MEETING ABSTRACTS

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A1

Antidepressant-like effects of benzodiazepine site inverse agonists in the rat forced swim test

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Background: There are three kinds of allosteric modulators acting through the benzodiazepine (BZ) binding site of the $GABA_A$ receptor: positive (agonist), neutral (antagonist), and negative (inverse agonist) modulators. Agonists and inverse agonists commonly exert bidirectional influences on observed behavioral parameters. In the present study we have investigated the modulation of behavioral responses to environmental novelty in two unconditioned paradigms: spontaneous locomotor activity (SLA) and forced swim test (FST), elicited by DMCM (methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate), a non-selective inverse agonist, in the dose range that previously did not produce anxiogenic effects and convulsions.

Methods: SLA in the test cage (40 x 25 x 35 cm) during 30 min was recorded automatically, beginning 20 min after i.p. injections (DMCM 0.1, 0.5 and 1 mg/kg) without any habituation. FST was performed in a glass cylinder, 45 cm high, 20 cm diameter filled with water up to a height of 20 cm, with a temperature of 21–23 °C. Male Wistar rats were exposed to two swimming sessions (an initial 15-min pretest session, followed 24 h later by a 5-min test session). The animals received i.p. 0.1, 0.5 and 1 mg/kg of DMCM or vehicle, 20 min before the test session. A rat was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line.

Results: ANOVA showed a significant effect of treatment on the total immobility time of the animals during 30 min of monitoring of spontaneous locomotor activity ($p < 0.05$). Namely, Dunnett's analysis showed that the highest applied dose of DMCM (1 mg/kg) exerted the activity-decreasing effect related to vehicle. In FST during the test session, ANOVA indicated statistically significant effects of DMCM ($p < 0.05$) on the average immobility time of animals. Dunnett's analysis showed that DMCM (1.0 mg/kg) significantly increased immoblity, but at the lowest applied dose DMCM (0.1 mg/kg) decreased immobility, and exerted acute antidepressant-like effects.

Conclusions: These data suggest that negative modulation at $GABA_A$ receptors might have triggered the acute antidepressant-like effects in rats and these effects were not confounded by locomotor influences. On the other hand, these effects are not straightforward, because they exert a kind of bimodal influence. Furthermore, these results encourage the synthesis of new BZ site ligands, aimed to possess more selective affinity/efficacy profiles.

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A2

Methadone-drugs interactions: possible causes of methadone-related deaths

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Background: Methadone is an effective analgesic and it is widely used to suppress withdrawal symptoms from other opiates. Its consumption is usually associated with concomitant drug use in heroin addicts, and this combination is a possible risk factor for lethal outcome. The aim of this study was to analyze characteristics of methadone-related deaths (MRDs), and to evaluate the concomitant use of the drugs that can contribute to methadone toxicity.

Methods: To investigate MRDs, a 10-year retrospective study was carried out (2001–2010) at the Institute of Forensic Medicine in Novi Sad, Clinical Centre of Vojvodina, Serbia. These data included age and sex of subjects, and drugs detected in *post-mortem* samples of blood and urine. Toxicological screening and quantification of drugs were carried out in blood and urine using gas chromatography-mass spectrometry. Methadone concentration in blood was defined to be lower than 200 µg/L, 200–1000 µg/l, or higher than 1000 µg/L.

Results: A total number of 40 MRDs was identified (19.1% of all deaths associated with fatal opiate-related poisoning). The median age of victims at the time of death was 31, whereas the majority of them (80%) were male. The concentration of methadone in blood and urine samples was quantified in 11 cases and in 9 of them it was lower than 200 µg/L (mean concentration 79.5 µg/L). In one case it was 245 µg/L, whereas in the other one methadone was detected only in urine in concentration of 1209 µg/L. In 7 cases only methadone was found (17.5 % of MRDs); 47.5% of MRDs was associated with other drugs – the average number of associated drugs was 3.5, while in blood samples of 35% of MRDs other illicit drugs were identified. The most frequent concomitants were one or more benzodiazepines (67.5% of MRDs), followed by antipsychotics (15%), tramadol (15%) and antidepressants (12.5%). The most commonly identified benzodiazepine was diazepam.

Conclusions: In MRDs a low methadone level combined with other drugs was most frequently noted. The mechanism of death cannot be attributed to particular pathway. The most detected concomitants were well-known inhibitors, inducers or metabolic substrates of CYP3A4 and CYP2D6 involved in metabolism of methadone.

Moreover, they can increase the risk of torsades de pointes and of respiratory depressant effects of methadone. Further studies could clarify the possible mechanism of death where methadone is used in combination with benzodiazepines in order to prevent MRDs.
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A3

Use of ACE inhibitors in Serbia in 2010

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Background: Cardiovascular diseases are the most frequent cause of morbidity and mortality in many countries. That explains why medications for the treatment of cardiovascular diseases are group of drugs with largest consumption, and ACE inhibitors take a large part in the consumption. The aim of this study was to analyze the consumption of ACE inhibitors in Serbia in 2010 compared with Norway, a country with a developed pharmacotherapeutic practice.
Methods: The data about the use of ACE inhibitors in Serbia were taken from the Agency for Drugs and Medical Devices of the Serbia. The data about drug consumption in Norway were taken from the official website of the Norwegian Institute of Public Health.
Results: In Serbia, the use of drugs of first choice in the treatment of hypertension was very uneven, where the consumption of ACE inhibitors was dominant. Opposed to this condition, the consumption of the first choice antihypertensive drugs was very balanced in Norway. Total consumption of ACE inhibitors in Serbia in 2010 year was 190.4 defined daily doses per 1000 inhabitants per day (DID) and total consumption of ACE inhibitors in Norway in 2010 year was 51.7 DID. During the analyzed year the largest use of plain ACE inhibitors in Serbia was for enalapril (81.0 DID), ramipril (34.2 DID), fosinopril (24.5 DID) and cilazapril (13.1 DID) and in Norway was ramipril (27.3 DID), enalapril (11.1 DID) and lisinopril (6.2 DID). In Serbia a significant part of the consumption of ACE inhibitors consisted of more expensive drugs such as fosinopril, cilazapril and quinapril, whereas these have not been used at all in Norway during that period.
Conclusions: In Serbia in the year 2010, ACE inhibitors and their fixed combination with diuretics are the most frequently used drugs within the group of drugs which is used for cardiovascular diseases treatment. The amount and structure of the utilized ACE inhibitors in Serbia is different in a lot of ways from the amount and structure of the utilized ACE inhibitors in Norway. Pharmacoeconomic analyses also show that large financial resources would be saved if the structure of the utilized ACE inhibitors in Serbia were more similar to the one in Norway.
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A4

Differences in the use of medicines for peptic ulcer and gastro-esophageal reflux disease between Serbia, Croatia and Sweden

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Background: The medicines for peptic ulcer and gastro-esophageal reflux disease (ATC subgroup A02B) are among the most commonly prescribed class of drugs. The aim of this study was to analyze the pattern of consumption of histamine H_2 receptor antagonists (H_2RAs) and proton pump inhibitors (PPIs) in Serbia in 2010 in comparison with Croatia and Sweden.
Methods: The data on the consumption of medicines have been provided from the databases of the national regulatory agencies. The results were expressed as the number of defined daily doses per 1000 inhabitants per day (DID). A qualitative analysis was carried out according to the drug utilization 90% (DU90%) approach.
Results: The overall consumption of medicines from A02B subgroup in 2010 in Serbia was 22.9 DID, whereas in Croatia and Sweden was 32.8 DID and 48.6 DID, respectively. In Serbia, H_2RAs accounted for 71.8% (16.5 DID) of medicines used within A02B subgroup, while in Croatia H_2RAs accounted for 37.3% (12.2 DID) and in Sweden 2.2% (1.1 DID). In the same year, the utilization of PPIs in Serbia (6.5 DID) was more than three times lower than in Croatia (20.6 DID) and more than seven times lower than in Sweden (47.3 DID). The bulk of prescription (DU90%) was made up of 3 (out of 7) medicines in Serbia, 5 (out of 8) medicines in Croatia and 5 (out of 14) medicines in Sweden. The most frequently used medicine from the A02B subgroup in Serbia was ranitidine (56.0%, i.e. 12.8 DID), in Croatia pantoprazole (36.5%, i.e.12.0 DID) and in Sweden omeprazole (81.3%, i.e. 39.0 DID).
Conclusions: The overall utilization of the medicines for peptic ulcer and gastro-esophageal reflux disease was notably lower in Serbia in comparison with Croatia and Sweden. Besides the quantity, the pattern of use showed remarkable differences. Most commonly used medicines from the A02B subgroup in Serbia were H_2RAs whereas in Croatia and Sweden were PPIs. These findings suggest that implementation of pharmacotherapeutic guidelines in Serbia is needed in order to achieve harmonization in prescribing practice.
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A5

Remarkably lower consumption of antidepressants in Serbia in comparison with Finland

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Background: Depression is an important health problem worldwide due to significant disability that it causes, reduction of quality of life, loss of work days, and even suicide. The aim of our survey was to evaluate the overall utilization and pattern of use of antidepressants in Serbia in a comparison with Finland in 2010 and to propose appropriate interventions in Serbia on the basis of the results obtained.
Methods: The data on utilization of antidepressant drugs (ATC group N06) for 2010 were retrieved from the annual reports of relevent public institutions in Serbia and Finland. The ATC/DDD methodology was applied and the results were expressed in defined daily doses per 1000 inhabitants per day (DID). As an indicator of the quality of drug prescribing, the Drug Utilization 90% (DU90%) method was used.
Results: An overall antidepressant consumption in 2010 appeared to be 6-fold lower in Serbia (11.7 DID) in comparison with Finland (68.8 DID). Selective serotonine reuptake inhibitors (SSRIs) accounted for the majority of antidepressant utilization in both countries (73.8% for Serbia and 63.9% for Finland). Apart from that,

the pattern of utilization of the most frequently used SSRI drugs was different between these countries. Sertraline accounted for the highest share in Serbia (5.6 DID) while citalopram and escitalopram are generally the most widely used drugs for the treatment of depression in Finland (17.5 DID and 11.8 DID respectively). In our country, the group of nonselective monoamine reuptake inhibitors (13.1%) took the second place that makes a notable difference in comparison with Finland where the second-ranked group was other antidepressants (N06AX) (29.2%).

Conclusions: The differences between selected countries in antidepressant utilization are partly consequential to different socioeconomic and health policy factors. The considerably lower utilization of antidepressants in Serbia implies possible underdiagnosing of affective disorders in general practice. To reduce the serious consequences that may be caused in that way, the early diagnosis and timely, adequate and effective management and treatment of depression is essential.

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A6

Considerable differences in the utilisation of antidiabetics between Serbia and Scandinavian countries

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Background: Diabetes mellitus is a major public health concern with devastating human, social and economic impact. It is increasing globally, affecting more than 180 million people worldwide. The objective of our study was to analyse the overall volume of use of antidiabetics in Serbia compared to Scandinavian countries (Sweden, Norway, Denmark), chosen for their rational and conservative prescription practice.

Methods: Data on consumption of antidiabetics (ATC group A10) in 2010 were extracted from the databases of the representative national authorities. Utilisation of these medicines was measured through the defined daily dose (DDD) unit and the results were expressed as DDD per 1000 inhabitants per day (DID).

Results: In 2010, antidiabetics were used at a similar rate in Serbia (47.3 DID) and Scandinavian countries (from 46.5 DID in Sweden to 47.67 DID in Norway), but the share of use of insulins (A10A) and oral antidiabetics (A10B) differed among the observed countries. The proportion of insulin in Serbia was 22.0% of all antidiabetics which is relatively low in comparison with Scandinavian countries (from 36.2% in Denmark to 50.8% in Sweden). Utilisation of long-acting insulins (A10AE) was much lower in Serbia (1.3 DID) compared to Scandinavian countries (range: 2.6–4.6 DID). The share of oral antidiabetics use also differed among these countries. In Serbia, sulfonylureas (A10BB), as a second-line treatment for type 2 diabetes, were used predominantly (55.6%) compared to metformin (44.1%). In Scandinavian countries, metformin, as preffered oral agent for type 2 diabetes and the only medicine from the biguanide class (A10BA), was used at a higher rate than in Serbia (from 51.2% in Denmark to 60.4% in Sweden). New medicinal products with effect on the incretin system (A10BH and A10BX) were also used at a higher rate in Scandinavian countries (range: 0.5–2.5 DID) in comparison to Serbia (0.002 DID).

Conclusions: This cross-national study has demonstrated large differences in the utilisation of various antidiabetics among observed countries that may be attributed to considerable variations in attitudes and habits, especially with regard to the management of type 2 diabetes.

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A7

The bile acid membrane receptor TGR5: a novel pharmacological target in metabolic syndrome

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Background: TGR5 (M-BAR, GPBAR or GPR131) is a plasma membrane-bound, G protein-coupled receptor for bile acids, expressed in many human cells. The aim of this study was to describe that targeting TGR5 could provide an exciting new pharmacological approach to improve different aspects of the metabolic syndrome in humans.

Methods: The data on pharmacological targeting of TGR5 have been provided from more than eighty review and original scientific articles, published from 2007 to 2012. The research was performed using the following key words: bile acids, TGR5, metabolism, diabetes, obesity.

Results: A dietary supplementation of bile acids (BAs) significantly reduced body weight in mice fed with a fat-rich diet. It was the consequence of the induction of deiodinase 2 (D2) through a TGR5/cAMP-mediated pathway. D2 is able to induce the conversion of inactive thyroxine (T4) into the active 3,5,3′-tri-iodothyronine (T3), which enhances the energy expenditure in brown adipose tissue (BAT) and skeletal muscle myoblasts. TGR5 induces glucagon-like peptide-1 (GLP-1) secretion in cultured mouse enteroendocrine STC-1 cells. This property contributes to beneficial effects of TGR5 on glucose metabolism and improves insulin sensitivity. TGR5 activation in mice decreased serum and liver triglyceride levels. The anti-inflammatory action of TGR5 in mouse macrophages attenuated the development of atherosclerotic lesions and could contribute to protective effects of TGR5 on liver steatosis.

Conclusions: TGR5 may be targeted by natural compounds as well as by synthetic agonists. Despite the fact that targeting TGR5 in animals brings great promise for metabolic syndrome treatment, multiple studies described the side effects of targeting TGR5 and further clinical studies are needed to evaluate and identify safe and efficient TGR5 agonists.

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A8

The $GABA_A$ receptor α2 subunit gene (GABRA2) is associated with alcohol-related behavior

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Background: γ-Aminobutyric acid type A ($GABA_A$) receptors, the major inhibitory neurotransmitter receptors in the brain, are implicated in the acute and chronic effects of alcohol, including tolerance, dependence and withdrawal. Various polymorphisms in the gene encoding the $GABA_A$ receptor α2 subunit (GABRA2) have been associated with alcoholism and with antisocial behavior in different populations of European ancestry. As early onset of alcoholism often reflects greater severity, including a higher risk for recurrence, comorbid antisocial personality disorder and conduct

disorder, Cloninger's classification distinguishes type II alcoholism with an early onset, elevated levels of antisocial behavior and delinquency, from the type I alcoholism with a late onset, neurotic symptoms and minimal criminality.

Methods: Genotyping of GABRA2 polymorphisms (rs567926, rs279858 and rs9291283) was performed in samples of 355 alcoholic patients of Croatian origin (280 males and 75 females) using TaqMan Real-Time allelic discrimination technique after extraction of DNA from whole blood. The results of allelic and haplotypic analysis were compared between alcohol-dependent subjects with a combination of early onset of alcohol abuse and presence of aggressive behavior corresponding to type II alcoholism subgroup, and individuals with the late onset of alcohol abuse and without aggression corresponding to type I alcoholism subgroup, according to Cloninger.

Results: Cloninger's Type I and Type II alcohol-dependent patients did not differ significantly in the frequency of the genotypes and alleles for rs567926, rs279858 or rs9291283. However, the G-T-G haplotype was more often present in the alcohol-dependent subjects with early onset of alcohol abuse and aggressive behavior, corresponding to the Cloninger type II alcoholism subgroup ($\chi^2 = 6.102$, $p = 0.013$).

Conclusions: Our results revealed a haplotypic association between the GABRA2 gene and a more severe form of alcoholism, characterized by the early onset of alcohol abuse and presence of aggressive behavior. These findings support an important role of $GABA_A$ receptors in the susceptibility to alcoholism and highlight them as potential targets for novel therapeutics in the treatment of alcohol dependence.

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A9

Protective effect of silymarin on doxorubicin-induced cardiotoxicity in rats

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Background: Silymarin, an extract of *Silybum marianum* seeds, possesses a broad spectrum of action: antifibrotic, antiinflammatory, lipid peroxidation-inhibiting and free radical-scavenging effects. It is a complex of five major compounds, and silibinin is the most biologically active component of the complex. The aim of this study was to investigate, evaluate and confirm the potential antioxidative effects of silymarin rich in silibinin.

Methods: White laboratory rats of Wistar type were used in this experiment. They were treated with saline, 1 ml/kg, orally; olive oil 1 ml/kg, orally; silymarin, 60 mg/kg, orally, every day; with doxorubicin, 1.66 mg/kg, intraperitoneally, every second day; and with the combination of silymarin and doxorubicin in the stated doses. The animals were anaesthetised with urethane and a prepared jugular vein was connected to an infusion pump with verapamil in the course of recording an electrocardiogram. Then, animals were sacrificed by cardiopunction and blood samples were taken to determine serum enzymes activity.

Results: The results show that the treatment with silymarin and doxorubicin in combination causes statistically significant increase of the verapamil dose necessary to produce first change (2.21:0.62, $p < 0.01$); continuous reaction (2.77:1.5, $p < 0.05$) and toxic effect (3.38:1.87, $p < 0.05$) in comparison to the groups treated with doxorubicin alone. The administration of silymarin prevented an increase of creatine kinase activity induced by doxorubicin (279:616; $p < 0.01$). The activity of lactate dehydrogenase was not significantlly different between groups. Silymarin also prevented the doxorubicin-induced increase in aspartate aminotransferase activity (349.5:279.5, $p < 0.05$).

Concusions: On the basis of the results it can be concluded that pro-oxidative activity of doxorubicin is significantly lower when it is administered in combination with silymarin.

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A10

Drug Information Unit, Medical Faculty of Novi Sad

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Background: In Serbia in general there are several ways to obtain necessary information on various drugs. Medical and pharmaceutical professionals gather information from the National Agency of Drugs and Medical Devices or from various publications such as British National Fromulary, Physicians Drug Reference etc. The general population can obtain information from their general practicioner (GP) or pharmacist. At the Department of Pharmacology and Clinical Pharmacology, Medical Faculty of Novi Sad, there exists a Drug Information Unit, a regional center offering drug information to both professionals and the general population in Vojvodina (approximately 1,600,000 inhabitants).

Methods: The client can require information by phone (more than 99.5% of all requests) or by e-mail. Interns in Clinical Pharmacology collect necessary data regarding the therapeutic problem (age and sex of the patient, other drugs taken, present diseases etc.). After solving the problem using the electronic databases or hardcopy sources, and upon the approval from the senior clinical pharmacologist, interns deliver the information to the client (both by phone and e-mail).

Results: About 3% of all requests are from the general population (usually questions on interactions, side effects, dosing and administration); the remaining requests are from health (20% from GPs, and 80% from specialists) or pharmaceutical professionals. Almost 30% of all of the requests of the health professionals are regarding possible drug–drug or drug–disease interactions. About 12% of requests are related to side effects of the administered drug. Pregnancy and lactation are subjects of interest in 15% of the overall number of requests. Maximal doses allowed and posology have a share of about 11%. The remaining information is concerning the pharmacokinetics of the drugs, first line drugs for certain diseases, dosing in children etc.

Conclusions: It can be concluded that the Drug Information Unit is a useful source of information for both professionals and the general population offering various information on different topics related to drugs.

