity, suggesting the involvement of intracellular targets of the compound. Preliminary fluorescence microscopy data links CRTH2 also to Bcl-xL regulation, but further studies are required to validate its participation in anti-apoptotic signalling.

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A2.6

Role of prostaglandin D_2 in equine allergic diseases

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Background: Similar to humans, horses develop skin and respiratory symptoms that are attributed to allergies. Thus, horses are a natural disease model, as they share similar immune response mechanisms with humans. Furthermore, there is a growing need for additional treatment options for allergic equine diseases, because of their widespread usage as domestic animals and their commitment in sport. Equine insect bite hypersensitivity (IBH)—an allergic disease of the skin—and heaves, also known as RAO (recurrent airway obstruction)—an allergic airway disease—are the most studied atopic diseases in horses and their prevalence has distinctly increased in the last decades. Lipid mediators play a crucial role in the pathogenesis of human allergic diseases and antagonism of leukotriene and prostaglandin (PG) D_2 receptors has been shown to be effective in the treatment of asthmatic and allergic patients. However, thus far, little is known about their therapeutic value in veterinary medicine. Our main goal was to explore the role of PGD_2 and its receptors CRTH2 and DP_1 in leukocytes of allergic (IBH and RAO) and non-allergic horses. We aimed to address the crucial questions if (i) the PGD_2/CRTH2-DP_1 axis represents a potential target for new therapeutic approaches, and if (ii) alterations in the PGD_2/CRTH2-DP_1 axis may serve as a new class of biomarkers.

Methods: Assays were performed in whole blood or polymorphonuclear cells were isolated from whole blood of allergic and non-allergic horses by density gradient centrifugation. The impact of PGD_2 on eosinophil and neutrophil responsiveness was evaluated in well-established assays of shape change and chemotaxis. In some experiments, cells were pretreated with selective antagonists for DP_1 (MK-0524; 1 µM) and CRTH2 (OC000459; 300 nM). Expression pattern of the PGD_2 receptors DP_1 and CRTH2 were characterized by flow cytometry. Differential blood count was performed with a standard hematology analyzer for human and veterinary use.

Results: The DP_1 receptor is expressed on equine eosinophils and neutrophils at a basal level and enhanced expression of DP_1 was found on eosinophils from allergic horses. While CRTH2 expression was generally found at higher basal levels, both neutrophils and eosinophils from allergic horses showed an increased expression. Eosinophils and neutrophils from allergic and non-allergic horses showed no difference in their shape-change capacity in response to PGD_2 (0.1–30 nM), but showed a distinct migratory activity towards this lipid mediator. Neutrophils and eosinophils from allergic horses showed an increased chemotactic response towards PGD_2 (30 nM) and an increased expression of PGD_2 receptors. In presence of the selective CRTH2 antagonists MK-0524 and OC000459 chemotaxis mediated by PGD_2 and the selective CRTH2 agonist 13,14-dihydro-15-keto-PGD_2 (DK-PGD_2) was attenuated. Generally, neutrophils of allergic horses exhibited a hyper-migratory phenotype also in response to other chemoattractants such as interleukin-8 and leukotriene B_4.

Discussion: The increased expression of DP_1 and CRTH2 and the enhanced response of eosinophils and neutrophils to PGD_2 in allergic horses suggest that the PGD_2/CRTH2-DP_1 activation axis has a pivotal role in mediating equine allergic responses and thus may represent a potential novel therapeutic and diagnostic target.

Molecular Pharmacology, Oncology and Toxicology

A3.1

Palbociclib treatment of *FLT3*-mutant AML cells reveals a kinase-dependent transcriptional regulation of *FLT3* and *PIM1* by CDK6

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Background: Up to 30% of patients with acute myeloid leukemia (AML) have constitutively activating internal tandem duplications (ITDs) of the FLT3 receptor tyrosine kinase (FLT3-ITD) on initial diagnosis, and additional patients may acquire them on relapse. Such mutations are associated with a poor prognosis and a shortened overall survival. FLT3 tyrosine kinase inhibitors (FLT3-TKI) are being developed as targeted therapy for *FLT3*-ITD$^+$ AML; however, their use is complicated: they provide short-term disease control but relapse invariably occurs within months, illustrating the need for additional therapeutic targets. PIM protein kinases are oncogenic targets expressed in AML cells.
Methods: We used human leukemic cells with wildtype *FLT3* and *FLT3*-ITD, respectively. Cell viability was analysed in a high-throughput drug screen. The molecular mechanism was deciphered biochemically, by ChIP experiments and via FACS stainings (*e.g.* PI, annexin V/7-AAD). The clinical relevance was addressed by single agents and combinatorial strategies. We also performed mouse xenograft experiments to test whether palbociclib represses FLT3-mediated leukemia *in vivo* in immune-compromised Rag2$^{-/-}\gamma c^{-/-}$ mice. We further evaluated the effects of palbociclib in a *FLT3*-ITD$^+$ subcutaneous tumor xenograft model. *PIM1* and *FLT3* gene expression was analysed ín tumor tissues. Primary human AML biopsies were used for further validation.
Results: The FDA-approved CDK4/6 kinase inhibitor palbociclib induced apoptosis of *FLT3*-ITD$^+$ AML cells. The drug toxicity was specific for *FLT3*-mutant cells and was ascribed to the transcriptional activity of CDK6 in a kinase-dependent manner: CDK6, but not its functional homolog CDK4, was bound to the promoters of *FLT3* and *PIM1*, factors at two signaling nodes that are critical for survival of the leukemic cells. Dual targeting with palbociclib and PIM1 inhibitors or palbociclib and FLT3 inhibitors resulted in synergistic cytotoxicity.
Discussion: Targeted CDK6 inhibitors harbor the potential to suppress the relapse after remission. Concomitantly targeting two critical signaling nodes in leukemogenesis could represent a therapeutic breakthrough, overcoming/preventing therapy resistance, thereby prolonging therapeutic efficacy.
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A3.2

A comparison of the β-adrenergic receptor antagonists landiolol and esmolol: receptor selectivity, partial agonism and pharmacochaperoning actions

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Background: Blockage of β_1-adrenergic receptors is one of the most effective treatments in cardiovascular medicine. Esmolol was introduced some three decades ago as a short-acting β_1-selective antagonist. Landiolol is a more recent addition. Here, we compared the two compounds for their selectivity for β_1-adrenergic receptors over β_2-adrenergic receptors, partial agonistic activity, signaling bias and pharmacochaperoning action by using HEK 293 cell lines, which heterologously expressed each human receptor subtype.
Methods: Receptor determination on the cell surface, phosphorylation of p44/p42 mitogen-activated protein kinase/extracellular signal-regulated kinase 1 and 2 (MAP kinase; ERK1/2), ligand affinity/selectivity for the receptors, and partial agonistic activity were examined by flow cytometry, immunoblotting, radioligand binding assays and [^{3}H]cAMP accumulation assay respectively.
Results: The affinity of landiolol for β_1-adrenergic receptors and β_2-adrenergic receptors was higher and lower than that of esmolol, respectively, resulting in an improved selectivity (216-fold *vs*. 30-fold). The principal metabolite of landiolol (M1) was also β_1-selective, but its affinity was very low; hence, it is unlikely to contribute to the action of landiolol *in vivo*. Both, landiolol and esmolol caused a very modest rise in cAMP levels but a robust increase in the phosphorylation of extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2) indicating that the two drugs exerted partial agonist activity with a signaling bias. If cells were incubated for ≥24 h in the presence of ≥1 μM esmolol, the levels of β_1-adrenergic, but not of β_2-adrenergic, receptors increased. This effect was contingent on export of the β_1 receptor from the endoplasmic reticulum and was not seen in the presence of landiolol.
Discussion: Based on these observations we conclude that landiolol offers the advantage of (i) improved selectivity and (ii) the absence of pharmacochaperoning activity, which sensitizes cells to rebound effects upon drug discontinuation.

A3.3

Development of a multi-dimensional screening model to investigate the immune-modulatory effects of extractables and leachables from packaging materials

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Background: The plastic manufacturing process is accompanied by the addition of a variety of chemical compounds to achieve the designated properties of the synthetics. These additives are not bound to the polymer matrix and, therefore, become available due to migration out of the plastic. Risk assessment of migrating packaging components into final products entails significant challenges for regulators and manufacturers. Apart from the analytical requirements

associated with the identification and quantification of extractables and leachables, adverse effects on human metabolism with regard to various impacts on human health are of particular concern. This work evaluates different extraction approaches requiring individual analytical techniques for the detection of very volatile, volatile and semi-volatile compounds.

Methods: Sampling included food contact materials, PVC medical devices and house dust. For determination of volatile and semi-volatile extractables, packaging materials and product containers were directly exposed to solvents with different polarities, *e.g.* hexane, methylene chloride, *tert*-methyl butyl ether, isopropanol and water over time (2 weeks to 6 months) at room temperature and/or thermal treatment with 60°C. Solvent extracts were concentrated and injected into a 6890 gas chromatograph (GC, Agilent Technologies) coupled with a single quadrupole mass spectrometer (MS, Agilent Technologies) operated at 70 eV. Migration studies targeting volatile and semi-volatile leachables in aqueous products thermally stressed at 60°C for two weeks were conducted. Additionally, direct static-headspace sampling of packaging materials and the aqueous sample material was performed using a 7697 headspace sampler (Agilent Technologies). For identification and quantification, a screening method with 210 reference substances including volatile and semi-volatile compounds *e.g.* phthalates, polycyclic aromatic hydrocarbons (PAH), linear hydrocarbons (C8–C40), solvents and individual reference standards was established.