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A11

Influence of different *Hypericum perforatum* L. preparations on pharmacokinetic and pharmacodynamic properties of pentobarbital, diazepam and paracetamol

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Background: Herb–drug interactions are an important safety concern and the study was conducted regarding the interaction between the natural top-selling antidepressant remedy *Hypericum perforatum* and conventional drugs.
Methods: This study examined the influence of acute pretreatment with different *H. perforatum* extracts on pentobarbital-induced sleeping time impairment of motor coordination caused by diazepam and paracetamol pharmacokinetics in mice. The preparations profile of St. John's wort was determined using RP-HPLC analysis. Ethanolic extract, aqueous extract, infusion, tablet and capsule of *H. perforatum* were used in the experiment.
Results: By quantitative HPLC analysis of active principles, it has been proved that *H. perforatum* ethanolic extract has the largest content of naphtodianthrones: hypericin (57.8 μg/ml) and pseudohypericin (155.4 μg/ml). Pretreatment with ethanolic extract of *H. perforatum* potentiated the hypnotic effect of pentobarbital and the impairment of motor coordination caused by diazepam to the greatest extent and also increased the paracetamol plasma concentration in comparison to the control group. These results were in correlation to naphtodianthrones concentrations.
Conclusions: The obtained results show a considerable influence of *H. perforatum* on pentobarbital and diazepam pharmacodynamics and paracetamol pharmacokinetics.
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A12

Acetaminophen changes paracellular transport activity through regulation of the tight junction protein in an intestinal barrier model

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Background: Drugs and diet interact with each other in mutually influencing their bioavailability. In our previous work we have shown that *N*-acetyl-*p*-aminophenol (APAP, acetaminophen) reduces the paracellular transport activities of itself and co-administered substances. The aim of this study was to find out how APAP reduces the bioavailability of small molecules which pass paracellularly, and which tight junction proteins are involved in the regulation of paracellular transport using a Caco-2 barrier model.
Methods: The Caco-2 *in vitro* model is widely used by the pharmaceutical industry to predict the absorption of orally administered drugs. To construct a Caco-2 barrier model, the Caco-2 cell line, a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells, was seeded onto inserts (Millipore, 0.4 μm pore size) for 21 days. After differentiation, the cells were treated topically with 10 mM APAP for 24 h. The cell transepithelial electrical resistance (TER) and capacitance (Ccl) were determined by two different impedance-measuring systems. The membrane permeability was tested by differently sized molecules: Lucifer Yellow (520 Da), 3–5 kDa and 40 kDa Fluorescein thiocarbamoyl (FITC)-dextran. The tight junction proteins (ZO-1, occludin) were investigated using Western blot analysis and immunofluorescence staining.
Results: APAP increased the TER value, *i.e.* membrane integrity, which correlated significantly with the decrease of permeability of the small molecules Lucifer Yellow (ca. 520 Da) and FITC-dextran (3–5 kDa) but not of large molecules (40 kDa FITC-dextran). The tight junction protein ZO-1 and its tyrosine phosphorylation were up-regulated after 24 h treatment with APAP.
Conclusions: Tight junction proteins play an important role in maintaining the intestinal barrier function. We assume that APAP affects the paracellular transport pathway through disassembly of the tight junction. We suppose that APAP leads to remodelling of the tight junction protein ZO-1 through changes in the level of protein expression and in tyrosine phosphorylation. APAP may reduce the bioavailability of co-administered substances through remodelling the tight junction by regulating ZO-1 and its tyrosine phosphorylation. As a result, the paracellular transport activity for small molecules is decreased as is net intestinal absorption.
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A13

The role of CB_2 receptor ligands in human eosinophil function

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Background: Eosinophils play a key role in allergic diseases such as bronchial asthma and atopic dermatitis. A prominent feature of these diseases is the accumulation of eosinophils in inflamed tissue induced by several chemoattractants like prostaglandin (PG) D_2 or eotaxins. After the discovery of the endocannabinoid system and investigation of several endogenous and synthetic ligands, evidence has accumulated that cannabinoids, especially CB_2 receptor ligands, may play a major role in mediating inflammatory responses. Elevated levels of 2-arachidonoylglycerol (2-AG; a CB_1/CB_2 agonist) were found in tissues of mouse models of allergic inflammation, suggesting a possible involvement in leukocyte recruitment.
Methods: Blood was sampled from healthy volunteers, erythrocytes were removed by dextran sedimentation and polymorphonuclear leukocytes were obtained via Histopaque gradients. For all assays eosinophils were further purified by negative magnetic isolation. Shape change was recorded immediately after stimulation on a FACSCalibur flow cytometer. For chemotaxis assays an AP48 microBoyden chamber was used and migration time was 1h at 37°C. Intracellular Ca^{2+} levels were analyzed by flow cytometry after treating eosinophils with Fluo-3-AM for 60min at room temperature.
Results: We found that CB_2 receptor agonists like the endocannabinoid 2-AG or the synthetic selective agonist JWH-133 significantly increased eosinophil responses in shape change assays induced by PGD_2 or eotaxin-1. The observed effects could be abolished by pretreatment with the selective CB_2 receptor antagonists SR144528 or AM630. As cytoskeletal changes are required for firm arrest and leukocyte diapedesis, transmigration assays were conducted subsequently, confirming the shape change data as eosinophil migration induced by PGD_2 was increased by pretreatment with JWH-133. Calcium flux assays showed Ca^{2+} mobilization induced by JWH-133 and the tendency to increase PGD_2-induced Ca^{2+} release.
Conclusions: We could show that JWH-133 can influence human eosinophil activation/migration via activation of CB_2 receptors as chemoattractant effects were significantly modulated, suggesting a possible pro-inflammatory role in allergic inflammation which may further lead to cannabinoid-based treatment options in allergic inflammatory diseases.
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A14

Laropiprant attenuates EP_3 and TP prostanoid receptor-mediated thrombus formation

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Background: The use of the lipid-lowering agent niacin is hampered by a frequent flush response which is largely mediated by prostaglandin (PG) D_2. Therefore, concomitant administration of the D-type prostanoid (DP) receptor antagonist laropiprant has been proposed to be a useful approach in preventing niacin-induced flush. However, antagonizing PGD_2, which is a potent inhibitor of platelet aggregation, might pose the risk of atherothrombotic events in cardiovascular disease. Therefore, we investigated the effects of laropiorant on platelet function.

Methods: Platelet aggregation assays were performed *ex vivo* using a platelet aggregation analyser (Aggregometer II). Blood from healthy human donors was used to obtain platelet-rich plasma. The expression of P-selectin and activation of glycoprotein IIb/IIIa was examined using CD62P and PAC1 antibodies, respectively, by direct flow cytometry. *In vitro* thrombus formation was assessed by flowing whole blood on collagen-coated Cellix biochips at −30 dyn/cm^2 using the Mirus nanopump.

Results: *In vitro* treatment of platelets with laropiprant prevented the inhibitory effects of PGD_2 on platelet function, *i.e.* platelet aggregation, P-selectin expression, activation of glycoprotein IIb/IIIa and thrombus formation. In contrast, laropiprant did not prevent the inhibitory effects of acetylsalicylic acid or niacin on thrombus formation. At higher concentrations, laropiprant by itself attenuated platelet activation induced by thromboxane (TP) and E-type prostanoid (EP)-3 receptor stimulation, as demonstrated in assays of platelet aggregation, P-selectin expression, and activation of glycoprotein IIb/IIIa. Inhibition of platelet function exerted by EP_4 or I-type prostanoid (IP) receptors was not affected by laropiprant.

Conclusions: These *in vitro* data suggest that niacin/laropiprant for the treatment of dyslipidemias might have a beneficial profile with respect to platelet function and thrombotic events in vascular disease.

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A15

Uremia alters HDL composition and cholesterol efflux capacity

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Background: Functional impairment of HDL may contribute to the excess cardiovascular mortality experienced by patients with renal disease, but the effect of advanced renal disease on the composition and function of HDL is not well understood.

Methods: Mass spectrometry and biochemical analyses were used to study alterations in the proteome and lipid composition of HDL isolated from patients on maintenance hemodialysis.

Results: We identified a significant increase in the amount of acute-phase protein serum amyloid A1, albumin, lipoprotein-associated phospholipase A_2, and apoC-III composing uremic HDL. Furthermore, uremic HDL contained reduced phospholipids and increased triglycerides and lysophospholipids. With regard to function, these changes impaired the ability of uremic HDL to promote cholesterol efflux from macrophages.

Conclusions: In summary, the altered composition of HDL in renal disease seems to inhibit the cardioprotective properties of HDL. Assessing HDL composition and function in renal disease may help to identify patients at increased risk for cardiovascular disease.

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A16

Alloxan-induced diabetes alters rat common carotid artery response to adenosine

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Background: It is well established that diabetes mellitus represents an important risk factor for endothelial dysfunction and associated cardiovascular events. Accordingly, vascular responsiveness of different isolated blood vessels was shown to be altered in experimental diabetes. The aim of this study was to investigate the effect of adenosine on intact or denuded isolated rat common carotid arteries obtained from healthy or diabetic rats.

Methods: The current study involved two groups of male Wistar rats (220–280 g): (1) healthy controls and (2) rats with alloxan-induced diabetes. Carotid arteries were extracted from rats, carefully dissected from surrounding tissue, cut into 4 mm-long rings and placed in an organ bath. The endothelium was removed from some rings by gently rubbing the intimal surface with stainless steel wire. Apart from the pharmacological verification, the presence of endothelial cells was confirmed by histological evaluation on randomly selected preparations. Concentration-response curves for adenosine (0.01–100 μM) were obtained in a cumulative fashion on serotonin-precontracted arteries.

Results: The adenosine-induced maximal relaxant response of rings with or without endothelium was similar in all investigated groups ($p > 0.05$), indicating an equi-effective action of adenosine irrespective of diabetes. The analysis of the median effective concentrations (pEC_{50}) showed that the response of intact or denuded vessels to adenosine was comparable but only within each group, thus confirming an endothelium-independent relaxation. On the other hand, the comparison of pEC_{50} values between healthy and diabetic animals showed a significant decrease of pEC_{50} ($p < 0.05$) in rats with alloxan-induced diabetes, which was also accompanied by a matching rightward shift of the cumulative concentration-response curves for adenosine.

Conclusions: Adenosine induced endothelium-independent relaxation of the rat common carotid artery, with comparable pharmacological efficacy in all investigated groups, yet with reduced pharmacological potency in diabetic rats. This confirms the initial hypothesis that diabetes alters the response of the rat common carotid artery to adenosine.

A17

A PET microdosing study with the P-glycoprotein inhibitor tariquidar

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Background: The adenosine triphosphate-binding cassette transporters P-glycoprotein (Pgp) and breast cancer resistance protein (BCRP) restrict absorption and body distribution and promote excretion of several clinically used drugs. Tariquidar (XR9576) is a potent third-generation dual Pgp and BCRP inhibitor, which is currently tested in clinical trials to overcome chemoresistance of tumors and to enhance brain distribution of Pgp/BCRP substrate drugs. We performed a positron emission tomography (PET) microdosing study with carbon-11-labelled tariquidar ([^{11}C]tariquidar) which aimed at assessing the brain distribution of [^{11}C]tariquidar in healthy volunteers.
Methods: Six healthy subjects received an i.v. bolus injection of approximately 400 MBq of [^{11}C]tariquidar containing less than 30 µg of unlabelled tariquidar. Then, dynamic brain PET scans and arterial blood sampling were performed. Radiolabelled metabolites of [^{11}C]tariquidar in plasma were measured with a solid-phase extraction/HPLC assay. Brain activity uptake was expressed as the ratio of the area under the whole brain grey matter time-activity curve to the area under the plasma time-activity curve from time 0 to 60 min ($AUC_{0–60\ brain}/AUC_{0–60\ plasma}$).
Results: Brain activity uptake was low after injection of [^{11}C]tariquidar with a mean $AUC_{0–60\ brain}/AUC_{0–60\ plasma}$ of 0.14 ± 0.03. At 60 min after radiotracer injection, 78 ± 12% of total radioactivity in plasma was in the form of unchanged parent radiotracer. Less than 1% of the total injected dose excreted in urine over 90 min.
Conclusions: Low brain uptake of radioactivity is consistent with tariquidar being, at microdoses, a dual substrate of Pgp and BCRP. [^{11}C]Tariquidar PET after inhibition of Pgp with unlabelled tariquidar may be a promising approach to selectively assess BCRP function at the human blood-brain barrier.
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A18

Role of perivascular adipose tissue in endothelial dysfunction of adipose triglyceride lipase-deficient mice

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Background: Perivascular adipose tissue (PVAT) has been recognized as an important factor in vascular biology due to its ability to produce a variety of vasoactive substances. In addition, it is regarded as an important source of proinflammatory mediators and reactive oxygen species (ROS). Experiments from our laboratory demonstrated that mice lacking adipose triglyceride lipase (ATGL), a crucial enzyme of triglyceride catabolism, suffer from severe micro- and macrovascular endothelial dysfunction. Since blood vessels of ATGL knockout mice (ATGL(−/−) mice) are surrounded by large amounts of PVAT, we investigated its potential contribution to the observed endothelial dysfunction.
Methods and Results: PVAT encompassing thoracic aortas of wild-type (WT) and ATGL(−/−) mice was isolated, characterized, and analyzed for protein and mRNA expression of different adipokines, inflammation markers, and sources of oxidative stress using real-time PCR and Western blot analysis, respectively. Knockout of ATGL caused a 7-fold increase in PVAT wet weight. While mRNA expression of adiponectin was reduced to about 50%, leptin mRNA was increased about 4-fold in ATGL deficiency. Adipose mRNA levels of the inflammation markers tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein 1 (MCP-1), and interleukin-6 (IL-6) were about 5-fold higher in ATGL-deficient PVAT. In addition, the NOX2/p67^{phox} complex was significantly upregulated at protein level. Heme oxygenase-1, which has been described protective against oxidative and inflammatory stress, was increased about 5-fold in ATGL deficiency. To distinguish between direct PVAT-mediated effects and those originating from the cardiac dysfunctional phenotype of the animals, we additionally analyzed tissue isolated from ATGL(−/−) mice with cardiomyocyte-specific overexpression of ATGL (rescued cardiac phenotype). Interestingly, the effect of ATGL knockout on TNF-α and leptin expression was reversible. By contrast, increased adipose NOX2/p67^{phox}, MCP-1 and IL-6 expression persisted even upon restoration of cardiac function.
Conclusions: Our data indicate that PVAT-derived inflammatory and NADPH oxidase-mediated oxidative stress might contribute to endothelial dysfunction in ATGL deficiency. The functional consequences of these findings are currently being investigated in our laboratory.
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A19

Characteristics of low affinity high capacity histamine uptake into neonatal rat astrocytes

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Background: The neurotransmitter histamine is synthesized from histidine in histaminergic neurons. Later on it is taken up into synaptic vesicles by the vesicular monoamine transporter 2 and released into the synaptic cleft upon depolarization stimuli. The released neurotransmitter is metabolised by the enzyme histamine N-methyltransferase (HNMT) producing tele-methylhistamine (tMH). In order to be enzymatically degraded or possibly recycled, histamine must be transported either into the presynaptic neuron or into surrounding glial cells. Unlike other neurotransmitters, the mechanism and the transporters by which the histamine content within the brain is regulated is currently unresolved.
Methods: We used primary cultures of neonatal rat astrocytes to determine kinetic properties of histamine uptake and HNMT and organic cation transporter (OCT) mRNA expression. In addition, we investigated the influence of different antidepressants and OCT inhibitors on histamine transport into astrocytes
Results: Specific uptake of [^{3}H]histamine increased in a time-, temperature- and Na^+-dependent and ouabain-sensitive manner. The Na^+-dependent [^{3}H]histamine uptake was saturable. The K_m

value for this process was around 100 M and V_{max} was 160 pmol/mg protein/min, resembling low-affinity high-capacity uptake 2, which might occur via OCT2, the OCT isoform expressed in astrocytes. [^{3}H]histamine uptake was inhibited only by amitriptyline and desipramine, whereas the histamine metabolite tMH affected both histamine transport and reverse transport from cultured astrocytes. On the other hand, neither decynium-22 nor corticosterone, known inhibitors of OCT, affected carrier-operated histamine transport.

Conclusions: Taken together, astrocytes can represent a major inactivation site for histamine, but some facts remain unresolved, such as the existence of specific histamine transporters, the involvement of non-selective transporters and a possible release of histamine and/or its metabolites from astrocytes.

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A20

The role of PGE_2 EP_4 receptors in the regulation of endothelial barrier function

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Background: Prostaglandin E_2 plays a crucial role in inflammation, including pain, fever and tumorigenesis. Inflammatory cells, fibroblasts and epithelium are the main source of PGE_2 throughout an immune response. There are four known receptors for PGE_2, E type prostanoid receptors 1–4 ($EP_{1,2,3,4}$). According to recent reports, the EP_4 receptor seems to be a potential target of therapeutic treatment for attenuating inflammation as well as increased endothelial permeability.

Methods: Primary human microvascular endothelial cells of the lung (HMVEC-L) were cultured and transfected using siRNA approach. The mRNA level of the EP_4 receptor was determined using RT-PCR. EP_4 receptor protein expression was evaluated via Western blotting and flow cytometry. Changes in electrical impedance were measured using an ECIS application. Morphological alterations were observed via immunofluorescent staining of β-catenin and F-actin. Cell cycle and apoptosis analysis were performed using flow cytometry.

Results: The pulmonary microvascular endothelial cells express the PGE_2 receptor EP_4, which was shown by flow cytometry and Western blotting. In endothelial cells, EP_4 receptor protein expression was down-regulated to less than 40% by using the siRNA transfection approach. Also, the mRNA level of the EP_4 receptor was significantly down-regulated below 15% using siRNA transfection. In the endothelial impedance measurements, the EP_4 agonist and PGE_2-induced barrier enhancement was significantly suppressed in EP_4 receptor-silenced cells. Morphological studies revealed that the thrombin-induced disruption of endothelial monolayers could be reversed by stimulation of EP_4 receptors. Cell-cell contacts were enhanced and stress fibre formation was prevented by pre-treatment with PGE_2 and an EP_4-selective agonist. PGE_2 and the EP_4 agonist did not induce any changes in the endothelial cell cycle; however, EP_4 receptor activation appeared to be protective against staurosporine-induced apoptosis. Apoptotic cells were determined in the sub-G_1 phase of the cell cycle.

Conclusions: These data suggest EP_4 receptor agonists as potential therapeutic intervention for diseases with increased vascular permeability such as acute lung injury.

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A21

Bioactivation of nitroglycerin is determined by the subcellular localization of aldehyde dehydrogenase-2

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Background: Aldehyde dehydrogenase-2 (ALDH2) was characterized as the main enzyme responsible for bioactivation of the antianginal drug nitroglycerin (GTN). We have recently shown that ALDH2 is mainly cytosolic in murine vascular tissue, challenging the general assumption that GTN bioactivation takes place in the mitochondrial matrix of vascular smooth muscle cells.

Objective: In the present study we investigated whether bioactivation of GTN is affected by the subcellular localization of ALDH2 using immortalized ALDH2-deficient aortic smooth muscle cells with selective overexpression of the enzyme in either cytosol or mitochondria. Furthermore we investigated a potential correlation between the relaxation potency of GTN and the distribution of ALDH2 in arterial blood vessels from different species as well as the subcellular distribution of the enzyme in several murine organs.

Methods and Results: Radio thin layer chromatography analysis showed that cytosolic overexpression of ALDH2 led to denitration rates up to 4 times higher than mitochondrial overexpression, suggesting a more efficient bioactivation by cytosolic ALDH2. Interestingly, denitration rates of smooth muscle cells were even higher in cells without functional mitochondria (Rho0 cells), suggesting possible adverse effects of mitochondria on the bioactivity of GTN. Quantitative immunoblotting revealed that ALDH2 is mainly cytosolic in murine, rat, guinea-pig and rabbit aortas as well as in porcine, bovine and human coronary arteries. A similar expression pattern was found in several murine organs, except liver. Cumulative concentration-response curves to GTN established by vasorelaxation studies were biphasic for aortas with more than 10% ALDH2 in mitochondria (mouse and rabbit), strengthening the hypothesis that mitochondrial GTN metabolism counteracts cytosolic bioactivation of the drug.

Conclusions: The data indicate that cytosolic expression is essential for GTN bioactivation in arterial blood vessels and aortic smooth muscle cells, presumably due to limited access of GTN to the mitochondrial matrix.