Results: Depending on solvent polarity, different patterns of volatile and semi-volatile compounds were extracted from packaging materials, infusion sets, dialysis tubes, syringes, glas vials with stoppers and house dust. Comparable results including material-specific components were obtained for the hexane and the ether extracts, whereas substances derived from the production process or the material composition such as butylhydroxytoluol, styrene, methyl-acrylates, diethylphthalate, diethylhexylphthalate, 2-ethyl-1-hexanol, diisobutylphthalate and 9-octadecanamide were determined. By means of direct HS-GC-MS analysis of the aqueous extract *e.g.* 2-methyl-pentane, 3-pentene-2-one and 2-octane, unknown but structurally related hydrocarbons with m/z 43, m/z 45, m/z 57, m/z 71, m/z 85, m/z 98, m/z 112 and m/z 127 were detected. Using the internal data analyses method, these compounds were also identified in the final products.

Discussion: Although phthalates were replaced by alternative plasticizers in recent years, these compounds may also enter the human body by inhalation, enteral or parenteral uptake and may exert severe side effects on hormone metabolism promoting breast cancer, infertility or allergy. These experiments serve as part of a holistic approach to effectively evaluate and minimize the risk of leachables present in final products with regard to improvements in product quality, patient safety and consumer acceptance covering the production process and life-cycle management. Further studies regarding the biological activity will be conducted, including a validated quantification of identified migration compounds together with an investigation of the dose-dependent biological effects by means of an *in vitro* cell culture model.

Neuropharmacology and Neurosciences

A4.1

Functional rescue of misfolded SLC6 transporters

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Background: The physiological role of proteins belonging to the solute carrier 6 (SLC6) family is to transport neurotransmitters, amino acids, and osmolytes such as betaine, taurine, and creatine. Their dysfunction has been linked to different neurological and psychiatric disorders. SLC6 transporters are proteins of twelve membrane-spanning helices and cytosolic amino and carboxy termini. Mutations in the coding sequences of SLC6 transporter genes are known to cause protein misfolding. The aim of our study is to characterise and rescue misfolded/dysfunctional SLC6 transporter mutants using pharmacological chaperones.

Methods: Mutations of interest were created by site-directed mutagenesis using the QuikChange™ kit (Stratagene). HEK 293 or Schneider cells were transfected with plasmids encoding the wild-type and mutant transporters using Lipofectamine 2000 (Invitrogen) and Effectene (Qiagen), respectively. Radioligand uptake, confocal laser scanning microscopy and immunoprecipitation experiments were performed to study the mutations on molecular level. dDAT-G108Q flies were treated with pifithrin-μ and noribogaine and total sleep was quantified to assess the effects of pharmacochaperoning.

Results: Mutations in *Drosophila melanogaster* DAT (dDAT) abolish dopamine uptake and lead to a sleepless phenotype in flies. We showed that a single-point mutation in dDAT (dDAT-G108Q) arises due to defective protein folding. The mutant exhibited no specific dopamine uptake in HEK 293 or Schneider cells. Moreover, dDAT-G108Q was retained in the endoplasmic reticulum (ER), which was illustrated both by confocal microscopy and co-localisation with the ER-resident chaperone calnexin. We also found that dDAT-G108Q associates with significantly higher levels of HSP70-1A and calnexin, compared to the wild-type dDAT. This interaction was markedly reduced upon treatment with noribogaine or pifithrin-μ. A combined action of the two compounds resulted in an additive effect, *i.e.* enhanced surface expression. The pharmacochaperoning action of noribogaine can be accounted for by its ability to stabilise the inward-facing conformation of DAT, whereas pifithrin-μ acts by inhibiting HSP70. Noribogaine and pifithrin-μ also restored sleep *in vivo* in dDAT-G108Q-expressing flies. Interestingly, in the human creatine transporter-1 (hCRT-1), a conservative mutation of the equivalent glycine residue (hCRT-1-G132V) is linked to severe mental retardation in children. We found that this mutant has approximately 10% of the activity of the wild-type hCRT-1, and confirmed that the dramatic loss of function is also due to protein misfolding and retention in the ER compartment.

Discussion: Evidently, this particular glycine residue plays a common, crucial role in protein folding in a variety of members of the SLC6 family. In fact, the glycine is absolutely conserved among all SLC6 transporters. It resides in the first intracellular loop, juxta-membrane to the second membrane-spanning helix, a region already known to be involved in the folding of the related serotonin transporter [1].

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A4.2

Acetylcholinesterase inhibition activity of some novel *s*-triazine derivatives

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Background: Alzheimer's disease is a chronic neurodegenerative disorder caused by overproduction and accumulation of abnormally folded amyloid beta peptide. Currently, there is no effective therapy for Alzheimer's disease, but five medicines are used to treat the cognitive disorder. Acetylcholinesterase inhibitors are one group of medicines that are used in treatment of Alzheimer's disease. Reduction in the activity of the cholinergic neurons is a well-known feature of this illness.
Methods: Six triazine derivatives were investigated using RP-HPTLC and CRP-18W/UV254 plates. Five different mobile phases were used: acetone–water (φ = 0.5–0.8; v/v), acetonitrile–water (φ = 0.5–0.9; v/v), methanol–water (φ = 0.65–0.95; v/v), 2-propanol–water (φ = 0.4–0.7; v/v), tetrahydrofuran–water (φ = 0.5–0.75; v/v), and the results were used in computational calculations in order to determine the pharmacokinetic profile of the examined molecules. Additional investigations with acetylcholinesterase of choosen triazines were performed using docking Vina.
Results: Calculated values for Pe_J (permeability in jejunum, pH = 6.5 [cm/s]), K_a (absorption rate constant [min^{-1}]) and logBB (logarithm of the blood–brain barrier partition coefficient) were correlated with R_M^0 parameters obtained by RP-HPTLC using multiple linear regression. Best results are shown in Table 1.
Discussion: It was confirmed previously that in the case of multivariate data analysis best models can be obtained by predicting Pe_J at a pH of 6.5 and therefore these permeability conditions were used. The absorption rate constant (K_a) represents the rate of absorption of a drug absorbed from its site of application, whereas K_a is usually used to express parameters for routes of application other than intravenous. As it was previously reported, *s*-triazines have the ability to act as modulators in Alzheimer's disease and therefore their entry via the BBB is crucial for expression of possible beneficial effects. All of the tested *s*-triazines showed good activity in comparison with donepezil as reference molecule but the authors gave special preference to compounds with better pharmacokinetic profiles. Six investigated compounds possess positive logBB values and good Pe_J and K_a results. One molecule is considered as a structure with excellent pharmacokinetic characteristics and better docking scores in comparison to donepezil.
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A4.3

K_V7 channels: potential targets for the antinociceptive action of paracetamol

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Background: Paracetamol/acetaminophen (APAP) is a widely used analgesic whose mechanism of action remains controversial. The postulated mechanisms include inhibition of cyclooxygenase enzymes, effects on the descending serotonergic pathways, and involvement of the endocannabinoid system through its metabolite AM404. A small fraction of paracetamol is converted into a reactive intermediate, NAPQI (*N*-acetyl-*p*-benzoquinone imine) by cytochrome P450 enzymes. The M current is characteristic of the neuronal subtypes of voltage-gated potassium channels (K_V7 family). Inhibition of M currents enhances neuronal excitability, while their augmentation causes neuronal silencing with established translational use in pain management and epilepsy. Therefore, effects of APAP and its metabolites on K_V7 channels were investigated.
Methods: tsA201 cells were transfected using plasmids coding for human K_V7 channels. Dorsal root ganglia (DRG) were dissected from 10-day-old rats and dorsal horn (DH) cultures were prepared from newborn rats. They were cultured at 37 °C / 5% CO_2 for 2 days and 21 days, respectively. Electrophysiological recordings were made using the perforated patch-clamp technique.
Results: NAPQI enhanced currents through recombinant homomeric $K_V7.2$ and $K_V7.5$ channels up to 250% and 400% of control, respectively, and inhibited currents through $K_V7.3$ homomers down to 40% of control; both effects were irreversible and concentration-dependent. With $K_V7.2/7.3$ and $K_V7.3/7.5$ heteromers, currents were enhanced to 120% and 250% of control, respectively, in a concentration-dependent manner up to 3 µM NAPQI and depressed at higher concentrations, the effect being irreversible. The tail current in DH and capsaicin-sensitive DRG neurons showed an enhancement up to 250% and 120% of control with 3 µM NAPQI, respectively. There was a significant decrease in the excitability of DRG neurons with 10 µM NAPQI. On application of 3 µM NAPQI for 10 minutes, currents through wild-type $K_V7.2$ homomers exhibited a biphasic enhancement with a plateau at 3 minutes and a further time-

A4.2: Table 1: Examined linear dependence of Pe_J, K_a and logBB values using the multiple linear regression method.