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A22

Nitric oxide signaling in adipose triglyceride lipase-deficient microvascular endothelial cells

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Background: Adipose triglyceride lipase (ATGL) has been characterized as key enzyme of mammalian triglyceride catabolism. Mice with global ATGL deficiency were previously described to suffer from lethal cardiac dysfunction that originates from defective peroxisome proliferator-activated receptor alpha (PPARα) signaling in the heart. Experiments from our laboratory demonstrated that endothelium-dependent micro- and macrovascular relaxation is severely blunted in those mice. The aim of the present study was to investigate this phenomenon on a cellular level.

Methods and Results: Microvascular endothelial cells were isolated from hearts of wild-type (WT) and ATGL(−/−) mice and

immortalized to create WT-MyEnd and ATGL(−/−)-MyEnd cells, respectively. Cells were cultured to passage 2–4 and characterized for different parameters of PPARα and NO signaling. Real-time PCR analysis revealed that PPARα mRNA expression was reduced more than 50% in ATGL(−/−)-MyEnd cells, which reflects well the situation in ATGL-deficient hearts. By contrast, mRNA expression of peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) was similar in WT and ATGL-deficient cells. Likewise, mRNA levels of different PPARα target genes were unaffected by knockout of ATGL. Protein expression and activity of endothelial nitric oxide synthase (eNOS) were measured by Western blot and conversion of L-[^{3}H]arginine into L-[^{3}H]citrulline, respectively. Both parameters were almost identical in WT-MyEnd and ATGL(−/−)-MyEnd cells. To investigate if accelerated breakdown of NO due to increased formation of reactive oxygen species (ROS) occurs in ATGL-deficient endothelial cells, we measured mRNA and protein expression of xanthine oxidase (XO) and NADPH oxidase isoforms NOX2 and NOX4. However, no differences in mRNA or protein expression were observed. A similar result was achieved in experiments measuring ROS formating in homogenates of WT and ATGL-deficient cells using lucigenin-enhanced chemiluminescence.

Conclusions: Our results indicate that, albeit impaired PPARα, NO signaling and bioavailability are not compromised by ATGL deficiency in microvascular endothelial cells. Currently, other mechanisms responsible for the observed endothelial dysfunction are investigated in our laboratory.

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A23

The atypical cannabinoid O-1602 shows antitumorigenic effects in colon cancer cells and reduces tumor growth in a colitis-associated colon cancer model

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Background: Cannabinoids and the endocannabinoid system play an important role the protection against inflammation and cancer. O-1602, a synthetic cannabinoid with antiinflammatory properties, has little affinity to classical cannabinoid receptors but shows cannabinoid-like effects. In the present study, we were interested whether O-1602 produces antitumorigenic effects in colon cancer cells and whether it could reduce tumorigenesis in the colon *in vivo*.

Methods: We used the cell lines HT-29 and SW480 to study the effect of O-1602 on viability and apoptosis in cancer cells. A mouse model of colitis-associated colon cancer was employed to study the effect of O-1602 on tumor growth *in vivo*.

Results: Viability of HT-29 and SW480 cells was decreased and apoptosis was promoted by O-1602 in a concentration-dependent manner (0.1–10 µM). In the mouse model, treatment with O-1602 (3 mg/kg, i.p., 12x, every second day during a period of 3 weeks) reduced tumor area by 50% and tumor incidence by 30%. Histological scoring showed a significant decrease in tumor load. In tumor tissue, O-1602 decreased levels of phosphorylated activator of transcription factor 3 (pSTAT3) by 50% and tumor necrosis factor alpha (TNF-α) by around 45%. Treatment with O-1602 led to a ten-fold increase in the expression of the tumor suppressor p53.

Conclusions: O-1602 exerts antitumorigenic effects by targeting colon cancer cells as well as proinflammatory pathways known to promote colitis-associated tumorigenesis, thus providing a novel insight into antitumorigenic mechanisms of atypical cannabinoids. As O-1602 is free of central sedation, it could be an interesting compound for the treatment of colon and possibly other cancers.

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A24

The relaxation of non-pregnant rat myometrium by resveratrol with participation of the NO–cGMP pathway

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Background: Resveratrol (RSV) is a phytoalexin produced by grapevines. The benefit of resveratrol to health is widely reported. Resveratrol has been found to promote vascular relaxation but its mechanism of action is unclear. Data about an influence of RSV on the contractility of smooth muscles of the uterus are not available. It has been claimed that NO promotes uterine relaxation by the elevation of cyclic GMP. It is generally accepted that NO increases the intracellular cGMP concentration through activation of solubile guanylate cyclase (sGC). The aim of our study were to investigate the effects of RSV on the contractility of rat uterus and to investigate the involvement of the NO–cGMP pathway in the relaxant effect of RSV on spontaneous rhythmic contractions (SRC) and phasic contractions provoked by oxytocin.

Methods: Uterine strips were obtained from virgin female Wistar rats in oestrus. Strips were mounted into organ bath for recording isometric tension in Krebs-Ringer solution. Experiments followed a multiple curve design. In order to test the involvement of the NO–cGMP pathway in the mechanism of action of RSV, a methylene blue (methylthionine chloride; MB) inhibitor of sGC was used.

Results: RSV induced a concentration-dependent relaxation of SRC with $pD_2 = 4.53$ and E_{max} of 89% and of contractions provoked by oxytocin with $pD_2 = 4.66$ and E_{max} of 94% ($p < 0.05$). MB (10 µM) antagonized the response to RSV in both oxytocin-induced contractions and SRC with $pD_2 = 4.31$ and $pD_2 = 4.24$, $E_{max} = 76\%$ and $E_{max} = 67\%$, respectively.

Conclusions: RSV is a uterine relaxant and can be used in tocolysis. The antagonism by MB of the RSV effect suggests that NO–cGMP pathways are involved in RSV action on the contractions of rat uterus. However, the relative resistance to MB of resveratrol's effects at concentrations higher than 30 µM indicated an additional mechanism of action.

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A25

Improving the low solubility of resveratrol

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Background: Resveratrol, a polyphenol mainly present in grapes and red wine, demonstrated interesting biomedical properties for its cardioprotective action due to inhibition of the oxidation of low-density lipoprotein (LDL) and of platelet aggregation, inhibitory effects on cancer promotion and propagation and anti-inflammatory activities. These potential therapeutic and prophylactic applications are limited by the low bioavailability caused by its physical properties. Additionally, resveratrol has low water solubility and stability making its clinical success a formidable technological and medical challenge. The aim of this work is to present results of improvement of solubility of resveratrol through micellar and liposomal incorporation.

Methods: Solubilization of resveratrol in six different bile acid solutions (cholic acid and its keto derivates) was investigated after 18 hours of mixing 2 mg of resveratrol in 2 ml of bile acid solutions in pH 7 buffer at room temperature. Liposome preparations containing pure resveratrol, resveratrol with vitamin C and resveratrol with vitamin E were prepared using the thin film hydration method. Resveratrol content was analyzed using HPLC with UV/DAD detection.

Results: The analysis of solubilization of resveratrol showed that keto derivatives of cholic acid have greater ability to solubilize resveratrol than cholic acid, and that this efficiency increases with the number of keto groups present in bile acid. The most effective acid for the solubilization of resveratrol was 3,7,12-triketocholic acid. Also, it has been shown that the efficiency of incorporation of resveratrol in liposomes increases with the amount of added resveratrol during preparation and the presence of vitamin C or E in the formulation, and that these preparations have satisfactory characteristics.

Conclusions: Experiments carried out in this study provide useful information for potential development of different dietary and pharmaceutical resveratrol products.

A26

Understanding subtype-selective allosteric modulation of $GABA_A$ receptors

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Background: The γ-aminobutyric acid type A ($GABA_A$) receptors are the major inhibitory neurotransmitter receptors of the central nervous system. Benzodiazepine (Bz)-site ligands bind at the α/γ interface and can enhance GABA-induced Cl^- currents. The efficacy of certain benzodiazepines strongly depends on the type of α(1,2,3,5) subunits in the receptors. Functionally selective compounds for α2/3 can be anxiolytic without having the side effect of sedation. The molecular basis for functional selectivity is investigated in this work.

Methods: Two-electrode voltage-clamp electrophysiology recordings were performed in wild-type and mutated receptors expressed in *Xenopus laevis* oocytes. Modelling, docking and molecular dynamics simulation studies of α1γ2 and α3γ2-containing receptors were performed to understand Bz-ligand interaction with the different α subunits.

Results: Electrophysiology recordings identified flumazenil as a null modulator in α1 and a weak plus modulator in α3-containing receptors. A sequence comparison between the α1 and α3 subunit revealed the residue R228 as unique for the α3 subunit among all α subunits. α3R228A-mutated receptors completely lost their ability to respond to flumazenil. This amino acid is part of the so-called loop C, a several-residues-spanning segment that forms part of the ligand-binding site with a highly variable sequence. The functionally α3-selective ligand flumazenil was docked into the α/γ interface. The flumazenil-bound state in the α1 subtype has already been studied previously [1] and was used for comparison. Our results indicate that the binding mode of flumazenil in α1 and α3-containing receptors is very similar.

Conclusions: The models made in this study show improved properties in certain variable segments that could not be resolved in the previously published models [1]. For understanding the role of α3R228, more models and docking computations have to be made on the basis of these improvements to explore possible conformations.

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A27

Herb-drug interactions: the influence of essential oil of caraway (*Carum carvi* L.) on the pharmacokinetics of paracetamol

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Background: Despite the widespread use of herbal medicines, documented herb-drug interactions are sparse. Caraway (*Carum carvi* L.) is an aromatic plant from the Apiaceae family, widely used to flavor foods, as addition to fragrances, and for medical preparations. This survey examined the effects of chronic caraway essential oil pretreatment on paracetamol pharmacokinetics in male mice.

Methods: The essential oil (EO) of caraway, prepared as emulsion for peroral use, was applied to male mice during 5 consecutive days. Paracetamol, in the dose of 200 mg/kg, was applied p.o. or i.p. 2 hours after the last EO dose. Blood samples for pharmacokinetic assay were collected from the tail vein before paracetamol intake and 10, 30, 60, 90 and 120 min thereafter. Blood concentrations of paracetamol were determined by HPLC and the pharmacokinetic parameters were calculated using the WinNonlin software.

Results: In the control group, pharmacokinetic parameters of paracetamol after p.o. and i.p. application were rather congruent. Caraway EO pretreatment induced a statistically significant augmentation of pharmacokinetic parameters (C_{max}, AUC, AUC_∞) of i.p. applied paracetamol, speaking in favor of enhanced body exposure to the drug. However, after p.o. application of paracetamol, the pharmacokinetic data showed a significant decrease compared to control values, indicating a decrease in drug presence in the organism.

Conclusions: The chronic intake of caraway EO influences the pharmacokinetic properties of both orally and intraperitoneally applied paracetamol. Further investigation of the exact pathway of this herb-drug interaction is needed, as well as the assessment of its real clinical significance.

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A28

The role of nitric oxide and endothelin on optic nerve head blood flow autoregulation

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Background: Autoregulation is defined as the ability of a vascular bed to keep its blood flow constant despite changes in perfusion pressure. While several studies have investigated choroidal blood flow regulation, only few data are available for the optic nerve head (ONH). The aim of the present study was to explore the potential role of a potent vasodilator (nitric oxide) and a potent vasoconstrictor (endothelin-1) in ONH autoregulation.
Methods: Two randomized, double-blind, placebo-controlled, cross-over studies were performed. Eighteen subjects received either a nitric oxide synthase (NOS) inhibitor (L-NMMA) or placebo. Fifteen subjects received either an endothelin ET_A receptor antagonist (BQ-123) or placebo on two trial days. Isometric exercise (squatting) was performed to increase ocular perfusion pressure (OPP). ONH blood flow (ONHBF) was measured continuously by means of laser Doppler flowmetry. OPP was calculated as ⅔ × (mean arterial pressure) − (intraocular pressure).
Results: During all experiments the response in ONHBF was less pronounced than the response in OPP indicating autoregulation. L-NMMA had no influence on the response of ONHBF to isometric exercise ($p = 0.27$). When BQ-123 was administered the increase in ONHBF during squatting was more pronounced than during placebo ($p < 0.01$) leading to a left-shift of the pressure/flow curve.
Conclusions: The present data confirm previously published observations that ONHBF shows some autoregulatory capacity during changes in OPP. Nitric oxide does not seem to be involved in the regulatory mechanisms during isometric exercise. In contrast, endothelin-1 seems to provide some of the vasoconstrictor tone that counteracts the increase in OPP during isometric exercise.
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A29

The role of potassium channels in the mechanism of vasodilatation of human umbilical vein induced by resveratrol

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Background: Resveratrol (RSV) is polyphenol present in various kinds of food which we consume on daily basis. In the last ten years there has been growing importance of RSV in the literature. It is well known that RSV has many different beneficial effects on human health. RSV is partly responsible for the cardiovascular benefits of red wine. However, the mechanism of vasodilatation induced by RSV is unclear. There are many target molecules of RSV which could play an important role in the mechanism of action of RSV. The aim of our study was to define the role of K^+ channels in the RSV-induced vasodilatation of human umbilical vein (HUV) denuded of endothelium.
Methods: HUV rings were precontracted with serotonin (5-HT) or with 100 mM K^+. Concentration-response curves were obtained by adding increasing concentrations of RSV, from 1 to 100 μM. In order to test the role of vascular K^+ channels in this vasorelaxation, various K^+ channels blockers were added to the organ bath 20 minutes before RSV.
Results: RSV induced concentration-dependent vasodilatation (EC_{50} = 16.5 μM). A selective blocker of ATP-sensitive K^+ channels, glibenclamide (10 mM), induced a significant shift to the right ($p < 0.05$) of the concentration-response curve for RSV (EC_{50} = 38.0 μM). 4-Aminopyridine (4-AP, 1 mM), a blocker of voltage-gated K^+ channels, also induced a significant shift to the right (EC_{50} = 49.0 μM, $p < 0.05$). Tetraethylamonium (TEA, 10 mM), which predominantly inhibits large conductance Ca^{2+}-activated K^+ channels, and barium chloride ($BaCl_2$, 1 mM), which blocks inward rectifier K^+ channels, antagonized the response to RSV (EC_{50} = 28.0 μM, $p < 0.05$ and EC_{50} = 50 μM, $p < 0.05$, respectively). Concentrations of RSV above 10 μM relaxed HUV rings bathed in a medium containing 100 mM K^+ (EC_{50} = 47 μM, $p < 0.05$).
Conclusions: These results suggest that RSV induces endothelium-independent vasorelaxation of HUV. K^+ channels are involved in the vasodilatation of HUV induced by RSV, when RSV is applied in concentrations up to 10 μM. However, it seems that RSV has an additional, K^+ channel-independent mechanism of action when applied in concentrations higher than 10 μM.

A30

The effect of chronic fluoxetine administration on anxiety-like behavior and expression of 5-HT-related proteins in rats with constitutively altered 5-HT homeostasis

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Background: Serotonin (5-HT), a monoamine neurotransmitter/neuromodulator widely distributed in the brain, plays an important role in variety of behaviors and behavioral disorders including anxiety and depression. Therapeutic effects of fluoxetine, a widely prescribed selective serotonin reuptake inhibitor (SSRI), include inhibition of 5-HT transporters (SERT) and desensitization of $5\text{-}HT_{1A}$ receptors which leads to the enhancement of 5-HT transmission. The patient's ability to respond to treatment with fluoxetine (and other SSRIs) is greatly variable and genetic SERT variants, which are believed to influence serotonergic neurotransmission, might influence interindividual variability in the pharamacotherapeutic response. In our research we use Wistar-Zagreb 5HT rats, an animal model with constitutively high or low SERT activity (termed high-5HT and low-5HT subline), developed by selective breeding toward extremes of this parameter in our laboratory. In addition to differential regulation of peripheral serotonin, 5HT-sublines also displayed constituve alteration in brain 5-HT homeostasis. Thus we have demonstrated previously that animals from the high-5HT subline exhibit increased anxiety-like behaviour; however, no measurable differences in baseline functionality or expression of $5\text{-}HT_{1A}$ receptors between sublines were found. Here, we examined the response of 5HT-sublines to the chronic administration of fluoxetine.
Methods: We treated male rats from high-5HT and low-5HT sublines with fluoxetine (6 mg/kg, i.p) for 27 days. Anxiety-like behavior was evaluated 24 h after the 23rd injection by means of the elevated-plus maze paradigm. The expression of SERT and $5\text{-}HT_{1A}$ receptors was assessed in frontal cortices, 48 h after the last

injection, using RT-PCR. At the same time point we also measured cortical 5-HT levels using an ELISA assay.

Results: Fluoxetine-induced reduction of anxiety-like behavior, measured as increased time spent in open arms, was only observed in high-5HT animals. Furthermore, chronic fluoxetine administration increased the expression of SERT in high-5HT animals and decreased it in animals from the low-5HT subline. The expression of cortical 5-HT_{1A} receptors was not affected in high-5HT animals, whereas in the low-5HT subline a reduction in expression was noted. Tissue levels of 5-HT in fluoxetine-treated animals were significantly higher in the high-5HT subline as compared to the low-5HT subline.

Conclusions: The present data demonstrate that fluoxetine-induced changes in 5-HT regulation exhibit clear differences between hypo- and hyperserotonergic rats. These results may contribute to the better understanding of the interindividual variability in the outcome of psychotherapy with serotonin-related drugs.

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A31

Pharmacological applications of natural peptide libraries

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Background: The diversity in nature has long been and still is one of the biggest resources of pharmaceutical lead compounds and many natural products often exhibit biological activity against unrelated biological targets, thus providing us with starting points for drug development. Natural peptides of great number and diversity occur in all organisms from plants to microbes to man. Examples for such rich and yet largely untapped libraries of bioactive compounds are animal venom peptides, insect peptide hormones or plant defense peptides [1]. Our goals are (i) to discover and characterize novel bioactive peptides, (ii) to screen their pharmacological activity *in vitro*, (iii) to synthesize optimized peptide compounds and (iv) to determine their potential as pre-clinical drug candidates.

Methods: As proof-of-concept we have used a genome-mining approach or mass spectrometry and peptidomics to determine the occurrence and molecular structure of naturally-occurring peptides and have investigated their pharmacological profile on human oxytocin and vasopressin receptors, representative members of the GPCR family, as well as their anti-proliferative activity on cells of the human immune system. Circular plant peptides have been identified as potent immunosuppressive agents and promise great potential as templates for pharmaceutical applications due to their enormous stability and sequence diversity [2]. On the other hand we are exploring the pharmacological potential of endogenous insect vasotocin-like peptide hormones and marine cone-snail venom peptides as receptor-subtype selective ligands for the treatment of a wide range of challenging, but yet untreatable diseases.

Discussion: Unlike small molecules, peptides are just at the beginning as potential drug sources and still face a range of significant drug development challenges including efficient drug delivery, oral bioavailability and central penetration. Nevertheless, the ease of synthesis, the vast natural abundance of bioactive peptides and their immense pharmacological potential are so convincing that it is just a question of time until peptides can be utilized as orall bioavailable or even CNS-penetrating drugs together with an efficient delivery platform preserving their selectivity and interaction with extracellular targets, yet simultaneously retaining stability to enzymatic degradation.

Acknowledgements: This work was funded by the Austrian Science Fund FWF (grants P22889 and P24743).

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A32

Low affinity histamine uptake into neonatal rat astrocytes does not involve OCT

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Background: Histamine is a double-protonated molecule with corresponding pK_a values of 5.8 and 9.4. Therefore, at physiological pH, histamine exists as an equilibrium mixture of tautomeric cations: the monocation making up 96%, the dication only 3% and the rest being nonprotonated histamine. As a protonated molecule histamine most probably uses a carrier protein in order to cross the cell membrane. In the present work we wanted to determine the kinetic properties of histamine uptake and the influence of other biogenic amines on its transport.

Methods: We performed histamine uptake assays in the model system of cultured neonatal rat astrocytes. The mRNA expression of organic cation transporters (OCTs) was determined by qPCR. Student's *t*-test was used for statistical analysis of uptake data.