	Modifier	Equation	r^2	F	p
Pe_J	THF/H_2O	$-5.076\times10^{-5}R_M^0 + 6.390\times10^{-7}\,TPSA - 3.372\times10^{-7}MW + 1.020\times10^{-3}$	0.962	111.635	5.478×10^{-8}
K_a	THF/H_2O	$-3.301\times10^{-3}R_M^0 + 1.366\times10^{-5}\,TPSA - 2.283\times10^{-5}MW + 0.071$	0.973	159.912	1.047×10^{-8}
logBB	2-Pro/H_2O	$0.797R_M^0 + 8.780\times10^{-3}\,TPSA - 7.710\times10^{-3}MW + 1.583$	0.840	23.721	7.281×10^{-8}

Equation parameters: R_M^0: retention constant; TPSA: total polar surface area; MW: molecular weight.

dependent which reached equilibrium at 7 minutes. In a single-point cysteine mutant of $K_V7.2$ (C106A), the first phase of current enhancement with 3 µM NAPQI was abolished. The triple cysteine mutant, $K_V7.2$ (CCC150–152AAA) mutant showed a biphasic inhibition with a residual current of 20% at the end of a 10-minute period. In the other single cysteine mutants, $K_V7.2$ (C169A) and $K_V7.2$ (C492A), currents were enhanced by 3 µM NAPQI akin to wild-type channels. The observed effects were irreversible. Paracetamol (1 mM) and AM404 (10 µM) had no effect on homomeric $K_V7.2$ and $K_V7.3$ currents.

Discussion: These results indicate that the analgesic action of paracetamol may involve an enhancement of K_V7 currents by NAPQI as an active metabolite.

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A4.4

Angiotensin II potentiates NMDA currents in layer V pyramidal cells of rat prefrontal cortex

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Background: NMDA-type glutamate receptors in the prefrontal cortex (PFC) are critically involved in cognitive functions and behaviour. Angiotensin II (Ang II) has been reported to influence learning and memory in behavioural tests. The aim of the present study was to investigate the effect(s) of Ang II on NMDA receptor function in layer V pyramidal cells of the PFC by means of patch clamp technique.

Methods: Brain slices were prepared from 10–11-day-old rats, and whole-cell patch clamp access was established in layer V pyramidal cell of the PFC. The cells were continuously superfused (2.5–3 ml/min) with artificial cerebrospinal fluid (aCSF). NMDA (30 µM), applied 3 times for 1.5 min with 10-min intervals between applications, induced inward currents ($T_{1–3}$). Ang II (or in some experiments Ang IV) was applied 5 min before and during T_3. If receptor antagonists or tetrodotoxin were tested, they were present in the aCSF during the whole experiment. In some experiments Ca^{2+} was omitted from the aCSF. The effects at T_3 were presented as T_3/T_2 ratio. More than ±15% change at T_3 compared to T_2 was considered as an effect. The T_3/T_2 ratios were summarized as mean ± SEM of *n* experiments. One-way ANOVA followed by Bonferroni's *t*-test were used for statistical analysis; $p < 0.05$ was considered statistically significant.

Results: Ang II (0.003–1 µM) significantly potentiated the NMDA currents in a subpopulation (30–50%) of the pyramidal cells. This potentiation was reversed by the AT_1 receptor antagonist eprosartan (1 µM), while the AT_2 receptor antagonist PD 123319 (5 µM) failed to influence the Ang II–NMDA interaction. Synaptic isolation of pyramidal neurons by a Ca^{2+}-free medium or tetrodotoxin (0.5 µM) abolished the augmentation of NMDA currents by Ang II. The dopamine D_1 receptor antagonist SCH 23390 (10 µM) interfered with the Ang II-induced potentiation, while the D_2 receptor antagonist sulpiride (20 µM) failed to influence it. In a subset of pyramidal neurons (30–35%) NMDA responses were significantly reduced in response to high concentrations of Ang II (1–3 µM). These effects were not influenced by Ang II receptor (AT_1 or AT_2) antagonists but were mimicked by Ang IV (0.1 µM). Ca^{2+}-free medium or tetrodotoxin failed to influence these inhibitory effects.

Discussion: Ang II administration resulted in dual effects on the NMDA receptors in layer V pyramidal cells of the rat PFC. At lower concentrations (0.003–1 µM) it potentiated the NMDA currents in a subpopulation of these cells. This stimulatory effect could be reversed by the AT_1 receptor antagonist eprosartan, the D_1 receptor antagonist SCH 23390 as well as by synaptic isolation of pyramidal neurons. It suggests that the activation of AT_1 receptors occurs on interneurons resulting in dopamine release from these neurons and this dopamine, by activating D_1 receptors, potentiates the NMDA currents in layer V pyramidal cells. Inhibition of NMDA currents in response to high concentration of Ang II (1–3 µM) in another subpopulation of the pyramidal neurons may be the consequence of conversion of Ang II to Ang IV.

A4.5

Elucidating the mechanism for low-efficacy substrate efflux at the human serotonin transporter

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Background: Amphetamines and their congeners induce serotonin and dopamine efflux from presynaptic neurons through the serotonin transporter (SERT) and the dopamine transporter (DAT), respectively. Pathological consumption of amphetamine leads to drug abuse and addiction. In an attempt to design medications with low abuse potential, some compounds belonging to phenethylamine library (PAL) were recently synthesized exhibiting low efficacy in inducing neurotransmitter efflux through SERT and DAT as compared to amphetamines, though mechanistic explanation for partial efflux is unknown. We hypothesize that these 'partial releasers' trap SERT and DAT in specific conformational states, a question we address using an electrophysiological approach to investigate the effect of PAL compounds on the transport cycle of SERT.

Methods: Conformational changes in SERT and DAT on substrate binding can be inferred from analysis of substrate-induced currents carried through the transporters. These currents through SERT are comprised of two components: (i) peak currents reflecting substrate-induced charge movement, and (ii) steady-state currents indicating Na^+-conducting state associated with K^+-bound inward-facing conformation visited by the transporter during transport cycle. These currents were measured by whole-cell patch clamping of HEK 293 cells stably expressing human SERT. Currents induced by PAL-1045 (a partial releaser for SERT and DAT) were compared to currents induced by serotonin (5-HT) and the 'complete releasers' PAL-287, PAL-1046 and *para*-chloroamphetamine (pCA).

Results: The amplitudes of steady-state currents through SERT decreased with application of increasing concentrations of PAL-287, PAL-1045 and PAL-1046, indicative of a biphasic concentration response. This suggests that PALs readily diffuse through the cell plasma membrane and display high affinity to both the outward- and inward-facing conformation of SERT. This was further confirmed when these steady-state currents were rescued by lowering the pH of the external bath solution from 7.4 to 5.5, a condition that decreases membrane diffusibility of PALs. Substrate-induced peak currents under different internal pipette conditions were used as kinetic read-outs for on- and off-rates of the PALs from the outward-open conformation of the transporters. Subsequent data from these experiments point to the partial releaser PAL-1045 having comparable on-rates to, but considerably slower off-rates from, the

outward-open conformation of SERT when compared to the complete releasers PAL-287 and PAL-1046.

Discussion: In their unprotonated state, PAL compounds readily diffuse through the membrane and bind with high affinity to both the inward- and outward-facing conformation of SERT. Tight binding of the partial releaser PAL-1045 to SERT, owing to fast on-rates and slow off-rates, slows down the transport cycle and precludes 5-HT binding to transporters in the inward-open conformation. This accounts for a reduction in substrate efflux and partial release.

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A4.6

Exocyst-dependent trafficking of the wild-type dopamine transporter

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Background: The transfer of material between organelles is mediated by carrier vesicles. Each vesicle transport reaction can be divided into four essential steps: vesicle budding, transport, tethering, and fusion. The exocyst is a multiprotein complex required by many membrane proteins for delivery to and insertion into the plasma membrane. Uptake through the dopamine transporter (DAT) represents the primary mechanism used to terminate dopaminergic transmission in the brain. However, little is known about the specialized trafficking of DAT towards the target membrane. DAT requires an intact C-terminal PDZ-binding motif to reach the cell surface, whereas the closely related serotonin transporter SERT does not. Here, we tested the hypothesis that DAT requires the exocyst for reaching the cell surface.

Methods: HEK 293 or CAD cells were transiently co-transfected with plasmids encoding the wild-type dopamine transporter (DAT) and serotonin transporter (SERT) along with different components of the exocyst, *i.e.* Exo70, Sec6 and Sec8, using jetPRIME (Polyplus). Radioligand uptake, confocal laser scanning microscopy and immunoprecipitation experiments were performed 48 h after transfection to study the effect of exocyst components on trafficking of DAT and SERT.

Results: DAT relied on the exocyst to reach the cell surface. Surprisingly, SERT did not require the exocyst complex to reach the cell surface, regardless of whether the experiments were performed in HEK 293 cells (a cell line of fibroblast origin) or in CAD cells (a Cath.a-cell-derived line of neuronal origin) membrane. We found that three components of the exocyst complex, Sec6, Sec8 and Exo70, separately control trafficking of DAT. Immuno blots also showed the effect of exocyst components on trafficking of DAT as compared to SERT as control.

Discussion: The exocyst mediates DAT targeting to the presynaptic membrane. Identification of proteins as DAT-interactors along with the molecular bases and physiological significance of such interactions will result in a better understanding the role DAT plays in regulating dopamine homeostasis in the brain.

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A4.7

First-in-class treatment of negative symptoms of schizophrenia

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Background: Cariprazine is an orally active and potent dopamine D_3/D_2 receptor partial agonist with preferential binding to D_3 receptors and partial agonist at serotonin 5-HT_{1A} receptors. Cariprazine is the first and only atypical antipsychotic that demonstrates this balanced dual engagement of the D_3 and D_2 receptor systems, which, next to its antipsychotic effect may confer additional benefits such as improvement in negative symptoms and enhanced cognition. Cariprazine therefore was developed in the EU for the treatment of schizophrenia and for the treatment of schizophrenia with predominant negative symptoms.