Results: Histamine uptake in neonatal rat astrocytes is a bidirectional process, which was found to be dependent on pH and Na^+, but not Cl^--dependent, with low affinity (K_m 116 μM) and high capacity (158 pmol/mg protein). The uptake was inhibited by millimolar concentrations of other biogenic amines (dopamine, noradrenaline and 5-hydroxytryptamine). The histamine metabolite tele-methylhistamine affected both directions of histamine uptake. In spite of the presence of OCT2 in neonatal astrocytes, the OCT inhibitors decynium-22 and corticosterone had no affect on histamine clearance.

Conclusions: Histamine is taken up into astrocytes by low-affinity high-capacity uptake, which involves transporter(s) other than OCT.

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A33

Influence of the 14-alkoxy group and the substitution in position 5 in *N*-methyl-14-alkoxymorphinan-6-ones on *in vitro* and *in vivo* pharmacological activities

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Background: Opioid analgesics are the cornerstone drugs for the treatment of moderate-to-severe pain. Morphine and other analgesics like fentanyl, oxycodone and oxymorphone activate the μ opioid (MOP) receptor, the main type targeted for pharmacotherapy of pain. These drugs share the same pharmacological profiles including severe adverse effects such as respiratory depression, constipation, tolerance and physical dependence. Chemical approaches towards the identification of novel MOP analgesics with reduced side effects include structural modifications of morphinan-6-ones in key positions that are important for binding, selectivity, potency and efficacy at opioid receptors. A representative example is the development of the 14-*O*-methyl-substituted derivative of the clinically used MOP analgesic oxymorphone, namely 14-*O*-methyloxymorphone, and its 5-methyl-substituted analogue, 14-methoxymetopon. The focus of the present work is on structure-activity relationship (SAR) studies and *in vitro* and *in vivo* pharmacological investigations on a series of opioid ligands differently substituted in positions 5 and 14 of the morphinan skeleton.
Methods: Radioligand binding assays were performed using rodent brain membranes. Mouse vas deferens (MVD) and guinea-pig ileum (GPI) bioassays, and [^{35}S]GTPγS functional assays with Chinese hamster ovary (CHO) cells expressing human opioid receptors were used to assess opioid agonism. Antinociceptive properties were established using hot-plate and writhing tests in mice after subcutaneous (s.c.) administration.
Results: Binding studies showed that all derivatives display affinities in the subnanomolar range at the MOP receptor and were MOP receptor-selective. In smooth muscle preparations and CHO cells transfected with MOP receptors they behaved as potent agonists. The differently substituted *N*-methylmorphinan-6-ones produced marked antinociceptive effects in mice when given s.c., being several-fold more potent than morphine. On the basis of the SAR that has emerged, certain modifications in the substitution pattern, e.g. introduction of an alkyl or arylalkyl group in position 14 and/or in position 5, result in interesting alterations in opioid activity by influencing the pharmacological properties of ligands interacting with opioid receptors. Analysis of the *in vitro* and *in vivo* opioid profile for this series of 14-alkoxymorphinans leads to an improved understanding of the relationship between affinity and/or selectivity for opioid receptors, agonist activity, antinociceptive potency and the nature of substituents in morphinans.
Conclusions: These results represent a useful and valuable outcome for the design and optimization of existing structural templates increasing the chance of identifying clinically useful analgesics for superior management of pain.
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A34

Plasma levels of an atropine/scopolamine mixture following ingestion of low doses as food contaminant

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Background: Mass poisoning with *Datura* alkaloids, present in contaminated buckwheat flour occurred in 2003 in Slovenia. A preliminary risk assessment for atropine and scopolamine in the flour was carried out. To obtain more accurate information, we performed a randomised, double-blind, placebo-controlled, crossover study in 20 healthy adult volunteers exposed to low doses of an atropine/scopolamine mixture as food contaminant.
Methods: The volunteers ingested a traditional Slovenian dish, "žganci", made of boiled buckwheat flour to which 5 doses of an atropine/scopolamine mixture in 2:1 ratio were added. Besides the control meal, the volunteers ingested the following doses of atropine/scopolamine mixture: 0.12 of atropine/0.10 of scopolamine (0.32 expressed as atropine) μg/kg body mass (bm), 0.37/0.29 (0.95) μg/kg bm, 1.22/0.95 (3.12), 3.58/2.81 (9.20) μg/kg bm and 12.10/9.50 (31.10) μg/kg bm. Changes in body temperature, heart rate, saliva and sweat secretion, pupil size and near-point vision as well as subjective symptoms were checked at regular intervals for up to 4 hours after the ingestion. To determine the low levels of each alkaloid in the plasma, samples of venous blood were taken before the ingestion and 30, 60, 120 and 240 minutes afterwards, and analysed by a validated liquid chromatography/tandem mass spectrometry. The study was approved by the national Medical Ethics Committee.
Results and Conclusions: The maximum effects on salivary and sweat secretion were observed at 90 minutes after ingestion and persisted up to 240 minutes. The maximum effect on heart rate was noted at 120 minutes and lasted until 165 minutes, whereas the maximum effect on pupil size and near-point vision was observed at 240 minutes after the ingestion. The maximum concentrations of alkaloids in the plasma were reached 120 minutes after the ingestion, whereas scopolamine had another smaller peak at 30 minutes. The maximum concentrations of atropine and scopolamine after the highest dose were 2.52 ± 0.19 μg/L and 0.49 ± 0.06 μg/L, respectively. Following the ingestion of the low-dose atropine/scopolamine mixture, the area under the curve (AUC_{0-4h}) of atropine was 2 to 5 times higher than the AUC_{0-4h} of scopolamine which may have been due to the differences in metabolism. The changes in the heart rate were to some degree associated with the maximum plasma concentrations, whereas the time course of the other endpoints was not.

A35

Potentially inappropriate medication use in the elderly in Montenegro

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Background: Potentially inappropriate medications (PIMs) continue to be prescribed and used as first-line treatment for the most vulnerable of older adults, despite evidence of poor outcomes from the use of PIMs in older adults. PIM use is an important and preventable safety concern in the care of elderly patients and has been associated with adverse drug reactions, hospitalization and mortality. The aim of this study was to estimate the prevalence of PIM use among the elderly in Montenegro in 2012.
Methods: The data about prescribed medications in the elderly were taken mostly from a data base of randomly selected general practitioners from various cities of Montenegro. Other sources of information were data bases of nursing homes. The study included information on 108 patients. PIMs were identified using the latest Beers criteria published in 2012. Results of our study were compared with similar researches that had been conducted in countries with a developed pharmacotherapeutic practice.
Results: The study showed that 41.7% of Montenegrin patients received at least one PIM. Moreover, 31.1% of them used two or more PIMs. The most frequent PIMs, independent of diagnosis or medical condition, were NSAIDs (18.5%), followed by

benzodiazepines (15.7%), digoxin (9.3%) and metoclopramide (3.7%). Potentially serious drug-drug interactions, according to the Beers criteria, were detected in 1% of all patients included in the study. Compared to this condition, for example, a study conducted in Sweden in 2005 [1] showed that the prevalence for PIMs was 17%; for anticholinergic drugs 6%, long-acting benzodiazepines 5%, psychotropic drugs 5% and potentially serious drug-drug interactions 4%. Logistic regression revealed no direct correlation between the total number of medications taken and the likelihood of receiving an inappropriate drug in Montenegrin patients.

Conclusions: Comparing the results of our study with results obtained in countries with a developed pharmacotherapeutic practice, it was shown that the use of PIMs in the elderly in Montenegro in 2012 was significantly higher. Based on the results of our study, the use of drugs among elderly in Montenegro, in most cases, doesn't conform to the principles of rational pharmacotherapy practice. This study emphasizes the need for continued provider education to inform prescribers of the potential risks of using certain medications in the elderly and to improve prescribing practices.

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A36

Tolerance to nitroglycerin through proteasomal degradation of aldehyde dehydrogenase-2 in a genetic mouse model of ascorbate deficiency

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Background: L-Gulonolactone oxidase-deficient mice (Gulo(−/−)) were used to study the effects of ascorbate deficiency on aortic relaxation and lowering of blood pressure by nitroglycerin (GTN). Special emphasis was given to changes in expression and activity of vascular aldehyde dehydrogenase-2 (ALDH2), which has been recently characterized as key enzyme of vascular GTN bioactivation.

Methods: Ascorbate deficiency was induced in Gulo(−/−) mice by ascorbate deprivation for 4 weeks. Some of the animals were concomitantly treated with the proteasome inhibitor bortezomib and effects were compared with ascorbate-supplemented Gulo(−/−), untreated or nitrate-tolerant wild-type mice. Aortic relaxation of the experimental groups to GTN, acetylcholine and the NO donor diethylamine NONOate was studied. Changes in mRNA and protein expression of vascular ALDH2 were quantified by real-time PCR and immunoblotting, respectively, and aortic GTN denitration rates were determined by thin layer chromatography. Ubiquitination of proteins was analyzed by immunoblotting.

Results: Like GTN treatment, ascorbate deprivation induced vascular tolerance to GTN that was associated with markedly decreased rates of GTN denitration. Ascorbate deficiency did not affect ALDH2 mRNA levels, but reduced ALDH2 protein expression and the total amount of ubiquitinated proteins to about 40% of wild-type controls. These effects were largely prevented by ascorbate supplementation or treating Gulo(−/−) mice with the 26S proteasome inhibitor bortezomib.

Conclusions: Our data indicate that ascorbate deficiency results in vascular tolerance to GTN via proteasomal degradation of ALDH2. The results support the view that impaired ALDH2-catalyzed metabolism of GTN contributes significantly to the development of vascular nitrate tolerance and reveal a hitherto unrecognized protective effect of ascorbate in the vasculature.

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A37

Vascular bioactivation of nitroglycerin: reaction mechanism revealed by crystal structure of aldehyde dehydrogenase-2

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Background: Aldehdyde dehydrogenase-2 (ALDH2) is essential for the detoxification of ethanol in the liver but also catalyzes vascular bioactivation of nitroglycerin (glycerol trinitrate, GTN) to its active metabolite nitric oxide (NO), which causes vasodilation through accumulation of cyclic GMP. The clinical use of GTN as a vasodilator is hampered by loss of efficacy after prolonged treatment, and there is strong evidence that this results from mechanism-based inactivation of ALDH2 by GTN. The precise mechanism of the ALDH2/GTN reaction as well as the identity of the inactivated enzyme species is still elusive.

Methods: To address these issues, we have determined the crystal structure of an ALDH2 mutant in complex with GTN. In addition, the 3D structures of a reaction intermediate and of the GTN-inactivated enzyme were resolved.

Results: GTN is bound to the active site by hydrogen bonds to the so-called oxyanion hole, to Gln268 to Ser301 and by hydrophobic interactions. It is held in a position ideal for the nucleophilic attack of the active site Cys302. After this nucleophilic attack, a thionitrate is formed and 1,2-glyceryl dinitrate is released. This thionitrate was observed in the second crystal structure. It is in a position similar to the corresponding atoms of GTN and stabilized by hydrogen bonds to the oxyanion hole. Finally, the structure of the inactivated enzyme species determined to a resolution of 1.7 Å agrees well with mass spectrometric results, suggesting that exposure to GTN causes in oxidation of the active site Cys302 to sulfinic acid.

Conclusions: Our results provide important new insights into the complex mechanism of ALDH2-catalyzed GTN bioactivation and the development of nitrate tolerance.

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A38

The anti-addiction drug ibogaine inhibits cardiac ion channels: a study to assess the drug's proarrhythmic potential

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Background: The plant alkaloid ibogaine has shown promising anti-addictive properties in animals and humans. Although not licensed as a therapeutic drug, and despite evidence that ibogaine may disturb the rhythm of the heart, this alkaloid is used as an anti-addiction drug in alternative medicine. We have recently reported

that therapeutic concentrations of ibogaine inhibit human ERG (hERG) potassium channels, and thereby uncovered a mechanism by which the drug may induce life-threatening cardiac arrhythmias.
Methods: Here, to assess the drug's proarrhythmic potential in more detail, we studied the effects of ibogaine and its congener 18-methoxycoronaridine (18-MC) on various cardiac voltage-gated ion channels by using the whole cell patch clamp technique. Besides heterologously expressed ion channels in TSA-201 cells, native channels in isolated mouse and guinea pig ventricular cardiomyocytes were also studied. Finally, we performed computer simulations to estimate drug effects on the human cardiac action potential (AP).
Results: We confirmed that heterologously expressed hERG currents are reduced by ibogaine in low micromolar concentrations (IC_{50}, 4 µM). Moreover, at higher concentration, the drug also reduced human $Na_V1.5$ sodium currents. Experiments on mouse cardiomyocytes confirmed that ibogaine also inhibits voltage-gated ion channels in their native environment. 18-MC also reduced cardiac ion currents, but less potently than ibogaine. Although blocking hERG channels, ibogaine did not prolong the AP in guinea-pig cardiomyocytes at low micromolar concentrations. Higher concentrations (>10 µM) even shortened the AP. Finally, implementation of ibogaine's inhibitory effects on ion channels in a computer model of a human ventricular cardiomyocyte suggested that calcium channel blockade by the drug counteracts the AP-prolonging effect generated by hERG inhibition.
Conclusions: Because ibogaine inhibits cardiac ion channels in therapeutic concentrations, the drug is potentially proarrhythmic. The risk of its administration, however, is possibly reduced by the fact that the drug also shows antiarrhythmic properties.
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A39

Adverse drug reactions and polypharmacy in cardiac patients

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Background: Adverse drug reactions (ADR) appear more frequently then what is actually reported and registered. The aim was to establish an intensive monitoring system and to analyze ADR in hospitalized patients.
Methods: The prospective study covered 200 patients hospitalized in the Cardiology Center, Clinical Center of Montenegro. ADR were recorded in the following way: patients were interviewed on the basis of a symptoms list and any signs which could point to eventual ADR. Secondly, lab tests and other available parameters were monitored.
Results: At the time when interviews took place, patients received on average 8.0 ± 2.6 medicines (2–17). In total, 67 patients (33%) had 75 ADR. Twenty one ADR (28%) are classified as serious. Fifty four ADR resulted in the recovery of the patient (72%), eight had as an outcome prolonged hospitalization (11%), another eight were life threatening (11%), while five ADR (6%) were the cause of the hospitalization. The most frequent ADR which had as an outcome admission to hospital were caused by digoxin (40%), prolonged hospital stay by furosemide (38%), while the most frequent registered ADR which were life threatening were caused by streptokinase (50%).
Conclusions: Monitoring ADR in patients using cardiovascular drugs is a matter of importance since this class of medicines is usually used by elderly patients with critical conditions and accompanying diseases. Considering increased use of cardiovascular drugs and limitations in pre-marketing trials for drug safety evaluation, post marketing evaluation of adverse drug reactions induced by this class of medicinal products seems necessary. Additional educational efforts could affirm the rationalization of the pharmacotherapy.

A40

Uremia-induced lysine modifications transform plasma albumin into a high-density lipoprotein receptor inhibitor

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Background: Protein damage induced by retained uremic solutes may be an important component in the pathophysiology of advanced renal disease. Albumin isolated from hemodialysis patients was recently shown to block high-density lipoprotein (HDL) receptor-mediated cholesterol uptake. However, post-translational modifications that render albumin a scavenger receptor class B type I (SR-BI) ligand are not known. We hypothesized that the elimination of positive charge through oxidation of albumin-lysine residues is required to generate recognition motifs for SR-BI. Since carbamylation and carboxymethylation are major lysine modifications *in vivo*, we aimed at investigating their influence on the binding properties of HD-albumin to SR-BI.
Methods: Albumin from HD patients and control subjects was isolated from serum by affinity chromatography. Mass spectrometry was used to study structurally defined lysine modifications on HD-albumin. Competition experiments (displacement of Alexa-labeled HDL) were performed to assess binding affinity of modified albumin to SR-BI.
Results: We identified a significant increase in 3-chlorotyrosine, carbamyllysine and carboxymethyllysine content on HD-albumin. Competition experiments revealed that chlorolysine and carbamyllysine mediate binding of AOPP-albumin to SR-BI whereas binding properties of carboxmethyllysine did not differ significantly from native albumin.
Conclusions: Oxidation and carbamylation of serum albumin generate relevant SR-BI antagonists in renal disease that may interfere with SR-BI-mediated reverse cholesterol transport. Displacement of HDL from its major receptor may result in decreased hepatic cholesterol uptake, depressed HDL metabolism and abnormal HDL composition and function. Dysfunctional reverse cholesterol transfer may contribute to the excessive cardiovascular mortality observed in patients suffering from renal disease.
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A41

Impaired L-type Ca^{2+} channel function in the dystrophic heart

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Background: Duchenne muscular dystrophy (DMD), caused by mutations in the dystrophin gene, is an inherited disease characterized by progressive muscle weakness and degeneration. Besides the relatively well described skeletal muscle degenerative processes, DMD is associated with cardiovascular complications including cardiomyopathy and cardiac arrhythmias. The current understanding of the patho-mechanisms is still very limited, but recent research suggests, that dysfunctional ion channels in dystrophic cardiomyocytes considerably contribute to the cardiovascular complications.
Methods: By using the whole cell patch clamp technique, the functional properties of voltage-gated L-type Ca^{2+} channels were studied in ventricular cardiomyocytes derived from normal and dystrophic mice. Physiological consequences were followed up by investigating action potentials and by comparing surface ECG recordings in wild-type and dystrophic mice. Besides the commonly used dystrophin-deficient mdx mouse model, this study is amongst the first to additionally include the dystrophin-utrophin double-deficient mouse model for DMD.
Results: We found that the voltage-dependent inactivation of L-type Ca^{2+} channels is significantly reduced in dystrophic cardiomyocytes. Moreover, in cardiomyocytes derived from dystrophic adult animals, current density levels are significantly increased. Action potential duration was not prolonged in dystrophic murine cardiomyocytes, but incorporating the observed reduction in current density into a computer model of a human cardiomyocyte resulted in a marked prolongation. Physiological relevance was further suggested by an acceleration of atrioventricular nodal conduction and a prolongation of ventricular repolarisation in the ECG.
Conclusions: L-type Ca^{2+} channels are significantly impaired in dystrophic cardiomyocytes and likely contribute to the cardiovascular complications associated with Duchenne muscular dystrophy.
Background: This work was supported by the Austrian Science Fund (FWF, grant P23060).

A42

Histone deacetylase inhibitors, glutamatergic drugs and deep brain stimulation rescue resistance to fear extinction in a genetic mouse model

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Background: Impaired extinction of fear is a hallmark of a variety of disabling anxiety disorders including panic disorder, post-traumatic stress disorder, social anxiety disorder and specific phobias. Therapeutic interventions that reverse deficits in fear extinction represent a tractable approach to treating these disorders. We recently revealed that 129S1/SvImJ (129S1) mice are unable to extinguish learned fear responses following 'normal' fear conditioning, establishing these mice as a clinically relevant model to identify extinction-facilitating targets.
Methods: 129S1 mice were subjected to multi-trial 'normal' and 'weak' cued fear conditioning/extinction paradigms and novel treatment strategies to rescue deficient extinction were tested.
Results: Results revealed that 'weak' fear conditioning permitted fear reduction during massed extinction training in 129S1 mice, but also revealed a specific deficiency in extinction memory consolidation/retrieval. Rescue of this impaired extinction consolidation/retrieval was achieved with D-cycloserine (*N*-methly-D-aspartate partial agonist) or MS-275 (histone deacetylase (HDAC) inhibitor), applied after extinction training. We next examined the ability of different drugs and non-pharmacological manipulations to rescue the extreme fear extinction deficit in 129S1 following 'normal' fear conditioning with the ultimate aim to produce low fear levels in extinction retrieval tests. Results showed that rescue of both impaired extinction acquisition and deficient extinction consolidation/retrieval was achieved with prior extinction training administration of valproic acid (a GABAergic enhancer and HDAC inhibitor) or AMN082 [metabotropic glutamate receptor 7 (mGlu7) agonist], while MS-275 or PEPA (AMPA receptor potentiator) failed to affect extinction acquisition in 129S1 mice. Lastly, deep brain stimulation (DBS) by applying high frequency stimulation to the nucleus accumbens (ventral striatum) during extinction training, indeed significantly reduced fear during extinction retrieval compared to sham stimulation controls.
Conclusions: Collectively, these data identify potential beneficial effects of various drug treatments and DBS, including those with HDAC inhibiting or mGlu7 agonism properties, as adjuncts to facilitate the outcome of exposure-based therapies for anxiety disorders.
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A43

Discovery and biological evaluation of a diphenethylamine derivative (HS665), a highly potent and selective κ opioid receptor agonist

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Background: Activation of the κ opioid (KOP) receptor results in antinociceptive actions, while it is not involved in the unwanted effects including respiratory depression, dependence or abuse liability, as in the case of the μ opioid (MOP) receptor. Therefore, KOP agonists appear to possess some advantages over the most widely used MOR analgesics. Besides the analgesic activity, KOP agonists have also shown other beneficial effects such as anti-pruritic, anti-arthritic, anti-inflammatory, and neuroprotective effects. At present, the main classes of available chemically distinct KOP agonists include benzomorphans, morphinans, arylacetamides, diterpenes and peptides. Herein, we present a new molecular scaffold for KOP ligands of the class of diphenethylamines and biological investigations on *in vitro* and *in vivo* opioid activities.
Methods: Synthesis of the novel KOP ligands was accomplished by multi-step syntheses. Chinese hamster ovary (CHO) cell membranes expressing human opioid receptors were used in radioligand binding and [^{35}S]GTPγS functional assays. Antinociceptive activities were assessed in mice using the writhing test.
Results: Several novel ligands were synthesized and pharmacologically evaluated. Among them, HS665 proved to be the derivative with the highest selectivity for the KOP receptor vs. the other two types, MOP and δ opioid (DOP) receptors (selectivity ratios KOP/MOP >1,000 and KOP/DOP >20,000), and KOP agonist

potency. *In vivo*, this derivative produced dose-dependent and significant antinociceptive actions in a mouse model of visceral pain (acetic acid-induced writhing) after subcutaneous administration, being equipotent to the standard KOP agonist U50,488. Antinociceptive effects of HS665 were reversed by the KOP-selective antagonist nor-binaltorphimine demonstrating a KOP receptor-mediated mechanism of the antinociceptive action. HS665 has also recently been prepared in tritium-labeled form ([^{3}H]HS665), which can be used as research tool to characterize KOP actions at the cellular and molecular level and to establish the *in vitro* opioid activity profile of new ligands.

Conclusions: This study shows that through appropriate molecular manipulations, a new class of ligands interacting with KOP has been identified, namely HS665 and derivatives thereof. Such novel KOP ligands, besides the scientific value as pharmacological tools, may also have the potential of emerging as novel therapeutics for human disease states.

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A44

Neurobiological correlates of successful deep brain stimulation in a mouse model of high trait affect

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Background: Recent evidence suggests that high-frequency deep brain stimulation of the nucleus accumbens (NAcb-DBS) may represent a novel therapeutic strategy for individuals suffering from treatment-resistant depression although the underlying mechanism of action remains largely unknown. Using a unique psychopathological mouse model of enhanced depression- and anxiety-like behavior (HAB) we investigated behavioral and neurobiological effects of NAcb-DBS.

Methods: HAB mice underwent either chronic treatment with different selective serotonin reuptake inhibitors (SSRIs) or stereotactic surgery to implant DBS electrodes into the NAcb. NAcb-DBS was applied for 1 h daily for seven consecutive days (130 Hz, 100 µA, 60 µs pulse width) and sham-stimulated animals were used as controls. Anxiety- and depression-related behaviors were assessed using established tests with predictive anxiolytic or antidepressant validity. Furthermore, the effects of NAcb-DBS on challenge-induced immediate early gene expression and hippocampal neurogenesis were investigated.

Results: Chronic SSRI treatment did not alter the enhanced depression-like behavior of HAB mice, while repeated, but not single, NAcb-DBS induced robust antidepressant and anxiolytic responses. Interestingly, NAcb-DBS did not affect behavior in normal depression/anxiety animals (NAB), suggesting a preferential effect of NAcb-DBS on pathophysiologically deranged systems. Antidepressant-like effects of NAcb-DBS were associated with normalization of challenge-induced dentate gyrus hypoactivity and modulation of neuronal activity in various brain regions implicated in stress and depression. Furthermore, NAcb-DBS enhanced the blunted hippocampal neurogenesis in HABs.

Conclusions: Taken together we show that the normalization of pathophysiologically enhanced depression-like behavior by repeated NAcb-DBS was associated with normalization of aberrant brain activity and rescue of impaired adult neurogenesis, indicating that DBS affects gene expression as well as neuronal plasticity in a defined, mood-associated network. Finally, it is suggested that SSRI-insensitive HAB mice represent a clinically relevant model for elucidating the neurobiological correlates underlying the observed behavioral effects of NAcb-DBS.

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A45

Dopamine transporter phosphorylation site threonine 53 regulates substrate reuptake and amphetamine-stimulated efflux

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Background: In the central nervous system, levels of extraneuronal dopamine are controlled primarily by the action of the dopamine transporter (DAT). Multiple signaling pathways regulate transport activity, substrate efflux, and other DAT functions through currently unknown mechanisms but presumably by oligomerization, protein-protein interactions and post-translational modification, such as phosphorylation. DAT is phosphorylated by protein kinase C within a serine cluster at the distal end of the cytoplasmic N-terminus, while recent work in model cells revealed proline-directed phosphorylation of rat DAT at membrane proximal residue Thr53. However, specific phosphorylation sites in native DAT under basal condition with associated functional properties have not been ascertained so far.

Methods: We (i) applied mass spectrometry in rodent striatal tissue and heterologous cell systems to identify *in vivo* phosphorylation sites (ii) generated a phospho-specific antibody (pT53Ab) for the confirmation of the identified phosphorylation site and for the determination of a stoichiometry of phosphorylation, involvement of PKC and phosphatase. Functional implications of this identified phosphorylation site have been tested by dopamine uptake and amphetamine-stimulated substrate efflux.

Results: Phosphorylation of Thr53 (pThr53), occurred with a stoichiometry of ~50% under basal condition in rat striatal tissue, was unambiguously identified by mass spectrometry and immunoassay with phospho-specific antibody. pThr53 was strongly increased by phorbol esters and protein phosphatase inhibitors. Mutations of Thr53 to alanine to mimic dephosphorylation reduced dopamine transport V_{max} and ablated amphetamine-induced substrate efflux.

Conclusions: DAT is constitutively phosphorylated at Thr53 and its phosphorylation/dephosphorylation status plays a role in the transport mechanism, particularly in dopamine uptake and amphetamine-stimulated substrate efflux.

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A46

Functional implications of K_V7 channel phosphorylation

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Background: The family of K_V7 potassium channels, particularly $K_V7.2$, $K_V7.3$, and $K_V7.5$ controls neuronal excitability. Numerous neurotransmitters acting via G protein-coupled receptors signaling via Ca^{2+}/calmodulin or depletion of membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) tightly regulate K_V7 channel function. Moreover, the phosphorylation of K_V7 channels has been proposed to play a crucial role. However, *in vivo* phosphorylation sites and their functional implications need to be determined.
Methods: To investigate the role of steady-state K_V7 channel phosphorylation, superior cervical ganglion (SCG) neurons were pretreated for 30 min with different kinase inhibitors (GW8510: 10 µM, SB415286: 1 µM, SB203580: 10 µM, H7: 10 µM) which block CDK5, GSK3, p38 MAPK, and PKC as well as PKA, respectively. Thereafter, oxotremorine M (OxoM) or bradykinin-induced inhibition of the M-currents (primarily through $K_V7.2/7.3$ heterotetramers) was tested.
Results: Inhibition of CDK5 shifted the concentration-response curve for OxoM to the left, but not that of bradykinin. Similarly, GW8510 treatment of tsA201 cells, heterologously expressing $K_V7.2$ channels and M_1 receptors, caused a leftward shift of the OxoM concentration-response relation. In mass-spectrometric studies, several phosphorylated amino acid residues in the C-terminus of native and heterologously expressed $K_V7.2$ channels were detected, 5 of them are located within the putative PIP_2 binding site. CDK5 was predicted to target serines S427 and S446. In contrast to S446, mutation of S427 to alanine significantly increased $K_V7.2$ channel sensitivity towards inhibition via M_1 receptors. Additionally, treatment with GW8510 failed to cause any further effect. Nevertheless, these alanine mutations did not influence the channel-voltage dependence.
Conclusions: Hence, phosphorylation of C-terminal serine residue 427 determines $K_V7.2$ modulation by M_1 muscarinic, but not by B_2 bradykinin receptors, suggesting that the phosphorylation state of S427 regulates the affinity of the $K_V7.2$ C-terminus for PIP_2.
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A47

Environmental enrichment and visceral inflammation regulate stress-induced c-Fos and NPY expression within the dentate gyrus

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Background: The dentate gyrus, a part of the hippocampal formation, is an important brain region regulating the central response to stress. Given that stress resilience depends on genetics and adaptive processes within the brain, the question arises as to whether the acute stress response is modified by chronic environmental or pathological conditions. Therefore we investigated, on the one hand, whether environmental enrichment (EE), a housing condition suggested to promote stress resilience, alters the acute stress response. On the other hand, we assessed whether visceral inflammation, known to be exacerbated by stress, also has an impact on the central stress response.
Methods: Mice were housed in a standard or enriched environment for 10 weeks. During week 10, mice were treated either with iodoacetamide (IAA, 0.1% in drinking water) to induce gastritis or dextran sulfate sodium (DSS, 2% in drinking water) to induce colitis; control mice received plain water. At the end of the treatment period the mice were exposed to water avoidance stress, a psychological stressor, for 30 min. Two hours later post-stress c-Fos expression was measured immunohistochemically and post-stress neuropeptide Y (NPY) expression was investigated in the dentate gyrus by quantitative *in situ* hybridization.
Results: Two-way ANOVA revealed that EE increased post-stress c-Fos expression within the dentate gyrus in control ($p = 0.003$) and gastritis ($p = 0.027$) animals but not in colitis animals ($n = 6–8$). Furthermore, an inhibitory effect of gastritis and colitis on stress-induced c-Fos expression was observed in mice under EE. NPY expression per neuron was altered by both EE ($p = 0.002$) and visceral inflammation ($p = 0.001$). Specifically, post-stress NPY expression was higher in mice under EE (254.1 ± 17.3 grains/neuron) compared to standard-housed mice (191.7 ± 13.7 grains/neuron), independently of treatment conditions, and NPY expression was higher in gastritis mice (275.1 ± 17.5 grains/neuron) compared to control animals (204.2 ± 22.2 grains/neuron), independently of housing conditions.
Conclusions: These results indicate that processing within the dentate gyrus of an acute stress exposure is distinctly altered by EE and gastrointestinal inflammation. EE facilitates, whereas gastritis and colitis blunt the stress-induced neuronal activation visualized by c-Fos. The EE-induced increase in the stress response is paralleled by an increase in the expression of NPY in the dentate gyrus. This shows that NPY is not only involved in the stress response but also participates in EE-evoked neuronal plasticity.
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A48

The differential effect of resveratrol on the renal artery of normal and diabetic rats

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Background: Resveratrol, a polyphenol present in red wine, is thought to be responsible for cardiovascular benefits associated with moderate wine consumption. The mechanisms by which resveratrol causes vasodilatation are uncertain. The aim of this study was to investigate the mechanisms of resveratrol-induced vasorelaxation of rat renal artery (RA) with endothelium in normal and diabetic rats.
Methods: Alloxan was used for the induction of diabetes in rats. Samples of RA were obtained from male Wistar rats and were mounted in an organ bath for recording isometric tension. The experiments followed a multiple curve design.
Results: Resveratrol relaxed RA of normal rats more potently than RA of rats with diabetes (EC_{50} 8 and 50 µM, respectively). L-NAME and methylene blue partly antagonized the relaxation of RA of normal animals only. A nonselective blocker of voltage-gated K^+ (K_V) channels, 4-aminopyridine (4-AP) partly inhibited the relaxation of RA of normal as well as of diabetic rats. However, margatoxin, a selective antagonist of $K_V1.x$ channels, completely antagonized the relaxation of RA of diabetic rats only. Glibenclamide, a highly

selective blocker of ATP-sensitive K^+ channels, did not block resveratrol-induced relaxation in both experimental models.
Conclusions: In conclusion, we have shown that resveratrol induces a strong endothelium-dependent relaxation of RA of normal rats, and that 4-AP-sensitive K^+ channels are involved in this relaxation. In diabetic rats, resveratrol induced NO-independent relaxation and maragtoxin-sensitive K^+ channels are involved.
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A49

Regulation of $Ca_V1.3$ Ca^{2+} channels in cochlear inner hair cells

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Background: $Ca_V1.3$ Ca^{2+} channels are voltage-gated L-type calcium channels, regulating many different physiological functions including neurotransmitter release in cochlear inner hair cells (IHCs) after sound-evoked stimulation. In IHCs, the $Ca_V1.3$ channel shows rapid activation after stimulation with very slow inactivation kinetics, whereas in other tissues (heart and brain) the channel underlies a very strong inactivation. The exact mechanism for the very slow inactivation observed in IHCs is not known so far. Interaction of the auxiliary β subunit with RIM2α, an active zone scaffolding protein, slows down calcium-dependent inactivation (CDI) and voltage-dependent inactivation (VDI); however, it does not fully account for the even slower inactivation kinetics observed in IHCs, as recently published by our lab. RIM binding proteins (RBPs), another group of active zone proteins, are known to interact with RIM and with the $Ca_V1.3$ α1 subunit C-terminus. We therefore hypothesized that interaction of the Ca^{2+} channel with both RIM and RBPs results in a large signaling complex that restrains gating transitions, leading to the slow inactivation kinetics of the $Ca_V1.3$ channel in IHCs.
Methods : In order to investigate whether RBP isoforms and RIM proteins are expressed in inner hair cells, we performed nested PCR. Complex formation of RIM and RBPs with the channel after co-expression in a recombinant system was demonstrated by immunofluorescence microscopy.
Results: So far we could detect RIM2α, RBP2 and RBP3 transcripts in immature and mature IHCs with nested PCR. Preliminary immunofluorescence data also show that RIM and RBPs form a complex that binds to channel-derived peptides.
Conclusions: Our results are in agreement with the hypothesis, justifying further experiments. Therefore we will show the co-localization of $Ca_V1.3$, RIM and RBP in ribbon synapses of IHCs (immunohistochemistry) and study the functional consequences of such complex formation by patch clamp recordings.
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A50

A mouse model to study the C-terminal regulation of $Ca_V1.3$ L-type calcium channels

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Background: $Ca_V1.3$ voltage-gated L-type Ca^{2+} channels (LTCCs) play an important role for hearing, cardiac pacemaking and neuronal excitability. The C-terminus of $Ca_V1.3$ tightly controls channel gating by an intramolecular protein interaction involving two putative α-helices (termed PCRD, DCRD), which form a C-terminal modulatory mechanism (CTM) only in full-length $Ca_V1.3$ variants. In short ($Ca_V1.3_{42A}$ and $Ca_V1.3_{43S}$) $Ca_V1.3$ α1 subunit splice variants CTM is absent which leads to profound changes in channel gating: activation occurs at more negative voltages and Ca^{2+}-dependent inactivation (CDI) is faster.
Methods: We quantified $Ca_V1.3$ splice variants by qPCR analysis and transcript scanning, using different mouse tissues. To assess the physiological relevance of CTM, we generated a mutant mouse strain in which CTM function is disrupted by an HA-tag ($Ca_V1.3$-$DCRD^{HA/HA}$ mice). We used anti-HA antibodies to detect the expression of the HA-tagged full length channel by Western blot analysis.
Results: The short variants $Ca_V1.3_{42A}$ (highest relative abundance in substantia nigra (SN) and ventral tegmental area (VTA)) and $Ca_V1.3_{43S}$ are both less abundant in mouse brain indicating that the full length form $Ca_V1.3_L$ comprises the most abundant form (about 50% of all transcripts). In mouse heart short transcripts are rare and $Ca_V1.3_L$ represents about 90% of all known transcripts. $Ca_V1.3$-$DCRD^{HA/HA}$ mice contain a homozygous interruption of the CTM by disrupting the DCRD helix with an HA-tag. We show that this induces "short" gating properties in this mutant full-length variant. Homozygous mice are viable and display no gross anatomical and functional abnormalities. Expression of the HA-tagged full-length channel could be detected in mouse whole brain membrane preparations. Heterozygous mice show no overt differences in locomotor activity during daytime.
Conclusions: We have successfully generated a mouse model which will enable us to study the physiological role of CTM function *in vivo*. It mimics the (permanent) pharmacological inhibition of CTM function and will thus allow predictions about its potential as a new drug target. Furthermore, the HA-tagged α1 subunit will provide a tool to specifically determine the expression of $Ca_V1.3_L$ channels with anti-HA antibodies in mouse tissues.
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A51

Statins reduce endogenous dolichol levels in the neuroblastoma cell line SH-SY5Y

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Background: Statins are derived from fungi metabolites and are able to inhibit HMG-CoA reductase in the mevalonate pathway. Based on this feature, statins are safely and successfully used in the treatment of cardiovascular diseases correlated with hypercholesterolemia. Additionally, several studies have shown that statins have also pleiotropic effects, like anti-inflammatory, anti-thrombogenic, and anti-proliferative actions. Previously, we were able to show that statins have the potential to directly inhibit the ATP-binding cassette transporter B1 (ABCB1) which plays a key role in the chemoresistance of several tumor types [1].
Methods: In add-back assays the simvastatin-induced caspase 3 activation was measured using fluorescent caspase 3 substrate. Similarly, glycosylation of ABCB1 was determined by Western blot

analysis. The endogenous dolichol levels were quantified by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) in SH-SY5Y cells.

Results: Simvastatin reduced the amount of the mature-glycosylated form (180 kDa) while increasing the core-glycosylated form (140 kDa) of ABCB1. However, this effect and the apoptosis induction by simvastatin were reversible by addition of dolichol in a time- and concentration-dependent manner. Furthermore, our HPLC analyses proved that endogenous dolichol levels were significantly decreased by simvastatin treatment for 48 hours. Effects of simvastatin concentrations as low as 0.1 µM were significant. Finally, activation of caspase 3 triggered by simvastatin was prevented by addition of dolichol.

Conclusions: Here we show that simvastatin is able to reduce dolichol levels in SH-SY5Y cells. The endogenous dolichol depletion caused by simvastatin might influence glycosylation process in the ER leading to alterations of glycosylation pattern of ABCB1. Moreover, our data suggest that this correlation between simvastatin and decreased endogenous dolichol levels also correlates with the apoptotic potential of simvastatin.

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A52

LRET-based distance measurements in the mammalian glutamate transporter EAAT3

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Background: EAAT3 (excitatory amino acid transporter 3) mediates the regulation of synaptic transmission by reuptake of glutamate in the synaptic cleft. It is distributed in neuronal membranes and is selectively enriched in the neurons of the hippocampus, cerebellum and the basal ganglia. It belongs to the family of soluble carrier family 1 member 1 (SLC1A1) and is expressed in kidney, a wide variety of epithelial tissues, brain and eyes.

Methods: The project utilizes the high-resolution crystal structure of GltPh, the bacterial orthologue to mammalian glutamate transporters. GltPh provides a structural framework for the determination of the helical movement in EAAT3. The structural rearrangement of the protein is caused by the helical movements which will be assessed by distance measurements using the technique of lanthanide resonance energy transfer (LRET). The protein will be expressed in *Xenopus laevis* oocytes. Lanthanide binding tags (LBT) will be inserted into the protein to chelate the lanthanide terbium which serves as the donor element. Cysteines will be reacted to an acceptor dye (bodipy FL).

Expected results: The measured distances will allow us to obtain new insights into the structure-function relationship of the glutamate transporters which can be further investigated using different substrates and inhibitors.

Discussion: The results obtained in this project will allow us to better understand pathophysiological conditions associated with mutations in EAAT3, for instance mutations causing human dicarboxylic aminoaciduria.