Methods: The cariprazine schizophrenia program included three short-term studies in acute exacerbations, one long-term maintenance-of-effect study, one special clinical study in patients with predominant negative symptoms of schizophrenia, and two long-term safety studies. The primary and secondary efficacy parameters in the short-term studies were change from baseline to end in the Positive and Negative Syndrome Scale (PANSS) total score and Clinical Global Impressions – Severity scale (CGI-S) score, respectively, compared to placebo. The primary efficacy parameter in the maintenance-of-effect study was the time to first relapse during the double-blind period in comparison to placebo. The primary and secondary efficacy parameters in the predominant-negative-symptom study were change from baseline to end in the PANSS factor score for negative symptoms (PANSS-FSNS) and in the Personal and Social Performance Scale (PSP), respectively, in comparison to risperidone.

Results: In each of the three short-term studies significant improvements were seen for cariprazine relative to placebo in the primary and secondary efficacy parameters, using analysis of covariance (ANCOVA) with last observation carried forward (LOCF) and mixed-effects model for repeated measures (MMRM) methods to fill the missing endpoint. Cariprazine demonstrated robust efficacy in maintaining the antipsychotic effect: by the end of the double-blind period 47.5% of placebo-treated patients and 24.8% of cariprazine-treated patients had a relapse of schizophrenia symptoms. Cariprazine demonstrated robust efficacy on predominant negative symptoms of schizophrenia in a dose range of 3–6 mg, with a target dose of 4.5 mg: There was a statistically significant difference ($p = 0.002$) in favour of cariprazine over risperidone for the primary efficacy parameter PANSS-FSNS and for the secondary efficacy parameter PSP ($p < 0.001$). The most common adverse events were akathisia, insomnia and headache; however, the last two occurred with similar frequency also in the placebo groups. Cariprazine had a favorable safety profile considering prolactin levels, weight gain and hematological parameters.

Discussion: Overall, the efficacy data gained show that cariprazine improves a broad range of schizophrenic symptoms in all stages of schizophrenia and has a similar safety profile compared to other marketed antipsychotics. However, cariprazine is unique in treating predominant negative symptoms of schizophrenia, a first-in-class approach in a field where there are no available therapies at the moment and which is considered to be a high unmet medical need, as it represents a burdensome and disabling disease for patients, caregivers and society.

A4.8

Neutralization of the voltage sensor in domain I strongly affects activation and inactivation gating in $Ca_V1.2$

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Background: Voltage-gated calcium influx through $Ca_V1.2$ regulates numerous cellular functions. These channels have four S4 segments in domains I–IV carrying between 5 and 6 charges, respectively. Here, we ask whether IS4–IVS4 differentially affect activation and inactivation of $Ca_V1.2$.
Methods: To probe their role in $Ca_V1.2$ gating processes we partially or completely replaced arginines and/or lysines in IS4–IVS4 by glutamines. HEK 293 cells were co-transfected with cDNAs encoding wild-type or mutant $Ca_V1.2$ α1 subunits with auxiliary β3a as well as α2–δ1 subunits. Currents were measured in "high barium" (20 mM) extracellular solution. Activation/inactivation of barium inward currents was analyzed using the patch clamp technique.
Results: Charge-neutralising point mutations in S4 segments shift the activation and inactivation curves by similar amounts on the voltage axis. Regression analysis of half-activation voltage *vs.* half-inactivation potential exhibits significant correlation ($r = 0.95$, $p < 0.015$). Substitution of charged residues in IS4 induced the strongest shifts of the activation/inactivation curves. $Ca_V1.2$ carrying only one charge in lower position (R4) opens at more negative potentials. Increasing the number of charged residues until four gradually moved the activation curve towards more depolarized voltages. Compared to domain I, mutations in domain II did not substantially affect the voltage dependence. Neutralization of the upper charge in IVS4 (R1: R1359Q) shifted the activation and inactivation curves like the IS4N+4 (K264Q) mutant (equal right shift of about 13.5 mV) while double mutants (***e.g.*** R1359Q/R1362Q) failed to form functional channels.
Discussion: Our data support the hypothesis that activation and inactivation in $Ca_V1.2$ are coupled processes. We hypothesise also that IS4 strongly stabilizes the closed and inactivated states, suggesting a key role of this segment in $Ca_V1.2$ activation and inactivation gating.
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A4.9

Swapping the selectivity of ring-substituted methcathinones for human dopamine and serotonin transporters

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Background: Dopamine and serotonin transporters (DAT and SERT, respectively) are members of the solute carrier 6 (SLC6) transporter family. Both are associated with a number of human disorders and, therefore, are important targets for clinically relevant therapeutics. In addition, DAT and SERT are directly involved in the action of psychostimulant drugs, including cathinones and their methylated derivatives. Methcathinone (MCAT) and congeners are structurally related to amphetamine-type drugs, which are characterized by high abuse potential. Evaluation of a large set of analogs showed that adding a bulky chemical group to the 4-position of the phenyl ring can change the selectivity between DAT and SERT in a synaptosomal release assay [1]. In particular, the serine in position 149 (S149) of the human DAT and the alanine in position 169 (A169) in the human SERT were proposed to act as key amino acids in the selectivity of these compounds [1,2]. However, no functional studies are present in the literature to corroborate this hypothesis. Therefore, we investigated the importance of both amino acids for the selectivity of 4-MCATs.
Methods: Sequence alignments indicate that residue S149 in hDAT is homologous to A169 in hSERT. Therefore, we decided to generate mutations to exchange both amino acids and test whether this change swapped transporter selectivity among 4-MCATs. The transporter mutants were transiently or stably expressed in HEK 293 cells to test the potency of these compound in uptake inhibition assays with the following 4-MCATs: MCAT, 4-OCH_3MCAT, 4-BrMCAT, 4-CF_3MCAT. In parallel, pharmacoinformatic approaches were used to dock the compounds in both the wild-type and mutant transporters. The combined approaches will help to examine the selectivity of 4-MCATs.
Results: A robust selectivity profile is maintained between the rat and the human transporters: for all the compounds tested, the DAT selectivity (calculated as SERT EC_{50}/DAT EC50 or as SERT IC_{50}/DAT IC_{50}) conforms with previous data obtained from a synaptosomal release assay [1]. The only exception was seen with 4-OCH_3MCAT. Surprisingly, no difference was found between the wild-type and mutant transporters for all the 4-MCATs tested, both in hDAT and hSERT. This suggests that the amino acids S149 in hDAT and A169 in hSERT are, on their own, not sufficient to determine selectivity of 4-MCAT analogs.
Discussion: Our results clearly show that swapping S149 of hDAT and A169 of hSERT is not sufficient to exchange the selectivity of 4-MCATs. This suggests that other residues, or more likely a combination of residues, are involved. Our conclusion is supported by pharmacoinformatics which suggests alternative mutations that can be combined with the mutations tested so far. Moreover, since the selectivity of 4-OCH_3MCAT does not agree with previous data obtained in rat brain synaptosomes, we plan to also investigate possible species differences. Our study aims at elucidating the structural basis for compound selectivity at DAT and SERT, which may provide important information for the synthesis of new therapeutic and possibly more selective compounds.
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A4.10

Somatostatin receptor subtype 4 (sst_4) regulates stress and depression-like behaviours in mouse models

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Background: Extensive evidence suggests a role of the inhibitory neuropeptide somatostatin released from a population of GABAergic interneurons in stress-regulation, anxiety and depression. However, very little information is available about its receptors ($sst_{1–5}$) mediating these effects. The sst_4 receptor is not involved in the endocrine actions of somatostatin, but it has potent anti-inflammatory and analgesic functions proposing drug development perspectives. Since it is expressed in several mood- and emotion-related brain areas, we investigated its role in stress regulation.

Methods: The role of the sst_4 receptor in the responses to acute and chronic stressors was examined with wild-type (*Sstr4*$^{+/+}$) and *Sstr4*-gene-deleted (*Sstr4*$^{-/-}$) mice, as well as with the selective agonist J-2156. Anxiety in acute stress situations was analysed in the elevated plus maze (EPM), while depression-like behaviour (immobility) was determined in the tail suspension (TST) and forced swim tests (FST). In a mild chronic variable stress (CVS) model, anhedonia was examined by the sucrose preference test (SPT), anxiety in the light–dark box test (LDB), while depression-like behaviour in the TST and FST. Endocrine responses to CVS were also investigated. Acute neuronal activation following TST was determined with Fos, while chronic neuronal activation in response to CVS with FosB immunohistochemistry in stress-related brain areas. Expression of sst_4 in the amygdala was detected using sst_4^{LacZ} immunostaining in *Sstr4*$^{-/-}$ mice.

Results: Anxiety in the EPM and depression-like behaviour in the FST were significantly greater in *Sstr4*$^{-/-}$ mice compared to wild types. J-2156 exerted an anxiolytic effect in the EPM and an antidepressant-like action in the TST. J-2156 enhanced the TST-induced Fos response in several brain areas such as the central (CeA) and basolateral amygdala (BLA), which is supported by strong sst_4^{LacZ} immunopositivity in these regions. *Sstr4*$^{-/-}$ mice showed greater susceptibility to mild CVS: it increased light preference in the LDB in wild-type mice, but not in *Sstr4*$^{-/-}$ ones. Immobility of *Sstr4*$^{-/-}$ mice in the TST increased after the CVS, and their greater baseline immobility compared to wild types decreased. However, anhedonia did not develop in this model and *Sstr4* deletion did not influence this parameter. CVS increased the adrenal weight to a greater extent in *Sstr4*$^{-/-}$ mice than in *Sstr4*$^{+/+}$ ones. The baseline plasma corticosterone concentration of *Sstr4*$^{-/-}$ animals was higher than in wild types, but it was not affected by the CVS. Expression of the chronic neuronal activation marker FosB increased in the CeA and BLA of *Sstr4*$^{-/-}$ mice following the CVS, but it did not change in these brain regions of wild types.

Discussion: These are the first data demonstrating that activation of the sst_4 receptor exerts anxiolytic and antidepressant-like effects in acute stress situations, as well as complex regulatory actions on chronic stress-induced behavioural and neuroendocrine alterations. The sst_4 receptor is present in the mouse CeA and BLA, where both its genetic deletion and selective activation influence acute and chronic neuronal responses to stress. These data suggest that sst_4 receptors in the amygdala play an important role in stress regulation.

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A4.11

Ca^{2+} transients measured in different hippocampal GABAergic interneurons using two-photon laser scanning microscopy

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Background: Althoug the morphology and physiology of different GABAergic interneurons (INs) have been extensively investigated, much less is known about their axonal properties. In particular, Ca^{2+} dynamics at individual bouton level have not been studied in anatomically indentified GABAergic INs, although it is known that the invasion of action potential (AP) transiently opens voltage-dependent Ca^{2+} channels resulting in transmitter release.

Methods: We combined patch-clamp recording in whole-cell mode with two-photon scanning microscopy to record Ca^{2+} signaling of dendrites and boutons of INs in response to somatic stimulation [1]. Through a patch pipette, INs were filled with high- or low-affinity Ca^{2+}-sensitive dyes (OGB-1, OGB-6F) and were labelled by biocytin for the purpose of *post hoc* anatomical identification. Grouping of INs occured due to their localization, axon distribution and firing properties.

Results: Based on their electrophysiologycal and morphologycal properties, INs were classified into three subgroups which were as follows: non-fast-spiking INs (NFS), dendritic-fast-spiking INs (DFS) and perisomatic-fast-spiking INs (PFS). Our results revealed a significant difference in the amplitude and the time course of Ca^{2+} transients between dendrite and *en passant* boutons of NFS INs; the amplitude of Ca^{2+} transients was much higher and the time course was faster in boutons. The same properties of boutonal Ca^{2+} transients were also significantly different in distinct IN types. The unperturbated values of $\Delta[Ca^{2+}]$ evoked by a single AP were 565, 214 and 147 nM in NFS, DFS and PFS INs, respectively (in more detail: [2]) and the collapse of the transients was sharper in DFS and PFS cells. It was also determined that the APs invade through the whole axonal arbor whithout being stuck in branching points, and lead to elevations in $[Ca^{2+}]$ in almost all *en passant* boutons.

Discussion: GABAeric INs play a pivotal role in controlling the activity of pyramidal cells, which are the major players in mutiple central nervous system disorders. Any drug that is able to boost the function of these INs could hold preventive or curative promises for a great variety of disorders.

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A4.12

Valerenic acid serves as a scaffold for novel $GABA_A$ receptor-modulating anticonvulsants derived from a ligand-based pharmacophore model

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Background: Valerenic acid (VA) is a sesquiterpenoid from the common valerian, a prevalently used herbal medicinal plant and displays β2/3 subunit-selective $GABA_A$ receptor-modulating properties. VA exerts anxiolytic and anticonvulsive effects without concomitant sedation combined with a promising pharmacokinetic profile, thus making this compound an interesting drug candidate. Despite recent progress in the total synthesis of VA, alternative compounds are of high interest as they provide a straightforward access towards development of novel drugs. This study focuses on the synthesis of simplified molecules maintaining subunit-selective properties based on the VA scaffold and their *in vitro* and *in vivo* characterization.
Methods: A small-focused library of novel, simplified VA analogues was suggested by a pharmacophore model based on the known β2/3 subunit-selective $GABA_A$ modulators VA and loreclezole. Their effect on GABA-induced chloride currents (I_{GABA}) through $GABA_A$ receptors composed of α1β1-3γ2S subunits expressed in *Xenopus laevis* oocytes was analysed by means of the two-microelectrode voltage clamp technique. Anticonvulsant activity was assessed by means of the pentylenetetrazole test (PTZ) conducted in C57BL/6N mice.
Results: Efficacy of I_{GABA} enhancement by derivatives AR-013, AR-016, SM-226-1 and SM-408-1a was comparable to that of VA, while a slightly reduced potency was observed. Compound SM-408-1b displayed significantly increased potency and efficacy compared to VA on α1β3γ2S receptors while activity on α1β1γ2S receptors was dramatically decreased similar to VA. PTZ-induced seizure threshold was shifted by SM-408-1b at concentrations of 0.1 mg/kg body weight indicating more potent anticonvulsant activity compared to VA. The other studied compounds were either less efficacious or did not display significant potentiation of I_{GABA} at concentrations ≥30 µM.
Discussion: By using a ligand-based pharmacophore model, novel, simplified structures with β2/3 selectivity comparable to that of VA were identified. One compound, SM-408-1b, maintained subunit selective properties of VA and may therefore serve as a starting point for the development of novel, selective $GABA_A$ receptor-modulating anticonvulsants.
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A4.13

Temporal lobe epilepsy and dynorphin: development of a gene therapy

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Background: Focal epilepsies are the most frequent type of adult onset epilepsies and pose a persistent challenge in medicine. Especially sclerotic types of mesial temporal lobe epilepsy (mTLE) display a high incidence of drug resistance. Certain patients benefit from surgical removal of the epileptogenic focus; however, a large cohort of patients cannot be successfully treated. Therefore improved treatment options are urgently needed. The importance of several neuropeptides as neuromodulators and potential antiepileptics is widely acknowledged; however, peptides hardly cross the blood–brain barrier. Considering this, the aim of this project was to investigate the effect of permanent over-expression of pro-dynorphin (pDyn) in a model of refractory TLE.
Methods: TLE was induced in male C57BL/6N mice by local injection of 1 nmol of kainic acid (KA) into the CA1 region of the left dorsal hippocampus. As in human mTLE, this model features hyperexcitability, cell loss, sprouting of mossy fibers, pharmacoresistance as well as spatial memory impairment. A recurrent pattern of epilepsy-like activity is established within 3 to 4 weeks after KA injection. rAAV serotype 1 vectors, either expressing pDyn or GFP, were injected into the epileptogenic focus at different time intervals after KA or into the dorsal hippocampus of naive animals in control experiments. An EEG setup was used to study seizure-like activities in mice overexpressing pDyn or GFP in the epileptogenic focus. Histological, neurochemical changes and pDyn expression were studied applying immunochemistry or *in situ* hybridization. Micro-dialysis was used to confirm the release on-demand of the viral vector-derived dynorphin. A panel of behavioral tests was performed to investigate learning and spatial memory, stress-coping ability, anxiety and depression-like behaviors.
Results: Over-expression of dynorphin in the epileptogenic focus, resulted in a marked decrease in hippocampal paroxysmal discharges, but also in a reduction of the number of generalized seizures. In contrast, the rAAV1-GFP control group displayed ongoing epileptic-like activity. Our data show that pDyn is expressed by neurons, is stimulation-dependently released, and does not induce an inflammatory response. Animals injected at a sub-chronic stage of epilepsy (*i.e.* 1 or 2 weeks after KA) with rAAV1-GFP or rAAV1-pDyn present no changes in anxiety, stress coping and depression-like behaviors. However, rAAV1-GFP-injected mice, like untreated epileptic mice and human patients, displayed impaired spatial memory and learning. In contrast, animals over-expressing pDyn showed normal spatial memory and learning up to 6 months after KA. In non-epileptic control animals the over-expression of pDyn or GFP, induced no alterations of anxiety, stress-coping and depression-like behaviors.
Discussion: Our data provide proof of principle that pDyn over-expression is able to rescue electrophysiological and functional characteristics and deficits observed in a drug-resistant TLE model. The long-term goal of our studies is to develop the preclinical model into a gene therapy for patients suffering from refractory mesial temporal lobe epilepsy and potentially other types of intractable, focal epilepsies.

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A4.14

A structural motif that may serve as sealing of the outer vestibule of voltage-gated Na^+ and Ca^{2+} channels

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Background: In voltage-gated Na^+ and Ca^{2+} channels the extracellular part of the pore is lined by infolding loops connecting transmembrane segments V and VI of each of the four domains ("P-loops"). A highly conserved part of the P-loops is a ring of four tryptophan residues, each contributed by one of the four domains. The crystal structure of the bacterial voltage-gated Na^+ channel Na_VAb suggests that these residues establish hydrogen bonds with amino acids of a neighbouring subunit thereby stapling together adjacent subunits at the selectivity filter. Recently, we have shown that replacement of the domain-IV tryptophane in the rat skeletal muscle Na^+ channel $rNa_V1.4$, W1531, by glycine produces an external access pathway for charged local anesthetics to the receptor site located at the internal cavity. This pathway is conductive both for ions and for larger organic molecules [1]. This finding supports a role of the ring of tryptophans in structural stabilization of the external vestibule as suggested by the Na_VAb crystal structure [2]. However, as opposed to the homotetrameric assembly of Na_VAb, eukaryotic Na^+ channels are composed of four non-identical domains. Thus, we wanted to explore whether mutations of the tryptophans homologous to $rNa_V1.4$-W1531 in domains I–III had similar effects as $rNa_V1.4$-W1531G.