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A53

Decrypting structural and functional changes in $LeuT_{Aa}$ at atomic level employing LRET

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Background: Neurotransmitter:sodium symporters (NSS) are integral membrane proteins that mediate the reuptake of monoamine neurotransmitters previously released into the synaptic cleft. They are of pharmacological significance because they are the target of many clinically important drugs. $LeuT_{Aa}$, a leucine/alanine transporter is a bacterial homolog to NSS. Crystal structures of $LeuT_{Aa}$ with open to outward, occluded and inward-facing states have already been resolved at high resolution. Hence, $LeuT_{Aa}$ serves as a good paradigm for exploring the structure-function relationship of NSS proteins.

Methods and results: To investigate the structure-function relation in LeuT at atomic level we employ lanthanide-based resonance energy transfer (LRET). LRET-based measurements require the introduction of an LBT (lanthanide binding tag) to accommodate terbium as the donor element and fluorophores chemically linked to a cysteine residue as the acceptor element. LBT tags and cysteine are introduced at selected positions in $LeuT_{Aa}$. Introduction of an LBT tag may lead to a functionally disturbed host protein. So to screen functional LBT-LeuT mutants we established the scintillation proximity assay in the lab. To date, after screening functional LBT mutants of $LeuT_{Aa}$, we have measured the intramolecular distances at atomic level of LeuT in micelles. In order to validate these distances we want to see the distance changes after reconstitution into liposomes, a more native environment that allows to establish a sodium gradient; this is important since the NSS operates along a chemical gradient.

Discussion: Our LRET measurements are expected to help us validate or propose models of substrate transport. Our future plan focusses on the reconstitution of $LeuT_{Aa}$ in liposomes to allow for distance measurements in a more native environment.

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A54

The N-terminus acts as a lever to support amphetamine-induced substrate efflux by the serotonin transporter

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Background: We have previously shown that a highly conserved threonine at position 81, in the amino terminus of SERT, plays a key role in SERT function, by driving the transporter into a state that supports amphetamine-induced efflux [1]. Truncation of the first 64 amino acids or tethering the N-terminus to an additional transmembrane helix both abolish amphetamine-induced efflux by SERT [1].

Methods: Alanine scanning mutagenesis was carried out along the N-terminal region of SERT to pinpoint the residues involved in maintaining amphetamine-induced efflux. Two residues at a time were replaced by alanine using a site-directed mutagenesis kit (Quikchange kit, Stratagene). The mutants were characterised by uptake, release and binding assays; surface expression was visualised by confocal microscopy. Conformational sensitivity of the N-terminus was examined by proteolytic cleavage. Tryptic digestion of membranes prepared from SERT-expressing cells was performed under different buffer conditions, in the absence or presence of various ligands, and detected by Western blotting.

Results: Although all mutant SERTs generated in this study were targeted to the plasma membrane, some exhibited dramatic reductions in amphetamine-induced efflux. Moreover, they were all active with respect to [^{3}H]serotonin uptake, showing no marked changes in the affinity or velocity of substrate uptake. The reduction in efflux did not result from impaired affinity of the mutants for amphetamines (shown by [^{3}H]imipramine binding assays). In outward-facing conformations of SERT, the N-terminus is less susceptible to proteolytic digestion, possibly because it is shielded upon accompanying structural rearrangements. In inward-facing states, it is less susceptible to cleavage only when amphetamines are bound.

Conclusions: The region encompassing residues 22–32 may be a pivot for the movement of the N-terminus allowing amphetamine-induced release to occur. The mutagenesis and proteolysis data are consistent with the N-terminus of SERT acting as a lever, promoting substrate release by a second moiety of the oligomer.

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A55

How does the carboxyl terminus assist folding and ER export of the serotonin transporter?

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Background: The serotonin transporter (SERT) is exported from the ER by recruiting SEC24C to its C-terminus [1]. The same region also provides a docking site for proteinaceous chaperones (HSP isoforms) that assist in folding [2]. Based on these observations, we postulate sequential exchange of the chaperone(s) for the COPII coat as a mechanism to prevent premature ER export of partially folded SERT.

Methods: SERT (including a series of double and truncation mutants along its C-terminus) and related transporters were screened for their SEC24 isoform dependence, examined by siRNA-induced SEC24 knock-down in HEK293 cells. The cells were transfected with Sec24 siRNAs and, 48 h later, with transporter plasmids (Lipofectamine, Invitrogen), and the effects were determined by substrate uptake and confocal microscopy. GST-fusion proteins comprising the C-terminus of wild-type and mutant SERTs were used for pull-down experiments carried out with SEC24C and SEC24D. To examine the role of heat shock protein (HSP) 90 in regulating the formation of COPII vesicles, we treated HEK293 cells expressing wild-type SERT and its RI-607,608-AA mutant (putative ER export motif site) with geldanamycin, prior to measuring the effect of HSP90 inhibition on transporter trafficking.

Results: A lysine residue (K610) residing near the putative ER export motif on SERT (RI-607,608) was replaced by tyrosine (Y), the equivalent residue found in NET and DAT, leading to a relaxed preference for SEC24 isoform recruitment; SERT-K610Y no longer relied solely on SEC24C, but rather SEC24D for ER export. We searched for HSP isoforms that bound within or close to the SEC24-binding site. These experiments revealed a role of HSP90 in relaying SERT to SEC24C.

Conclusions: We show that the preference for SEC24 isoforms can be altered upon mutation of a single residue on the cargo protein. In SERT, lysine 610 appears to have a role in the interaction with SEC24C. We base our conclusion on the following evidence: (i) GST pull-downs showing an interaction with SEC24D, rather than SEC24C, (ii) siRNA-induced knock down of SEC24 isoforms A–D and (iii) co-expression with dominant negative SEC24C/SEC24D mutants, leading to reduced surface expression of the transporter.

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A56

Calmodulin kinase II regulates amphetamine-induced reverse transport in dopamine and serotonin transporters

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Background: Monoamine transporters such as the dopamine transporter (DAT) and the serotonin transporter (SERT) mediate the reuptake of previously released monoamines dopamine (DA) and serotonin from the synaptic cleft; thereby, these transporters regulate the monoamine content available for synaptic transmission. Certain stimuli, such as changes in ionic composition of the extracellular fluid or psychostimulants (e.g. amphetamines) are able to induce outward transport and thus increase extracellular monoamine concentrations. Influx and efflux of substrate are thought to be asymmetrical processes regulated by intracellular kinases. It has been demonstrated that removal of N-terminal serines ablates amphetamine-induced reverse transport in the DAT. Furthermore, the Ca^{2+}/calmodulin-dependent protein kinase II a (aCaMKII) can bind to the DAT C-terminus and phosphorylate N-terminal serines. Pharmacological inhibition of aCaMKII dramatically reduces amphetamine-induced efflux both in cells stably transfected with the human DAT as well as in rat striatal slices. Here, we test whether aCaMKII-regulation of amphetamine-induced reverse transport of monoamines is affected in mice with mutations in the aCaMKII gene.

Methods: Methods used were: release assays in mouse brain preparations, radioligand binding and uptake experiments, immunoprecipitations, surface biotinylation, mass spectrometry,

primary cultures of dopaminergic and serotonergic neurons, immunocytochemistry and behavioural pharmacology.

Results: We show here that aCaMKII regulates amphetamine-induced DAT-mediated efflux in mice with various mutations in the aCaMKII gene. Mice lacking aCaMKII or having a permanently self-inhibited aCaMKII (aCaMKII^{T305D}) display significantly reduced amphetamine-induced substrate efflux. A similar finding was observed in a mouse model of Angelman Syndrome, a neurogenetic disease characterized by motor impairments and autism spectrum disorders. Angelman Syndrome mice have a reduced aCaMKII activity and show comparable impairments in DAT function to aCaMKII mutants. This suggests that DAT-mediated dopaminergic signalling is affected in Angelman Syndrome. We further show that aCaMKII regulates the closely related SERT: both pharmacological inhibition and genetic disruption of aCaMKII significantly attenuates *p*-chloro-amphetamine-induced SERT-mediated serotonin efflux in transiently transfected cells and mouse brain preparations.

Conclusions: aCaMKII exerts an important modulatory role in amphetamine-induced DAT- and SERT-mediated substrate efflux. The finding that efflux is also affected in Angelman Syndrome mice might help in the understanding of the underlying pathophysiology. Symptoms of human Angelman Syndrome patients include movement impairments and autism spectrum disorders, conditions which are associated with dopaminergic and serotonergic malfunction.

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A57

Deciphering structural rearrangements during transport process in the bacterial transporter GltPh, homolog to mammalian glutamate transporter

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Background: Glutamate transporters are integral membrane proteins that catalyze the concentrative uptake of glutamate from the synapse by harnessing pre-existing ion gradients. In the central nervous system glutamate transporters are essential for normal development and function; they also are implicated in stroke, epilepsy and neurodegenerative diseases. The crystal structure of a eukaryotic glutamate transporter homologue from *Pyrococcus horikoshii*, is available at various conformations providing a structural framework for the determination of substrate and inhibitor binding to the transporter. In this study we aim to measure structural changes upon transport using lanthanide resonance energy transfer (LRET).

Methods: Site-directed mutagenesis was employed to insert genetically encoded lanthanide binding tags (LBT) into the the protein to perform LRET measurements. Thus generated LBT mutants were expressed and purified, and the functionality of the mutants was assessed by radioligand binding assay.

Results: Models for insertion of LBT were derived from the available crystal structures of the transporter. The wild-type and mutant proteins were expressed and purified using affinity column chromatography. Donor decay signals were recorded for LBT insertion mutants to confirm the insertion of tags. Furthermore, radioligand binding assays were performed with the mutants and they were found to be functional.

Conclusions: Taken together these mutants serve as the starting point to probe the conformational changes that were observed in previously solved crystal structures in reconstituted proteoliposomes. This could help us to integrate the structure-function relationship in the mammalian counterparts.

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A58

Peptide YY and neuropeptide Y in regulation of pain and spatial learning and memory

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Background: Peptide YY (PYY) and neuropeptide Y (NPY), two members of the pancreatic polypeptide-fold family of biologically active peptides, play important roles in the regulation of food intake, energy homeostasis and emotional-affective processes. While NPY also participates in nociceptive processing and cognition, the implication of PYY in pain, learning and memory has been little studied. Therefore, male wild-type, PYY knockout (PYY(−/−)), and NPY plus PYY double knockout (NPY(−/−);PYY(−/−)) mice were studied for their sensitivity to noxious heat in the plantar test and for their spatial learning and memory performance in the Barnes maze.

Methods: In the plantar test mice were habituated in small compartments for 1 h. Subsequently the plantar side of the right and left hind paw was exposed to an infrared source through a glass plate (cut off time: 15 s). The withdrawal response was assessed at two infrared intensities, and the withdrawal latency recorded. In the Barnes maze test, mice were placed in the middle of a circular maze with 20 holes. The task of the mice was to find a target hole with an escape box within a preset time. The animals received 4 training runs on the first day and 3 trainings daily for the following 3 days. The first probe trial was performed on day 5 and the second probe trial on day 12, to check short-term and long-term memory, respectively. Target hole visits, preference (target hole visits/total visits) and latency (time taken to reach the target hole) were measured.

Results: In the plantar test at the higher infrared intensity, PYY(−/−) and NPY(−/−);PYY(−/−) mice presented with a significant decrease ($p < 0.01$ and $p < 0.05$) in withdrawal latency when compared with wild-type mice. At the lower infrared intensity, the withdrawal latency of PYY(−/−) mice was also significantly shorter ($p < 0.01$) than that measured in wild-type mice, whereas NPY(−/−);PYY(−/−) mice did not significantly differ from wild-type mice. In the Barnes maze test, significant differences were only found during probe trial 1 when NPY(−/−);PYY(−/−) mice visited the target hole less often ($p < 0.01$) than wild-type mice. In addition, the total number of hole visits made by NPY(−/−);PYY(−/−) mice was significantly ($p < 0.01$) lower than that made by wild-type animals.

Conclusions: The most important observation of this study is that genetic deletion of the gut hormone PYY decreases the pain threshold to noxious heat. Additional knockout of the neuropeptide NPY, which is known to play a role in nociception, did not increase the hyperalgesic effect of PYY knockout. In contrast, the impact of PYY and NPY deletion on the performance in the Barnes maze was modest, and the decrease in target hole visits was probably due to reduced locomotion and did not reflect an impairment of short term memory.

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A59

Identifying forces that stabilize the oligomeric state of bacterial homologs of neurotransmitter transporters

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Background: Neurotransmitter transporters of the SLC1 and SCL6 family are found on presynaptic neurons and on glia cells. The function of these transporters is the termination of neurotransmission by the rapid removal of the neurotransmitter molecules from the synaptic cleft. These transporters couple substrate transport to ion gradients of sodium and chloride. Almost all of the eucaryotic transporters have been described to function as oligomers. However, the forces stabilizing the oligomeric state are not well understood. No crystal structures of eukaryotic transporters are available, but recently crystal structures of bacterial homologs thereof have been solved: GltPh (SLC 1 family) was found as a trimer, LeuT (SLC6 family) was crystallized as a dimer. These homologous crystal structures allow rationalizing on the driving forces that stabilize the eukaryotic counterparts.
Methods: The crystal structures of LeuT and GltPh were obtained from the Protein Data Bank (PDB). We identified the interfaces between the protomers and analyzed hydrogen bonding, hydrophobic and hydrophilic interactions as well as size and width of the interface area.
Results: We investigated the protein-protein interfaces between the transporter protomers and identified the dominant forces that stabilize the oligomer. These consist of hydrophobic interactions between the aliphatic side chains within the interface and of polar interactions by hydrogen bonds between hydroxyl groups.
Conclusions: The contributions of different forces to the stability of oligomer assemblies vary between proteins. While the hydrophobic mismatch is a prominent contributor to the stability of the GltPh transporter, it plays a minor role for LeuT, where helix packing and aromatic interactions seem to dominate.
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A60

A conformational change of the domain IV S6 segment of the voltage-gated sodium channel during inactivation

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Background: In voltage-gated Na^+ channels the S6 transmembrane segment of domain IV (DIV-S6) is part of the lining of the inner part of the pore. It is of pivotal importance for inactivation gating. We recently showed that amino acid I1581 of DIV-S6 ($rNa_V1.4$ amino acid numbering) is extraordinarily sensitive to both local and distal mutations suggesting a unique role in coupling of voltage-sensor movements to conformational changes in the pore. To date the only structural information relevant to voltage-gated Na^+ channels can be derived from the recently crystallized bacterial channel Na_VAb. In this structure the amino acid homologous to I1581 faces the lipid phase and is in close spacial relationship to the voltage-sensing apparatus. If this arrangement holds true for the eukaryotic Na^+ channel then site 1581 should not be exposed to bulk solution.
Methods: The following methods were used: site-directed mutagenesis of amino acids in the S6 segment of domain IV of the $rNa_V1.4$ channel; heterologous expression of the constructs in tsA 201 cells and *Xenopus laevis* oocytes; exploration of the kinetic effects of the mutations and gating sensitivity of the amino acid residue in the S6 segment of domain IV of the $rNa_V1.4$ channel by whole-cell patch clamp and two-electrode voltage clamp technique.
Results: We tested the hypothesis by replacing I1581 by a titrable histidine. In wild-type channels changing the pH of the bulk solution from 7.4 to 8.2 had no effect on the voltage-dependence of fast inactivation. However, in I1581H the same change in pH resulted in a 9.51 ± 1.98 mV hyperpolarizing shift ($p < 0.05$) of the voltage-dependence of fast inactivation.
Conclusions: The data suggest that during inactivation site 1581 is at least partially exposed to the bulk solution and not completely embedded in the lipid phase. The DIV-S6 segment may undergo a conformational change during inactivation, most likely a rotational movement, which allows access of external protons to site 1581.
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Functional and physical interactions between P2Y receptors and ion channels

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Background: Neuronal P2Y receptors, i.e. nucleotide-sensitive G protein-coupled receptors (GPCRs), are known to control various voltage-gated ion channels, in particular K_V7 K^+ and $Ca_V2.2$ Ca^{2+} channels. The differential modulation of these ion channels via GPCRs was shown to rely on the presence or absence of scaffolding proteins such as AKAP79/150 and NHERF-2. Since scaffold proteins are believed to bring GPCRs and ion channels in close proximity to guarantee efficient G protein-mediated modulation, this project evaluates whether a tight contact between P2Y receptors and ion channels is a prerequisite for their functional interaction.
Methods: $P2Y_1$ or $P2Y_{12}$ receptors with fluorescent tags (CFP or YFP) were expressed together with fluorescently labeled $K_V7.2/7.3$ or $Ca_V2.2$ channels in tsA 201 cells and the channel modulation by nucleotides was determined by measuring the according currents. To evaluate the behavior of the receptors and channels in the membrane, fluorescence recovery after photobleaching (FRAP) was determined by confocal laser microscopy.
Results: Activation of $P2Y_1$ but not of $P2Y_{12}$ receptors by ADP inhibited the K^+ currents in a concentration-dependent manner by up to $20.5 \pm 1.9\%$. Conversely, activation of both, $P2Y_1$ and $P2Y_{12}$ receptors, reduced the Ca^{2+} currents by up to $60.1 \pm 7.4\%$ and $76.3 \pm 4.2\%$, respectively. In initial FRAP experiments, the YFP-labeled receptors showed similar half-times of around 80 seconds. Upon coexpression of the $P2Y_1$ receptor with the K_V7 channel the half-time increased significantly ($p < 0.009$) to 116 seconds compared to single expression of receptor or channel only. In the case of $P2Y_{12}$, coexpression with K_V7 showed no significant change compared to $P2Y_{12}$ or K_V7 alone.
Conclusions: These findings suggest that distinct ion channels are modulated by different P2Y receptors. K_V7 currents are inhibited by $P2Y_1$ whereas $Ca_V2.2$ currents are reduced by both $P2Y_1$ and $P2Y_{12}$. Additionally, FRAP data show that the presence of K_V7 slows down the movement of $P2Y_1$ in the membrane, but not that of $P2Y_{12}$. This suggests that there is a physical interaction between $P2Y_1$ and

K_V7 which is not present between $P2Y_{12}$ and K_V7. The influence on movement of $P2Y_1$ and $P2Y_{12}$ receptors in the membrane by the presence $Ca_V2.2$ remains to be elucidated.

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A62

Does pulmonary surfactant generally affect antimicrobial activity?

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Background: Activity of antimicrobial agents may be affected by pulmonary surfactant. Notably, it was reported that the clinical efficacy of daptomycin is significantly impaired in pneumonia in spite of bacterial susceptibility *in vitro*. This study set out to assess the impact of pulmonary surfactant *in vitro* on bacterial killing of other antibiotics used for treatment of pneumonia.
Methods: Time-kill curves of daptomycin, doripenem, linezolid, moxifloxacin, and tigecycline were determined for *Staphylococcus aureus* ATCC 29213 and of colistin, doripenem and moxifloxacin for a clinical isolate of *Pseudomonas aeruginosa* at concentrations above or equal to the respective MICs. All experiments were performed over 24 h in Mueller-Hinton broth (MHB) and in MHB enriched with porcine surfactant at a concentration of 1 mg/mL (MHB_{surf}).
Results: As expected, daptomycin was not bactericidal in presence of surfactant at concentrations up to 64 times the MIC. In MHB_{surf} a higher concentration of moxifloxacin (16x) was needed than in MHB (2x MIC) to achieve sustained bacterial killing of *S. aureus*. In contrast, killing of *P. aeruginosa* by moxifloxacin was not affected by surfactant. A slightly higher concentration of doripenem (8x) was needed in MHB_{surf} to achieve sustained antimicrobial killing against *S. aureus* than in MHB (4x MIC). However, killing was faster in MHB_{surf}. Similarly, initial killing of *S. aureus* by tigecycline was faster in MHB_{surf} than in MHB while after 24 hours no difference in bacterial counts was observed between MHB and MHB_{surf}. For linezolid no significant effects were observed by adding surfactant. Likewise, surfactant had no significant influence on the activity of colistin and doripenem against *P. aeruginosa*.
Conclusions: The activity of moxifloxacin against *S. aureus* was reduced *in vitro* by addition of surfactant whereas this effect could not be observed against *P. aeruginosa*. Interestingly, antimicrobial killing by several antibiotics of Gram-positive *S. aureus*, but not of Gram-negative *P. aeruginosa* tended to be faster in presence of surfactant. Thus, apart from daptomycin, pulmonary surfactant is also capable of influencing the bacterial killing kinetics of several other antibiotics. The clinical relevance of these *in vitro* findings for pneumonia patients is currently unclear, and should be carefully evaluated.