Methods: To this end we expressed the mutations W402G, W756G and W1239G in tsA201 cells using transient transfection, and tested the mutations by means of the whole-cell patch clamp technique.

Results: Unlike wild-type channels, all tested mutations were blocked by external application of the membrane-impermeable derivative of lidocaine, QX222. This suggests that the tested mutations created an external access pathway for QX222 to its binding site in the internal cavity of the channel.

Discussion: Thus, similar to the prokaryotic Na_VAb the ring of tryptophans may serve to stabilize the structure of the external vestibule in eukaryotic Na^+ channels.

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A4.15

Derivatives of the class I antiarrhythmic agent sparteine act as irreversible Na^+ channel blockers

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Background: Blockers of voltage-gated Na^+ channels are in clinical use mainly as antiepileptics, antiarrhythmics and as local anesthetic agents (LAs). The use of these drugs as LAs is frequently limited by short duration of action and systemic toxicity. Such limitations could be overcome by agents that give rise to irreversible block of neuronal Na^+ channels. Here we show that attachment of aromatic residues at position 2 of the class I antiarrhythmic agent sparteine (Fig. 1) gives rise to long-lasting inhibition of Na^+ currents.

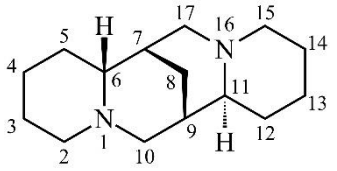

Figure 1: Sparteine

Methods: The compounds were tested by means of whole-cell patch clamp technique (manually as well as using an automated device, Cytopatch™) on tsA201 and HEK 293 cells, transfected with cardiac, neuronal, skeletal-muscle and brain isoforms of voltage-gated Na^+ channels. Na^+ currents were evoked by 20 ms depolarizations to −20 mV at 2 Hz from a holding potential of −140 mV.

Results: At a concentration of 300 µM, sparteine reduced currents through $Na_V1.4$ channels by ~20%. Block developed over 20 s and was completely reversible upon washout. By contrast, with all tested sparteine derivatives block developed over several minutes. With aliphatic substitutions at position 2, application of 10 µM produced a slowly reversible current reduction of ~10%. However, with aromatic substitutions at position 2, application of 10 µM gave rise to complete current reduction which could not be removed by a 20 min washout phase. No obvious isoform preference of the tested compounds could be detected.

Discussion: We conclude that attachment of aromatic residues as position 2 of sparteine produces irreversible blockers of voltage-gated Na^+ channels. Clinically, such agents could be used as long-lasting LAs.

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A4.16

Control of sensory neuron excitability by serotonin involves $5\text{-}HT_{2C}$ receptors and calcium-activated chloride channels

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Background: Serotonin (5-HT) is a constituent of the so-called "inflammatory soup" that sensitizes nociceptors during inflammation. Nevertheless, receptors and signalling mechanisms that mediate an excitation of dorsal root ganglion (DRG) neurons by 5-HT remain controversial. Therefore, capsaicin-sensitive nociceptive neurons dissociated from rat DRGs were used to investigate effects of 5-HT on membrane excitability and currents through ligand- as well as voltage-gated ion channels.
Methods: Using the perforated patch clamp technique in voltage- and current-clamp mode on primary cultures of rat DRG neurons, effects of 5-HT receptor ligands on membrane potential and excitability, as well as their effects on currents through K_V7, TTX-sensitive Na^+ channels, TRPV1, and Ca^{2+}-activated Cl^- channels were investigated.
Results: In 58% of the neurons tested, 5-HT increased action potential firing, an effect that was abolished by the 5-HT_2 receptor antagonist ritanserin, but not by the 5-HT_3 antagonist tropisetron. Unlike other algogenic mediators, such as PGE_2 and bradykinin, 5-HT did not affect currents through TTX-resistant Na^+ channels or K_V7 K^+ channels. In all neurons investigated, 5-HT potentiated capsaicin-evoked currents through TRPV1 channels, an effect that was attenuated by antagonists at 5-HT_{2A} (4F 4PP), 5-HT_{2B} (SB 204741), as well as 5-HT_{2C} (RS 102221) receptors. 5-HT triggered slowly arising inward currents in 53% of the neurons. This effect was antagonized by the 5-HT_{2C} receptor blocker only, and the current was prevented by an inhibitor of Ca^{2+}-activated chloride channels (CaCC). The 5-HT-induced increase in action potential firing was also abolished by this CaCC blocker and by the TRPV1 inhibitor capsazepine. Amongst the subtype-selective 5-HT_2 antagonists, only RS 102221 (5-HT_{2C}-selectively) counteracted the rise in action potential firing elicited by 5-HT.
Discussion: These results show that 5-HT excites DRG neurons mainly via 5-HT_{2C} receptors which concomitantly mediate a sensitization of TRPV1 channels and an opening of CaCCs.

A4.17

The role of SERT N-terminus in amphetamine-induced efflux

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Background: The serotonin transporter (SERT) mediates the re-uptake of 5-HT from the synaptic cleft and thus replenishes vesicular stores by operating in tandem with the vesicular monoamine transporter. Drugs such as amphetamines induce a reverse transport or efflux of 5-HT. Truncation of the N-terminus of SERT or its tethering to the membrane diminishes amphetamine-induced reverse transport. Here we explored the mechanistic basis for the role of the N-terminus in supporting amphetamine-induced reverse transport.
Methods: Membranes were prepared from HEK 293 cells stably expressing SERT (N-terminally tagged with YFP) and incubated in buffers of different ionic composition to stabilize the outward or inward conformation and in the absence and presence of para-chloroamphetamine (pCA). These membranes were subjected to limited tryptic digestion. Removal of the N- and C-termini was tracked by immunoblotting for GFP and a monoclonal antibody directed against a C-terminal epitope of SERT, respectively. In addition, mutant versions of SERT were generated, in which the N-terminus was truncated by 22, 32 and 42 residues (SERT-Δ22, -Δ32 and -Δ42). These were transiently expressed in HEK 293 cells to examine their ability to transport substrate in the forward and reverse mode and to bind inhibitors. Finally, the transport cycle was explored in real time by measuring substrate-induced capacitive currents and transport-associated currents in the whole-cell patch clamp configuration.
Results: In the presence of pCA, the N-terminus of SERT was protected against proteolytic cleavage by trypsin, indicating that binding of amphetamines to the hydrophobic core changes the conformation of the N-terminus. After transient expression, SERT-Δ22, -Δ32 and -Δ42 did not differ in their ability to support inward transport of 5-HT and in their ability to bind radiolabelled imipramine. However, amphetamine-induced release of preloaded serotonin was significantly reduced in SERT-Δ32 and -Δ42. The electrophysiological recordings indicated that SERT-Δ32 was less likely to operate in the reverse mode than SERT-Δ22 or wild-type SERT.
Discussion: The observations are consistent with the lever arm hypothesis, which posits that (i) amphetamine-induced release relies on the oligomeric structure of the transporter, and that (ii) the communication between the individual moieties is achieved by the N-terminus.
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A4.18

The role of hydrogen sulfide in autonomic nervous system

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Background: Hydrogen sulfide (H_2S) is a toxic gas also produced in mammalian tissues where it can exert various functions as gasotransmitter, such as opening of smooth muscle K_{ATP} channels resulting in vasorelaxation. Recently, H_2S was found to be synthesized and released in sympathetic ganglia and to potentiate ganglionic transmission [1], but the underlying mechanisms remained unclear.
Methods: Primary cultures of rat superior cervical ganglion (SCG) were used to determine release of previously incorporated [^{3}H]noradrenaline and to measure membrane potential and ion currents via the perforated patch clamp technique.
Results: In radiotracer release experiments, basal tritium overflow as well as outflow triggered by either electrical fields or depolarizing K^+ concentrations were enhanced by 0.1 to 1 mM of the H_2S donor NaHS in a concentration-dependent manner. In electrophysiological experiments, NaHS hyperpolarized the SCG membrane potential and reduced action potential firing, probably by direct activation of K_{ATP} channels. Supporting this hypothesis, we found that pre-inhibition of K_{ATP} channels attenuated the NaHS effect on action potential firing and membrane potential. In SCG neurons, hyperpolarization of membrane potential can be caused as well by an enhancement of currents through K_V7 channels [2]. Unexpectedly, NaHS inhibited currents through K_V7 channels in a concentration-dependent manner, whether endogenously expressed in SCG neurons or heterologously expressed in tsA cells. In addition, since H_2S potentiates ganglionic transmission [1] we studied the possible effect of NaHS on cholinergic miniature excitatory postsynaptic currents (mEPSC) in long-time cultured SCG neurons and found that NaHS increased their frequency, thus indicating an increase in the probability of acetylcholine release.
Discussion: These results show that H_2S hyperpolarizes SCG neurons and reduces membrane excitability. Since diazoxide, a K_{ATP} channel opener, shared this action and K_{ATP} blockers prevented the

effects of H_2S, we conclude that the effect on membrane excitability was caused by an opening of K_{ATP} channels. In addition, H_2S inhibited K_V7 channels and increased the frequency of cholinergic mEPSCs. These excitatory actions most likely underlie the previously observed increase in ganglionic transmission.

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A4.19

Glycine transporter 1 inhibitors may exert neuroprotective effects in hypoxic retina

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Background: Glutamate released from photoreceptors and bipolar neurons exerts its effect on various glutamate receptors in the neural circuitry of the retina. Thus, GluN1/GluN2A-type N-methyl-D-aspartate (NMDA) receptors may be present in the synapses of ON and OFF bipolar and ganglion cells, whereas extrasynaptic GluN1/GluN2B-type NMDA receptors are expressed in ON ganglion cells. GluN1/GluN2A and GluN1/GluN2B receptors, using D-serine and glycine as co-agonists, mediate neuroprotection and neurodegeneration, respectively. In the retina, glycine is involved in synaptic glycinergic and NMDA receptor-mediated glutamatergic neurotranmission and it may also diffuse into the extrasynaptic space by spillover mechanisms activating extrasynaptic GluN1/GluN2B receptors. As extrasynaptic glycine concentration is known to be controlled by nonsynaptic glycine transporter 1 (GLYT1), we have studied the regulatory role of this carrier in energy-deprived retina in which an excessive amount of glycine is released pathologically, activating GluN1/GluN2B receptors and thereby leading to retinal neurodegeneration.

Methods: Chickens or rats were anesthetized, decapitated, the eyeballs were removed and the posterior eyeballs containing the retinae were used. The eyeballs were loaded with [^{3}H]glycine, and its release and that retained in the tissue were determined by the superfusion technique. Oxygen and glucose deprivation (OGD) was used for stimulation of [^{3}H]glycine release; drugs were added to the superfusion buffer. In some experiments, the eyeballs with the retinae were immersion-fixed, embedded and sectioned. The retinae were stained with hematoxylin-eosin, or immunocytochemical labeling was carried out with goat anti-GLYT1 primary antibody and conjugated donkey anti-goat IgG applied as a secondary antibody.

Results: (1) OGD reduced [^{3}H]glycine uptake and evoked an increase in [^{3}H]glycine release in the retina. This release was independent on external Ca^{2+}. (2) The OGD-induced [^{3}H]glycine release was inhibited by sarcosine-containing (NFPS, Org-24461) and non-sarcosine-based GLYT1 inhibitors (Merck 13-h, SSR-504734). The release inhibition related to the GLYT1-inhibitory potency of the drugs tested. (3) The substrate-type GLYT1 inhibitor sarcosine enhanced [^{3}H]glycine release by an external-Ca^{2+}-independent mechanism and this effect was also reversed by GLYT1 inhibitors. (4) Intensive staining for GLYT1 was found in the inner nuclear and plexiform layers of the retina. Expression of GLYT1 was visable in glycinergic amacrine cells and GFAP-immunoreactive Müller glia cells.

Discussion: We have found that glycine is excessively released from amacrine cells and/or Müller cells in energy-compromised retinae. This release may occur with parallel release of glutamate resulting in overstimulation of GluN1/GluN2B receptors expressed in ON ganglion cells, and neurodegeneration of these cells may develop. Inhibitors of GLYT1 reduced the excessive glycine release evoked by OGD, and lack of receptor co-agonist may suspend pathological overactivation of GluN1/GluN2B receptors responsive to hypoxic degeneration of ON ganglion cells. The possible neuroprotective effects of GLYT1 inhibitors in the retina is now under investigation in our laboratory.

A4.20

Hypothermia prevents ischemic condition to release [^{3}H]dopamine from rat parietal cortex slices in a Ca^{2+}-independent manner

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Background: Stroke is the number two cause of death worldwide, and considering a further increase in the future in life expectancy, it may soon become the leading cause of death. In human brain during stroke an ischemic condition occurs. There is convincing evidence that a massive release of neurotransmitters occurs in the brain during ischemia. When glucose and oxygen are withdrawn (oxygen-glucose deprivation, OGD) the Ca^{2+}-independent release of various transmitters (noradrenalin/dopamine/glutamate) from *in vitro* slice preparations was increased and mediated via reverse operation of transporters. In addition, evidence showed that the excessive amount of released neurotransmitters and some of their metabolites are neurotoxic.

Methods: (1) Using a superfusion microvolume chamber [1] we loaded the rat parietal cortex slices with [^{3}H]dopamine ([^{3}H]DA) in Krebs solution and measured the transmitter release at rest and in response to field stimulation (2 Hz). The fractional release of [^{3}H]DA was measured by liquid scintillation counting and the distributions of [^{3}H]DA and its metabolites was determined using HPLC. A thermoelectric device (Frigomix) was used to quickly change the temperature. (2) "Stroke" was produced by transient occlusion of middle cerebral artery (TMCAO) in rats and after 24 hours they were decapitated. [^{3}H]DA release was measured from parietal cortex slices at various temperatures.

Results: (1) Removal of oxygen and glucose enhanced the resting and inhibited the stimulation-evoked release of radioactivity. We found that lowering the temperature (32°C and 27°C) reduced or prevented the release of [^{3}H]DA induced by ischemia (OGD) obtained at 37°C. Removal of Ca^{2+} from the Krebs solution and addition of

1mM EGTA failed to change the release of [^{3}H]DA evoked by OGD indicating that the release was external calcium-independent. (2) Lowering the temperature prevented or reduced the effect of OGD on DA release in parietal slices prepared from both control and operated sites of the brain taken from rats after *in vivo* TMCA occlusion.

Discussion: In our *in vitro* and *in vivo* model of ischemia we studied the effect of hypothermia on the release of [^{3}H]DA evoked by OGD from rat parietal cortex slice preparations. In summary, it seems likely that the application of hypothermic treatment in order to reduce the neurotoxic effect of an ischemic insult may have therapeutic importance. In the future, we are planning to test a device capable to quickly reduce the local temperature in animal experiments. Local hypothermia seems to be an alternative treatment for post-stroke patients or for those who suffer from spinal cord injury.

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A4.21

Neuroinflammation and brain injury: recent lessons and novel mechanisms

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Background: Inflammation is an important contributor to brain injury, but the mechanisms involved are improperly defined. Microglia, the main inflammatory cells in the brain become activated in various brain diseases, but their functional role in neuronal injury remains controversial.

Methods: Using selective microglia manipulation approaches, imaging, transgenic models and advanced microscopy, inflammatory actions through which microglia shape neuronal activity and injury can be investigated. Early inflammatory changes including microglia–neuron interactions, neuronal calcium responses, blood–brain barrier (BBB) injury, oxidative stress and perfusion changes are investigated in real time with two-photon and SPECT imaging or with super-resolution microscopy after cerebral ischemia and in other models of neuroinflammation.

Results: Microglia react rapidly to early changes in neuronal calcium responses, BBB injury and oxidative stress. Dysregulation of neuronal calcium responses is observed within 30 min after the onset of ischemia in the absence of functional microglia, leading to larger brain injury. We show that BBB injury after acute cerebrovascular events can be detected much earlier (within 2 h) than by using histology. Changes at the capillary/small vessel level as assessed by two-photon imaging show a good correlation with BBB injury seen in full brain hemispheres based on SPECT imaging studies. However, successful reperfusion after cerebral ischemia is followed by spontaneously occurring perfusion deficits later, which is further impaired by preceding systemic inflammation. Systemic inflammation also leads to larger BBB injury, which is apparent as early as 2 h after the onset of ischemia and is associated with impaired functional outcome.

Discussion: Understanding central and systemic inflammatory mechanisms is essential for the development of novel diagnostic and therapeutic tools in brain diseases.

A4.22

Different oxidative phosphorylation patterns in healthy mouse brain regions and alteration of oxidative phosphorylation in the epileptic mouse brain

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Background: Mitochondrial dysfunction is common in neurological diseases. Frequently, a regional specificity in vulnerability can be observed. The aim of this study was to understand how mitochondrial failure in particular brain regions contributes to specific pathological conditions.

Methods: We optimized protocols to study oxidative phosphorylation by means of high-resolution respirometry in defined brain regions of heathly mouse brains. With these methods at hand, we investigated alterations in oxidative phosphorylation during the development of epilepsy. For this purpose, we applied the well-established kainic acid model of mesial temporal lobe epilepsy in mice.

Results: In naïve mouse brains, complex I (CI)-linked respiration was highest in motor cortex. Complex II (CII)-linked respiration was especially high in the striatum. In the kainic acid (KA) model of temporal lobe epilepsy in mice, absolute CI- and CII-linked oxygen consumption as well as electron transport system (ETS) capacity were decreased in the injected dorsal hippocampus 2 days after KA. When normalized to ETS capacity, CII-linked respiration was significantly increased compared to controls. Three weeks after KA injection CII-linked oxygen consumption remained elevated when normalized to ETS capacity.

Discussion: The presented high-resolution respirometry protocols allow detailed analyses of oxidative phosphorylation in small amounts of specific tissues (about 2 mg). This allowed the comparison of different brain tissues implicated in neurological diseases of the healthy mouse and in disease models. We observed marked differences in oxidative phosphorylation patterns in healthy mouse brain regions and present alterations in oxidative phosphorylation in a model of epilepsy.

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Pharmacoepidemiology and Pharmacovigilance

A5.1

Content of home pharmacies and self-medication practice in households in Novi Sad, Serbia

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Background: Data regarding the contents of drugs in households and inclination of patients toward self-initiated treatment are scarce. The aims of this study were to analyze the volume and structure of drugs in households and intended self-medication in a general population.