A63

Pharmacochaperoning of the ER-retained A_1 adenosine receptor

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Background: The A_1 adenosine receptor is a member of the rhodopsin-related subfamily of GPCRs. Point mutations in the conserved NPxxY(x)5,6F motif at the junction of helix 7 and the C-terminus disrupt surface targeting of the receptor and result in its intracellular retention. This trafficking arrest can be overcome by addition of receptor ligands (pharmacochaperoning) that stabilize the receptor fold and thus promote surface expression. The mutants serve as a tool to explore a ramification of the retaliatory metabolite complex: hypoxia leads to intracellular accumulation of adenosine (by breakdown of ATP and by inhibition of adenosine kinase). Intracellular and extracellular adenosine levels are in equilibrium because of the action of the equilibrative nucleoside transporters. Extracellular adenosine dampens cellular metabolism by acting on inhibitory A_1 adenosine receptors and thus counteracts the impact of hypoxia. If adenosine also pharmacochaperoned A_1 adenosine receptors during hypoxia, it would enhance its effectiveness as a protective agent.
Methods: We created cell lines that stably expressed A_1 receptor Y288A with either a C-terminal YFP or an N-terminal FLAG-epitope fused to a streptactin peptide. These cell lines were incubated with a combination of inhibitors to test whether manipulations of intracellular adenosine levels increased the accumulation of the receptor. The A_1 antagonist DPCPX was used as an internal control. To mimic hypoxia, the cells were incubated for 24 hours under 5% O_2 content conditions. Radioligand binding and Western blotting was performed to determine the level of mature, binding competent receptors.
Results: Inhibition of adenosine kinase, adenosine deaminase and equilibrative nucleoside transporters enhanced the accumulation of binding competent receptors. The action of these inhibitors was as effective as DPCPX in pharmacochaperoning the receptor. The receptors acquired a mature glycosylation status indicative of export from the ER and delivery to the Golgi, and they reached the cell surface. Moreover, the action of the enzyme inhibitor cocktail could also be elicited by hypoxia.
Conclusions: Accumulation of intracellular adenosine elicits a pharmacochaperoning effect. Accordingly, the retaliatory metabolite concept may be extended to include a pharmacochaperoning (”physiochaperoning”) action of adenosine.
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A64

Statins impact on epigenetics of tumor cells

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Background: Histones are basic proteins and are modified by diverse post-translation modifications such as acetylation, methylation, phosphorylation and ubiquitinylation. These epigenetic modifications are important regulatory processes in proliferation, survival, differentiation and motility. The epigenetic gene regulation occurs in DNA methylation at DNA level and histone modification or chromatin remodelling at protein level. Several enzymes, like DNA methyltransferases (DNMTs), histone methyltransferases (HMTs), histone demethylases (HDMs), histone acetyltransferases (HATs) and histone deacetylases (HDACs), are able to modify the chromatin. The histone modifications lead to alterations in chromatin and form heterochromatin or euchromatin, which activates or silences transcription. Statins are used successfully in the treatment of hypercholesterolemia and inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. HMG-CoA reductase is the rate-limiting enzyme of the mevalonate pathway. The synthesis of isoprenoids such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) is reduced by statins by inhibition of the HMG-CoA reductase. These isoprenoid intermediates are involved in post-translational modifications of Ras,

Rho and Rac, which are typical for G proteins and have crucial roles in cancer cells.

Methods: Western blot analysis was performed to quantify acetylation status of histones in SH-SY5Y and RD cell lines.

Results: SH-SY5Y and RD cells were treated and incubated with increasing concentrations of simvastatin. The lysates were separated in a cytosolic and nuclear fraction. acetylated proteins were detected mainly in the nuclear fraction. Interestingly, the pattern of acetylation did not change very much upon statin exposure in the cytosol. However, in the nuclear fraction simvastatin extracts were acetylated to a greater extent.

Conclusions: The data presented here suggest that simvastatin can affect histones and their post-translational modifications. Enhanced acetylation of nuclear proteins induced by simvastatin might be interpreted as an inhibition of a HDAC activity or/and as an increased HAT-mediated acetylation in SH-SY5Y and RD cells.

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A65

Therapeutic potential of a novel multifunctional iron chelator on cognitive decicits and insulin degrading enzyme expression in a rat model of sporadic Alzheimer's disease

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Background: There is a need in modern pharmacology for a representative animal model which should accurately mimic sporadic Alzheimer's disease (sAD), the prevailing type of dementia in humans, and thus could be suitable for novel drug testing. Rats treated intracerebroventricularly with the betacytotoxic agent streptozotocin (STZ-icv), have been proposed recently as a non-transgenic sAD model which demonstrates AD-like pathology features at cognitive, neurochemical and structural level. In addition to the cognitive deficits, pathological accumulation of amyloid β (Aβ) peptide is one of the neuropathological hallmarks of sAD, and a growing body of evidence suggests the involvement of insulin degrading enzyme (IDE), responsible for Aβ degradation, in sAD pathophysiology. We have explored the time course of cognitive deficits and hippocampal (HPC) IDE expression in the STZ-icv rat model of sAD, and the therapeutic potential of the novel multifunctional iron-chelating drug M30 to improve these deficits.

Methods: Adult Male Wistar rats were injected bilaterally icv with STZ (0.3, 1 and 3 mg/kg) or vehicle and sacrificed one week, or one, three, six and nine months after the treatment. Two groups of STZ-icv (3 mg/kg)-injected rats were additionally subjected to an 11-week oral M30 treatment (2 and 10 mg/kg, 3x per week) beginning 10 days after the STZ-icv treatment. Cognitive deficits were measured by the Morris water maze swimming test (MWM) and the passive avoidance test (PA). IDE protein expression in HPC was measured by SDS-PAGE electrophoresis/immunoblotting. Data were analysed by the Kruskal-Wallis and the Mann-Whitney U test ($p < 0.05$).

Results: STZ-icv rats exhibited significant dose- and time-dependent cognitive deficits in the PA test (40–90%), while IDE protein expression was found to be decreased not earlier than one month after the STZ-icv administration (−56%), persisting decreased untill six months (−26%). Treatment with the high M30 dose improved STZ-icv-induced cognitive deficits, observed as a decreased number of mistakes in the MWM test (−60%) and increased latency time in the PA test (+300%). Treatment with both M30 doses significantly increased IDE protein expression in comparison with the STZ-icv treatment alone (low dose +19%, high dose +37%).

Conclusions: The STZ-icv rat model demonstrates long-term cognitive deficits and decreased hippocampal IDE protein expression which tend to correlate mutually. Chronic M30 treatment, initiated after the development of cognitive deficits, significantly improves the cognitive deficits as well as decreases IDE protein expression in the STZ-icv rat model of sAD, suggesting that multifunctional iron-chelating drugs might have a therapeutic potential in sAD treatment.

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A66

IL-6-mediated migration of human metastatic melanoma cells is reduced by simvastatin treatment

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Background: In 1987 the HMG-CoA reductase inhibitors, statins, were first marketed and are now widely used as well-tolerated therapeutics for hypercholesterolemia. High interleukin 6 (IL-6) plasma levels in melanoma patients are linked to a higher tumour burden and reduced overall survival. We have recently shown that simvastatin triggers apoptosis in human metastatic melanoma cells which is associated with concentration-dependent changes in autocrine IL-6 secretion. Here, we investigated IL-6 signalling with respect to proliferation and migration in human metastatic melanoma cells under statin application.

Methods: For this approach, human metastatic melanoma cells (A375, 518a2) were used for quantification of surface expression of the IL-6 receptor (IL-6-R/gp130) and for cell cycle with FACS analysis. Additionally, migration assays were carried out with simvastatin and/or IL-6 administration over time.

Results: Increasing concentrations of simvastatin led to morphological changes and detachment of the melanoma cells. After reseeding of the detached cells, A375 cells had a normal cell cycle profile while 518a2 cells underwent apoptosis resulting in cell death. Moreover, simvastatin treatment enhanced the surface expression of the IL-6-R and the gp130 subunit in a time- and concentration-dependent manner. Both cell lines responded to IL-6 treatment with increased proliferation and migration which was inhibited by simvastatin.

Conclusion: We demonstrate that simvastatin up-regulates the IL-6 pathway on the level of the heteromeric IL-6 receptor. Although increased IL-6 receptor expression would imply a stronger IL-6 signal, this is not seen in the presence of simvastatin. A novel therapeutic concept for simvastatin may emerge from the suppression of the IL-6-mediated proliferation and migration in metastatic melanoma cells.

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A67

Pharmacological stimulation of murine and human hematopoetic stem cells

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Background: Hematopoietic stem cell (HSC) transplantation is a standard procedure in the treatment of hematological disorders and also applicable to support aggressive chemotherapy in cancer. In practice, the clinical outcome is often limited by inefficient bone marrow (BM) engraftment or by low numbers of available stem cells. It would therefore be desirable to enhance engraftment by pharmacological stimulation. HSC require several signals for successful migration into the bone marrow. One of these signals is provided by stimulation of $G\alpha_s$ [1]. Pretreatment with prostaglandin (PG) E_2 enhances engraftment via activation of $G\alpha_s$-coupled EP_2 and EP_4 receptors [2]. Treprostinil is a stable analogue of prostacyclin/PGI_2, which acts via IP, EP_2 and EP_4 receptors and is approved for treatment of pulmonary hypertension. Here we tested the hypothesis that treprostinil stimulates stem cell engraftment.
Methods: Generation of murine bone marrow-derived HSCs: Undifferentiated HSC (lineage-negative, Lin− cells) were isolated from murine BM, separated by MACS (magnetic-assisted cell sorting) and characterized by fluorescence-activated cell sorting (FACS). BM transplantation: Lin− cells were pretreated *in vitro* in the absence and presence of 10 µM treprostinil alone or in combination with 30 µM FSK for 1 h at 37°C. The cellular threshold for successful transplantation was determined by titrating the number of HSCs required to allow for survival of lethally irradiated recipient mice. Engraftment and transplantation efficiency was determined by the analysis of white blood cell counts.
Results: Trepostinil triggered a concentration-dependent accumulation of cAMP in murine Lin− cells with an estimated EC_{50} in the range of 0.3 µM. A treprostini-induced cAMP accumulation was also observed in human HSCs, which were also shown to express the mRNA encoding IP, EP_2 and EP_4 receptors. Treprostinil enhanced engraftment of HSCs; this conclusion is based on the following observations: (i) mice injected with treprostinil-pretreated Lin− cells had significantly higher levels of circulating white blood cells as compared to those receiving vehicle-treated Lin− cells ($p < 0.05$, unpaired t-test); (ii) when pretreated and untreated Lin− cells were mixed to compete for BM reconstitution, the pretreatment with treprostinil increased transplantation efficiency 1.5–3-fold; (iii) *in vitro* pretreatment of HSCs with trepostinil reduced the minimum number of cells required to rescue a mouse; (iv) engraftment of HSCs was further enhanced when treprostinil was also administered after injection of the pretreated HSCs.
Conclusions: Treprostinil is suitable for improving haematopoetic stem cell transplantaion. The observations allow for designing a protocol to test the compound in a clinical trial.

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A68

Which conformation does the ABC transporter P-glycoprotein adopt in the physiological membrane environment?

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Background: The human genome contains 48 members of the ABC protein family. We focus on the multidrug resistance transporter P-glycoprotein (P-gp, ABCB1), which is expressed at the blood-brain-barrier, in intestine, kidney, liver and macrophages. The first structure of an ABC exporter was from *Staphylococcus aureus* and showed a twisted architecture. The same fold was observed in MsbA, mouse P-glycoprotein and the human mitochondrial ABCB10 transporter. Although ABC exporters have now been crystallized in several conformations, uncertainty remained with respect to the physiological conformation because they seem not to be fully compatible with all biochemical evidence.
Methods: We applied homology modeling and MD simulations to determine the equilibrium conformation of the membrane-inserted transporter to test the hypothesis whether the observed conformations might be a consequence of the crystallization procedure or conditions. We inserted the transporter model into a pre-equilibrated membrane and carried out equilibrium simulations.
Results and conclusions: In equilibrium we observe the wings to come close, which is in compliance with experimental observations. Water becomes expelled from the hydrophobic region and the open passage between the water-filled pore and the cell exterior closes. Our results indicate that the closed conformation is energetically more favourable.
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A69

Biophysical characterization of $Ca_V1.4$ L-type calcium channel mutants causing congenital stationary night blindness type 2 in humans

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Background: $Ca_V1.4$ L-type calcium channels show unique biophysical properties such as slow inactivation due to the lack of calcium-dependent inactivation (CDI). These properties make $Ca_V1.4$ channels appropriate candidates for triggering persistent glutamate release at retinal photoreceptor cell synapses. Mutations in the CACNA1F gene encoding for the $Ca_V1.4$ α1 subunit are described in patients with X-linked congenital stationary night blindness type 2 (CSNB2). Impaired transmission between rod photoreceptor cells and second-order neurons manifests as night blindness and various other visual symptoms in the affected individuals.
Methods: The aim of this study was to investigate the functional properties of $Ca_V1.4$ mutants L849P and R1816stop compared to wild-type (WT) in transiently transfected tsA 201 cells (+β3,+α2δ-1) via whole-cell patch clamp technique using 15 mM Ba^{2+} and Ca^{2+} as charge carrier. For statistics, either Mann-Whitney (two groups) or

Kruskal-Wallis test and Dunn's Post hoc test (multiple comparison) were used.

Results: L849P was mainly characterized by a reduced current density (pA/pF: WT: −16.3 ± 1.6 (n = 38), L849P: −2.5 ± 0.3 (n = 12), $p < 0.0001$; Ca^{2+}), only minor, not significant ($p > 0.05$) changes in the voltage-dependent activation properties were observed. In presence of the dihydropyridin-activator BayK8644 (5 µM) the current density was increased ~10-fold ($p < 0.001$). The fold-increase in current density was comparable to WT. As expected R1816stop, which lacks an intrinsic C-terminal modulator (CTM), exhibited CDI (f-value: WT: 0.11 ± 0.03 (n = 8); R1816stop: 0.63 ± 0.02 (n = 22)) and shifted the voltage-dependence of activation to more negative voltages ($V0.5_{act}$ in mV: WT: 1.8 ± 0.3 (n = 74), R1816stop: −12.3 ± 0.3 (n = 23)). In presence of the $Ca_V1.4$-CTM; comprising the last 122 C-terminal residues WT conditions were fully restored, e.g. $V0.5_{act}$ 2.2 ± 1.0 mV (n = 14) ($p < 0.0001$).

Conclusions: We assume that the reduced current density observed in mutant L849P derives from decreased channel expression, which might be explained by a folding defect of the $Ca_V1.4$ channel protein rather than a reduced open probability. Moreover, the fact that the functional phenotype of the R1816stop can be rescued bears a potential pharmacotherapeutic concept based to the C-terminal modulatory mechanism present in $Ca_V1.4$ channels.

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A70

New structural determinants of charged local anaesthetic block of voltage-gated sodium channels

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Background: Some blockers of voltage-gated Na^+ and Ca^{2+} channels are assumed to pass through the membrane and then bind to amino acids in the internal vestibule by access from the internal side of the membrane. However, in the heart isoform of the voltage-gated Na^+ channel, in L-type calcium channels and in T-type calcium channels an additional external access pathway (EAP) through the protein has been suggested. Furthermore, in voltage-gated Na^+ channels (Na_V) mutations at a specific site in the middle of the domain IV transmembrane segment 6 (site 1575 in $rNa_V1.4$, 1760 in $rNa_V1.4$) open an EAP for QX-222, a permanently charged, hydrophilic lidocaine analogue. Recently, the first crystal structure of a Na_V was published [1]. In this bacterial channel structure (Na_VAb) the side chain homologous to $rNa_V1.4$ I1575 (I202 in Na_VAb) is in close contact with a pore-loop sidechain, homologous to $rNa_V1.4$ W1531 (W179 in Na_VAb). In contrast, in all currently available structural homology models of Na_V, W1531 is not in contact with I1575. If W1531 were positioned as suggested in the Na_VAb structure then a reduction in the length of the side chain at this site would be predicted to open the EAP. To test this hypothesis we generated the mutations W1531A and W1531G and tested these constructs for block by external QX-222.

Methods: Whole-cell patch clamp measurements were done on TsA 201 cells transiently transfected with plasmids coding the $rNa_V1.4$ α subunit and its mutants, the sodium channel β1 subunit and GFP. Block levels were derived at 2 Hz stimulation frequency from a holding potential of −120 mV.

Results: Mutations W1531A and W1531G were found to be sensitive to extracellular QX-222 (block: 20.6 ± 2% and 17.7 ± 3.5%, respectively).

Conclusions: Our results indicate that position 1531 is an important part of the EAP in $rNa_V1.4$, as predicted from the crystal structure of Na_VAb. Thus the bacterial channel Na_VAb appears to share important structural motifs with eukaryotic sodium channels.

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A71

Bacterial peptidoglycan primes the immune system leading to increased sickness in response to lipopolysaccharide

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Background: While the effects of the bacterial component and Toll-like receptor-4 (TLR4) agonist, lipopolysaccharide (LPS), on affective behaviour is well described, there is a lack of data concerning the effects of peptidoglycans on behaviour. Amongst others, peptidoglycans activate the intracellular receptors nucleotide-binding oligomerization domain 1 (NOD1) and NOD2. Here, the effects of the NOD1 activator FK565 and NOD2 activator muramyl dipeptide (MDP) were investigated with respect to parameters of immune activation and behaviour.

Methods: Male C57BL/6N mice received an intraperitoneal injection of FK565 (0.003 mg/kg), MDP (3 mg/kg) or sterile saline (0.9% NaCl) and an additional injection of LPS (0.83 mg/kg) or sterile saline 4 hours after the first injection. Body weight and rectal temperature were monitored throughout the study. Exploratory and anxiety-like behaviour was evaluated with the open field test (OFT) 1 day and with the step-down test 2 days after treatment. Spleen weight, an index of immune activation, was measured on the third day after sacrifice of mice.

Results: While none of the single treatments induced changes of body temperature, combined treatment with FK565+LPS and MDP+LPS caused a decrease of body temperature 4.5 hours post-treatment. A loss of body weight could be observed in the LPS, FK565+LPS and MDP+LPS-treated groups on day 1 and 2, while FK565 and MDP alone had no effect on body weight. On the third day post-treatment, the weight loss was gone in the LPS treated group, but was still evident in the groups receiving the double treatments. In the OFT, only treatment with FK565+LPS or MDP+LPS decreased travelling distance and visits to the central area. Likewise, in the step-down test only the double-treated mice presented an increased latency. LPS alone and in combination with FK565 or MDP increased spleen weight while FK565 and MDP alone were without effect.

Conclusions: The present results reveal that administration of a NOD1 or NOD2 activator alone fails to induce any systemic signs of immune activation, sickness (weight loss) and behavioural disturbance. In contrast, mice primed with either FK565 or MDP display increased anxiogenic and immune reactions to LPS. These findings indicate that NOD and TLR-4 agonists synergize *in vivo* in causing immune activation and sickness behaviour.