Methods: The study was prospective, comparative and randomized. Data were collected from households in the municipality of Novi Sad in the period of 8 months (December 2011 – July 2012). In order to obtain a sample of 383 households, which was calculated on the basis of pilot study conducted in the municipality of Novi Sad in 2010, 1008 households were contacted (response rate 38.0%). The first part of the survey was collecting data when the researcher conducted a review and analysis of all drugs in the household. In the second part drugs were classified according to the ATC classification and then analyzed whether they were OTC (over the counter) or POM (prescription-only medications) and whether they were obtained on prescription or bought without prescription. Households with and without children younger than 12 and elderly households were analyzed separately.
Results: The average number of drug packages per household was 11.5 ± 5.8. The highest percentage of prescription-only medications was in elderly households (71%), while the highest percentage of OTC drugs was in households with children (51%). In households with and without children the most common prescription-only medications were anti-rheumatic and anti-inflammatory products (24.6% and 17.7%, respectively) and antibiotics for systemic use (14.7% and 13.2%, respectively), while in elderly households the most common drugs were agents acting on the renin–angiotensin system (11.5%) and psycholeptics (9.4%). More than half of the drugs in the household were purchased on patients' own initiative (53.9%). The highest percentage of drugs purchased without prescription was in households with children (45.0%), while the lowest percentage was in elderly households (16.8%). The most common purchased groups of OTC medicines were analgesics (41.8%) and nasal preparations (14.4%), while the most often bought prescription only medications were antirheumatic and anti-inflammatory products (41.5%) and systemic antibiotics (12.4%).
Discussion: A large number of drugs in some surveyed households may be explained by excessive prescribing, noncompliance with recommended treatment and oversized drug packages. Despite the regulation prohibiting off-prescription sales of antibiotics, the second most common group of POM bought without prescription encountered in households were antibiotics, following anti-inflammatory and anti-rheumatic products. Similar results were recorded in a Croatian research of home pharmacies [1]. Self-medication with POM was observed at a higher rate in households with children younger than 12 years of age, while the tendency towards self-medication was the lowest in households with elderly population. This is also comparable with results of a Belgian study [2]. In conclusion, our survey indicated that self-medication with prescription drugs appears to be a rather common practice.
Acknowledgements: This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia, project no. 41012.

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A5.2

A retrospective study of international pharmacovigilance safety reports on irreversible injection-site reactions after subcutaneous administration of Sayana®

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Background: Sayana® was introduced as the first depot medroxy-progesteron acetate (DMPA)-containing contraceptive, administered via subcutaneous injection. In 2014 and 2015 the regional pharmaco-vigilance centre of Zurich (RPVC Zurich) received 11 anonymised reports of severe and persistent local reactions after Sayana® administration, which were classified as lipodystrophies, atrophies or persisting indurations. In this retrospective study, we analysed global individual case safety reports (ICSRs) of this adverse drug reaction (ADR).
Methods: International, national and regional ICSRs associated with Sayana® administration were analysed. Data on ADRs were retrieved from the WHO Global Database VigiBase™ and were analysed statistically. For the local reports additional demographic data, drug administration information, duration of Sayana® treatment, latency time of the ADR, its course, severity and outcome were collected.
Results: Worldwide, 398 ICSRs associated with Sayana® were registered in the database until 2016-0 1-01. After selecting all cases possibly related to a severe or persistent local reaction, 355 reported corresponding terms, corresponding 323 (81.4%) international ICSRs remained for analysis. Of these, 91.6% ($n = 296$) were categorized as serious. The majority of the reactions ($n = 193$; 54.4%) did not recover (*e.g.* atrophy, fat necrosis or lipoatrophy). In the 67 Swiss ICSRs, 77 ADRs were reported using 10 different terms including severe or persistent local reactions like lipodystrophy, atrophy or fat necrosis. Of these, 32 patients (47.8%) did not recover. All 11 regional cases reported to the RPVC Zurich were categorized as serious ADRs. For the majority of the patients ($n = 7$; 63.6%) the interval between the application of Sayana® and the development of the lipodystrophy was 2–4 months. Most of the reactions were irreversible ($n = 9$; 81.8%). One patient even required plastic surgery.
Discussion: Administration-site reactions during Sayana® treatment do occur frequently. Persistent local injection site reactions such as lipodystrophy, fat tissue necrosis or atrophy do occur under subcutaneous Sayana® use. These reactions were recently integrated in the Swiss product information. Patients should be informed and advised about these potentially irreversible effects at the injection site.

A5.3

Savings potential in public expenses on pharmaceuticals in Austria, 2011–2014

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Background: The rise in public expenses on pharmaceuticals is of global concern. Nations and social insurance authorities have taken

measures to cut costs, among them the Austrian authorities. In contrast to other countries, the measures by the Austrian authorities grant a high grade of freedom of choice of drugs to the prescriber. This report is based on precise data of public expenses on pharmaceuticals and calculations at substance level (ATC level 5).

Methods: Data from all prescriptions filled in Austrian pharmacies on public expense by outpatients (2006–2012) were obtained from the Main Association of Austrian Social Security Institutions (Hauptverband der österreichischen Sozialversicherungsträger). Savings potential was calculated at ATC-5 level (substance level) taking into account recommendations of authorities and the status of the medical evidence. Calculations were performed by replacing expensive brands with more cost effective brands or by replacing substances with low grades of evidence by substances with high-grade evidence and choosing cost-effective brands.

Results: Public expenses on prescriptions filled in Austrian pharmacies by outpatients (2006–2012) rose from €2.45bn in 2011 to €2.67bn in 2014. A cummulative saving potential of over €2.53bn was calculated over the years 2011–2014 (2011: €675.4m, 2012: €656.9m, 2013: €585.2m, 2014: €616.1m). In 2014, for drugs acting on the CNS, the savings potential was €169.15m, for drugs acting on the cardiovascular system, the savings potential was €107.61m alone. Savings potential data are available at ATC-5 (substance) level.

Discussion: This report points out the enormous possible savings potential that could have been achieved by careful implementation of the authorities' and state-of the-art medical recommendations. The savings potential data are based on calculations from all prescriptions filled in Austrian pharmacies on public expense by outpatients (2006–2012) and therfore differ substantially from previous estimations. However, a more cautious prescription practice could provide an even greater savings potential.

A5.4

Prescription of psychostimulants in Austria, 2006–2014

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Background: Psychostimulants are drugs acting on the central nervous system, intended to improve mental and/or physical functions such as alertness and wakefulness. Today's most important medical indication lies in the treatment of the attention deficit and hyperactivity disorder (ADHD), a condition that is subject to controversial discussion regarding prevalence, (under)diagnosis and (over)treatment. This study analyses the prescription data of stimulants in Austria in the years 2006 to 2014.

Methods: Data from all prescriptions filled in Austrian pharmacies on public expense by outpatients (2006–2012) were obtained from the Main Association of Austrian Social Security Institutions (Hauptverband der österreichischen Sozialversicherungsträger). Prescriptions and costs of CNS stimulants were analysed in detail using WHO drug statistic methologies.

Results: Prescriptions filled in Austrian pharmacies on public expense by outpatients steadily rose from 770,000 (2006) to over 2,000,000 defined daily doses in 2014, public expenses on psychostimulants rose from €1,780,000 to €5,120,000. Deducted from these crude figures and population data, 4 children/adolescents (5–19 years of age) out of 1,000 received a one-year pharmacotherapy with psychostimulants in 2014.

Discussion: Use of and treatment with psychostimulants bear a history of unfavourable side effects (especially after long-term use), abuse, misuse and revocation of approvals. ADHD itself, its diagnosis and treatment have always been controversial. Austrian prescription data demonstrate a generally cautious precription practice with—in contrast to other countries—worrisome constant increase in prescription numbers.

Training and Education

A6.1

Certification of medical specialists in pharmacology and toxicology by the European Certified Pharmacologists (EuCP) Programme

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Background: The LifeTrain framework [1], part of the EU's Innovative Medicines Initiative's [2] training and education network EMTAIN [3], has been founded in order to promote projects of continuous professional development (CPD) in all areas of medicines research, from basic science through clinical development to pharmacovigilance, recognising the importance of life-long learning in the biomedical sciences. The EuCP Programme [4] is a joint initiative by EPHAR, the Federation of European Pharmacological Societies, and EACPT, the European Association for Clinical Pharmacology and Therapeutics, to identify individuals working in the field of pharmacology whose practical skills, experiences and especially knowledge cover the entire breadth of the discipline.

Methods: EACPT and EPHAR have called on their national member societies of to establish certification schemes according to the EuCP Programme. Upon accreditation by the EuCP Committee, these societies shall invite individual members fulfilling the EuCP criteria to apply for certification. Following a positive evaluation, the society submits a summary of the evaluation to the EPHAR/EACPT EuCP Committee, which, following a final review, issues the certification for a period of five years. Re-certification requires documented CPD activities as specified in the national EuCP programme.

Results: APHAR has set up the first national scheme accredited with the EuCP Programme, based on the Austrian Medical Association's diploma for specialists in pharmacology and toxicology. Pharmacologists holding this diploma may apply for certification, supporting their application with documents as suggested in the APHAR guidelines for EuCP certification [5]. The EuCP Commission of EACPT and EPHAR has recently approved the first Austrian pharmacologists who have applied for certification.

Discussion: The EuCP certification guarantees a Europe-wide common high standard and qualifies certified individuals for tasks and positions where an extensive knowledge of the entire discipline is required, such as in academic or industrial executive positions, in medical and scientific steering boards, for decision-making and advisory functions in the public health system, in public agencies or in industry. Certification programs for clinical pharmacologists and for pharmacologists with a non-medical background are in preparation.

Links

1. http://www.lifetrain.eu
2. http://www.imi.europa.eu
3. http://www.emtrain.eu
4. http://www.eucp-certification.org
5. http://www.aphar.at/eucp

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