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A72

Soluble osteopontin concentrations in serum and ascites of women with advanced serous ovarian cancer

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Background: Despite advances in surgery and combination chemotherapy, ovarian cancer is first in terms of death rates of gynaecological malignancies. More than 90% of ovarian cancers arise from surface epithelium and the serous histological subtype is the most commonly diagnosed epithelial ovarian carcinoma. Extensive seeding of the peritoneal cavity by tumour cells is often associated with ascites, particularly in advanced, high-grade serous carcinomas. Currently CA-125 is the most widely used biomarker in the evaluation and management of women with epithelial ovarian cancer. However, in approximately 15% of these patients CA-125 is not indicative of disease status or progression. Therefore, an alternative tumour marker would be useful. Osteopontin is a secreted, integrin-binding glykophosphoprotein which is overexpressed in ovarian cancer cells and thus may serve as a serum biomarker. By combining the data from blood and fluid from the proximity of the tumour we might be more likely to discover a protein biomarker secreted from the tumour rather than deriving from another part of the body.
Methods: We analysed twenty patients treated at the Department of Gynaecology, Ljubljana, divided into two groups: controls (without adnexal pathology) and patients with advanced serous ovarian cancer (International Federation of Gynecology and Obstetrics (FIGO) stage III and IV). Both serum and free peritoneal fluid including ascites were collected and examined. Preoperative osteopontin concentrations were determined using the FlowCytomix Simplex kit (eBioscience). FlowCytomix Pro 2.4 (eBioscience) was used for data analysis.
Results: Patients with advanced ovarian cancer had significantly increased serum osteopontin concentration vs. controls ($p < 0.013$) and increased concentration of osteopontin in ascites vs. peritoneal fluid from control patients ($p < 0.001$).
Conclusions: Our preliminary results suggest that osteopontin might represent an effective biomarker associated with advanced serous ovarian cancer due to its elevated levels in both serum and ascites. The potential utility of osteopontin determination in monitoring women with CA-125-negative disease is worthy of exploration. However, larger prospective trials will be needed to assess the ability of serum osteopontin to provide diagnostic and prognostic information or indications of treatment response.
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A73

Expression of organic cation transporter 3 (SLC22A3) and plasma membrane monoamine transporter (SLC29A4) in human umbilical vein endothelial cells and their relevance for histamine uptake

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Background: Increased plasma histamine levels lead to pathological events. Endothelial cells actively participate in histamine clearance by promoting its uptake via yet unidentified carriers, thus limiting histamine effects. The organic cation transporter 3 (OCT3) and plasma membrane monoamine transporter (PMAT) are the two most prominent transporters for endogenous monoamines. OCT3 and PMAT show Na^+/K^+-ATPase independency. Both are highly sensitive to inhibition by the isocyanine compound, decynium-22. However, OCT3 is highly sensitive to corticosteron, whereas PMAT is not. We showed in the past that decynium-22 inhibits histamine uptake in cultured human umbilical vascular endothelial cells (HUVEC). In the present study we identified the expression of OCT3 and PMAT in freshly isolated and cultured HUVEC as well as some characteristics of histamine uptake in cultured HUVEC such as ouabain and corticosteron sensitivity.
Methods: We used freshly isolated and cultured HUVEC for determination of mRNA levels of hOCT3 and hPMAT transporters by real-time PCR. For the histamine uptake studies we used cultured HUVEC and determined [^{3}H]histamine uptake.
Results: OCT3 and PMAT are expressed in freshly isolated HUVEC as well as in primary HUVEC culture. Ouabain (0.1 mM) had no influence on uptake of histamine. Corticosteron inhibited the uptake of histamine in HUVEC, however the effect was observed only in mM concentration.
Conclusions: Our results suggest that because of low sensitivity of histamine uptake to corticosteron, expression of OCT3 in HUVEC is probably not relevant to histamine uptake in these cells, while PMAT expression is worthy of further examination.
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Characterization of colistin tissue pharmacokinetics by microdialysis

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Background: Colistin is an important antimicrobial treatment option against multidrug-resistant Gram-negative bacteria. However, colistin is a large and chemically complex molecule and information on its ability to penetrate into tissues remains sparse. Thus, the present work investigated the ability of microdialysis (μD) to assess pharmacokinetics (PK) of colistin in the interstitium of soft tissues, i.e. at a potential site of infection.
Methods: *In vitro:* Colistin recovery for linear CMA 66 μD probes with a molecular weight cut-off of 100 kDa was assessed through forward and reverse μD for different colistin concentrations. *In vivo:* Three male healthy volunteers received a single intravenous dose of 2.5 million international units of the inactive prodrug colistin methanesulfonate. Colistin concentrations in plasma and in μD samples obtained from two probes inserted into subcutaneous adipose tissue of the thigh were determined. Retrodialysis was used for probe calibration. In both settings, μD was performed with and without addition of albumin to perfusion solutions and colistin was quantified by liquid chromatography/tandem mass spectrometry (LC-MS/MS).
Results: *In vitro*, colistin recovery was constant over time and showed mean recovery values of 52 ± 3 and 71 ± 8% for forward and reverse μD, respectively. *In vivo*, recovery of colistin was 43 ± 15%. In both settings, colistin recovery was not improved by addition of albumin to μD perfusion solutions. Due to small volumes,

reliable quantification of colistin was not possible in some µD samples, yet maximum concentrations in adipose tissue were relatively high (0.76 ± 0.21 µg/mL) compared with those in plasma (1.2 ± 0.43 µg/mL) attesting for extra-vascular distribution.
Conclusions: The present data demonstrate the feasibility of µD for evaluation of colistin tissue pharmacokinetics and show opportunities for optimization of experimental setting.

A75

The galanin system in depression and antidepressant treatment: focus on the locus coeruleus

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Background: Our knowledge about central changes underlying depressive disorders is still incomplete, but disturbances in monoaminergic neurotransmission are involved. There is also increasing evidence for a possible role of the neuropeptide galanin and its three G protein-coupled receptors in the pathophysiology and treatment of depression [1].
Methods: Using *in situ* hybridization we investigated whether transcriptional processes in the galanin system may be involved in the heightened depression-like behaviour of HAB rats selectively bred for high trait anxiety as compared with their low anxiety/depression (LAB) counterparts [2] and in the treatment responses to established antidepressant drugs. Subsequently, the modulation of depression-related behaviour by intra-cerebrally applied galaninergic ligands was studied in HAB and LAB rats.
Results: The abundance of galanin mRNA was increased in the paraventricular hypothalamus, the central amygdala and the locus coeruleus (LC), but not in the dorsal raphe of HAB as compared to LAB animals. Conversely, long-term (42 days, p.o.) treatment with either desipramine, paroxetine or tranylcypromine caused a general reduction in galanin mRNA expression in the locus coeruleus (LC) of unselected rats indicating a common response to antidepressant drug treatment while in the paraventricular hypothalamus galanin mRNA was increased by tranylcypromine only. This observed common effect of the antidepressants on galanin mRNA in the LC is in contrast to the finding in the HAB model raising the exciting possibility that altered coerulear galanin mRNA expression may be associated with depression-related behaviour. Indeed, intra-LC galanin caused a pronounced increase in the immobility of LAB rats indicating enhanced depression-like behaviour while a galanin receptor antagonist reduced immobility in HAB rats.
Conclusions: The present data suggest that depression-like behaviours can be altered by interference with the galanin system and, thus, the galanin system may represent an interesting target for novel antidepressant pharmacotherapy. In particular, its modulation in the LC, where galanin highly coexists with noradrenaline, appears to be critical.
Acknowledgements: Supported by the Austrian Science Fund FWF and a Young Investigator Funding of the University of Innsbruck.

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A76

ABC transporters of the liver fluke *Fasciola hepatica*

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Background: The liver fluke *Fasciola hepatica* is one of the most important parasites affecting animal health all over the world, causing the so called liver fluke disease (fascioliasis). Infections of mammals can only occur via larvae of the fluke, which live on plants in moistly grassland. Therefore infections of humans are rather rare, but infections of animal stock still lead to large financial losses. The drugs of choice used in the treatment of infected animals are benzimidazoles like triclabendazole, which prevent microtubule formation. During the last decades more and more flukes resistant to these drugs have been found. Beside mutations in the target proteins (β-tubulin), detoxificationmechanisms via ABC (ATP-binding cassette) transporters are thought to be responsible for these resistances. Up to date little is known about proteins of the fluke. We therefore try to isolate yet unknown proteins, focusing mainly on ABC transporters, of the fluke, to investigate their potency as putative new drug targets.
Methods: Adult flukes were collected from bovine liver at a slaughter house in lower Austria. An ABC transporter of *Fasciola hepatica* was cloned from isolated fluke RNA. Heterologous expression was used for further characterization of the transporter in various cell lines. Polyclonal antibodies were raised to allow localization of the transporter by immunfluorescence microscopy.
Results and conclusions: We could clone a full length ABC transporter consisting of twelve transmembrane domains, showing high homology to members of the B-type family of ABC transporters. Heterologous expression of this *Fasciola hepatica* ABC transporter showed mainly intracellular localization in various cell lines, including mammalian and insect cell lines. Some members of the B-family of ABC transporters, like the mitochondrial transporters, ABCB10, are known to be expressed intracellularly. We have not determined a substrate of the transporter yet, but if the expression pattern holds true for the subcellular expression of the native fluke transporter, this transporter can not be responsible for resistances to anthelmintic drugs in the liver fluke. We have two other ABC transporters identified by sequence comparison and cloned from fluke RNA which will be analyzed in similar ways.
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A77

The influence of alpha-melanocortin enantiomers on acetaminophen-induced hepatis in mice

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Background: L-alpha-Melanocortin is a strong inhibitor of inflammation. It is a promising new anti-inflammatory and hepatoprotective peptide. Consequently, its melanocortin receptors (MC_1, MC_3, MC_4 and MC_5) could be possible targets for the development of new antiinflammatory drugs for chronic inflammatory liver disease. For a long time it has been believed that only the L-enantiomers of amino acids are present in higher

animals, but recent investigations show that D-amino acids also exhibit physiological effects *in vivo*, despite their very small quantities. The aim of this study was to compare hepatoprotective effects of L-alpha-melanocortin and D-alpha-melanocortin using the acetaminophen model of chemical liver damage in male CBA mice.
Methods: Tested substances were applied intraperitoneally 60 minutes prior to the intragastric application of acetaminophen (150 mg/kg). Animals were sacrificed 24 hours after the administration of acetaminophen. The criteria for monitoring hepatoprotective effects of the tested substances were biochemical parameters (AST and ALT) and histopathological analysis.
Results: The results obtained by the histopathological analysis and biochemical findings show potent hepatoprotective and anti-inflammatory effects of L-alpha-melanocortin in the liver, and suggest the possibility of modulating liver inflammation by means of melanocortin molecules and related receptors. D-alpha-melanocortin did not show any hepatoprotective effects *in vivo*.
Conclusions: Our results show that peptide enantiomerism influences the protective effects of alpha-melanocortin peptides *in vivo*. This concept may be used to modulate peptide function *in vivo* and antibody binding assay *in vitro*.
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A78

The NBD-NBD interface is not the sole determinant for transport in ABC transporters

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Background: The ABC (ATP-binding cassette) transporter superfamily constitutes one of the largest classes of membrane transporters. ABCB1 contains two functional nucleotide binding sites (NBSs) at the interface of the two nucleotide binding domains (NBDs), whereas ABCB11 has one degenerate ATP binding site. According to the structural alignments, ABCB1 and ABCB11 differ by only four residues in the NBD-NBD interface, all of them located at NBS1: E556M, G1178R, Q1180E and S474E. It has been shown that a mutation of the Walker B glutamate (E556) abolishes steady-state ATP hydrolysis and drug transport activities of ABCB1 [1]. We tested the hypothesis that function may be restored in ABCB1 when NBS1 is engineered on the basis of ABCB11.
Methods: These four residues were mutated in ABCB1 according to ABCB11. Wild-type or mutant ABCB1-transfected cells were used to measure rhodamine 123 transport by flow cytometry. First-order rate constants corresponding to efflux rate were plotted as a function of ABCB1 expression, which was determined by MRK16 staining.
Results: The E556M mutation of the catalytic glutamate resulted in loss of transport function. While the double mutation in the LSGGQ motif (G1178R, Q1180E) reduced transport to below 20%, no measurable rhodamine 123 efflux was observed in either the triple mutant (E556M, G1178R, Q1180E) or the quadruple mutant (E556M, G1178R, Q1180E, S474E).
Conclusions: Engineering of ABCB1 NBS1 to mimic ABCB11 NBS1 yields a non-functional transporter, indicating that the NBD-NBD interface is not the sole determinant for transport in ABC exporters.
Acknowledgements: We thank the Austrian Science Fund (FWF) for financial support (FWF project P23319).

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A79

Activity of semicarbazide-sensitive amine oxidase in guinea-pig tissues is not affected by metformin

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Background: Semicarbazide-sensitive amine oxidase (SSAO) is an enzyme that performs oxidative deamination of primary amines to aldehyde, ammonia and hydrogen peroxide. It appears that these products may have important signalling function in the regulation of cell development and glucose homeostasis. Much attention has been given to raised plasma SSAO activity in diabetic patients, both types 1 and 2. We were interested whether the peroral antidiabetic drug metformin affects SSAO activity *in vitro*. In this study we used metformin because it is usually the first-line medication used for treatment of type 2 diabetes.
Methods: First we studied the activity of SSAO in 15 different guinea-pig tissues with radiochemical methods. Two of 15 tissues (liver and kidney) were also used for the *in vitro* experiment, in which preincubation with metformin or the SSAO inhibitor semicarbazide was performed. Tissue homogenates were used for protein quantification using the Bradford method and for determination of SSAO activity using radioactive [^{14}C]benzylamine as substrate. Since benzylamine is also a substrate for MAO-B, the MAO-B inhibitor deprenyl was added to the incubation mix. After 30 min of incubation at 37°C in sodium phosphate buffer the reaction was stopped by addition of perchloric acid. The product [^{14}C]benzaldehyde was extracted into toluene containing 0.6% 2,5-diphenyloxazol. We measured radioactivity in the organic phase with a liquid scintillation counter.
Results: The highest concentration of SSAO was determined in the gastrointestinal system, especially in the liver (32 ± 4.0 mU/mg protein). Relatively high SSAO activity was found also in the spleen (47 ± 4.8 mU/mg protein), abdominal artery (39 ± 8.5 mU/mg protein), skin (32 ± 9.0 mU/mg protein), kidney (33 ± 4.7 mU/mg protein) and lung (26 ± 2.6 mU/mg protein). Smaller amounts of SSAO were present in pancreas and brain. Our results indicated that metformin had no effect on SSAO activity *in vitro*. As expected, semicarbazide decreased SSAO activity in a concentration-dependent manner.
Conclusions: SSAO appears to have an important role in the gastrointestinal system of the guinea-pig. The high SSAO concentration in the liver is probably due to the detoxifying function of this tissue. The relatively high SSAO levels in the lung could protect the animals from inhaled methylamine and other volatile amines. We conclude that SSAO activity is probably not affected during treatment with the antidiabetic drug metformin.
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A80

Real-time uptake of fluorescent ASP+ via the organic cation transporter 3

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Background: The organic cation transporter 3 (OCT3) shows a broad expression pattern in the nervous system and has been detected in glial cells and neurons. Here, OCT3 serves as an additional high-capacity, low-affinity reuptake system for monoamine neurotransmitters such as norepinephrine, serotonin and dopamine, and therefore OCT-mediated uptake has been termed “uptake 2”.
Methods: We used the mouse and human isoforms of OCT3 and stably expressed them in HEK 293 cells. We measured OCT3-mediated uptake of the fluorescent substrate 4-Di-1-ASP (4-(4-(dimethylamino)styryl)-*N*-methylpyridinium (ASP+) in real-time. Uptake of tritiated 1-methyl-4-phenylpyridinium was measured in comparison to the fluorescent uptake measurements. We used mass spectrometry to assess the phosphorylation status of OCT3.
Results: We show that ASP+ is selectively taken up via OCT3 in real time. ASP+ uptake allows for sensitive assessment of transport via OCT3 and hence, we exploited the mode of action of several OCT3 substrates and transport inhibitors such as the stress hormone corticosterone. All results with the fluorescent ASP+ are in line with previously published reports. Finally, we tested if OCT-mediated uptake is sensitive to phosphorylation and observed that GF109203X inhibited uptake.
Conclusions: Uptake by OCT3 can be measured with the fluorescent ligand ASP+; this uptake is comparable to the uptake of radioactively labeled MPP+. Uptake inhibition by corticosterone was comparable using either ASP+ or MPP+ and similar to inhibition of protein kinase C.
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A81

Restricted collision coupling of the adenosine A_{2A} receptor is due to its agonist-induced confinement in the membrane

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Background: The A_{2A} adenosine receptor is of interest because of several reasons. (i) It is a frequently blocked pharmacological target, because it is the site of action of caffeine. (ii) It has a long C-terminus that provides a docking site for several proteins, which direct the fate of the receptor from its synthesis to its lysosomal degradation. (iii) The A_{2A} receptor can only promote activation of a limited number of available G_s molecules. This coupling mode was termed restricted collision coupling. (iv) Most G protein-coupled receptors carry one or several cysteine residues in their C-terminus which is subject to palmitoylation to anchor and stabilize the amphipathic helix 8; the A_{2A} receptor lacks this palmitoylation site. We explored the hypothesis that there is a causal link between the absence of a palmitoyl moiety and restricted collision coupling.
Methods: We constructed a mutant A_{2A} receptor, R309C, which underwent palmitoylation as verified by both mass spectrometry and metabolic labeling. Radioligand binding, cAMP accumulation and Western blotting were performed to determine its signaling properties. Using single particle tracking of quantum dot-labeled receptors we compared diffusivity and diffusion mode of wild-type and mutant A_{2A} receptors.
Results: In contrast to the wild-type receptor, the concentration-response curve for agonist-induced cAMP accumulation was shifted to the left with increasing expression levels of A_{2A} receptor R309C. Single particle tracking demonstrated that agonist activation resulted in a decline in mean square displacement of both receptors, but the drop was substantially more pronounced for the wild-type receptor. In addition, in the agonist-bound state, the wild-type receptor was frequently subject to confinement events; these were rarely seen with the palmitoylated A_{2A} receptor R309C.
Conclusions: Taken together, the observations link restricted collision coupling to diffusion limits imposed by the absence of a palmitoyl moiety in the C-terminus of the A_{2A} receptor.
Acknowledgements: This work was supported by the Austrian Sciences Fund (FWF) and the Medical University of Vienna.

A82

NKP-1339, a first-in-class anticancer drug showing mild side effects and activity in patients suffering from advanced refractory cancer

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Background: NKP-1339 is one of the most promising investigational non-platinum metal drugs in clinical development against solid malignancies. Recently, NKP-1339 was evaluated in a clinical phase I trial regarding its safety, tolerability, maximum tolerated dose, pharmacokinetics, and pharmacodynamics. The high tumor targeting potential of NKP-1339 is probably based on delivery to tumor sites by serum proteins such as albumin and transferrin as well as on the activation of the compound in the reductive tumor milieu. The reduction of ruthenium(III) to ruthenium(II) is favoured under hypoxic condition (frequently occurring in solid tumors) and is followed by severe disruption of the cellular redox balance and induction of apoptosis via the mitochondrial pathway.
Methods: In the recently completed clinical phase I trial 34 patients with advanced solid tumors were enrolled for dose escalation [1]. NKP-1339 was infused on day 1, 8, and 15 of 28-day cycles. To gain further insight in the mechanism of action, protein expression studies in cancer cells exposed *in vitro* were performed.
Results: The maximum tolerated dose of NKP-1339 was determined at 625 mg/m^2, and the most common drug-related side effects were nausea, vomiting, and fatigue. A long-lasting partial response was observed in 1 patient with a gastro-intestinal neuroendocrine tumor (NET), and 7 patients having experienced stable disease, including NET (2), non-small-cell lung cancer (2), colorectal cancer (1), sarcoma (1), and cancer of unknown primary (1). Moreover, NKP-1339 was found to down-regulate in cancer cell lines the ER chaperon GRP78, a key regulator of the unfolded protein response, which is associated with intrinsic and chemotherapy-induced resistance.
Conclusions: The very limited side effects of NKP-1339 and the activity observed in a variety of tumors so far are very promising. Further clinical phase I drug combination studies and single agent phase II studies are planned.

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A83

TRPC3 overexpression promotes angiotensin II-induced cardiac dysfunction

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Background: TRPC3 was recently demonstrated as a player in pathogenesis of cardiac hypertrophy, while the potential pro-arrhythmogenic role of TRPC3 is incompletely understood. Using a TRPC3 transgenic overexpression mouse model, we examined the involvement of TRPC3 in cardiac actions of angiotensin II (AngII).
Methods: AngII effects on cardiac functions were characterized in Langendorff perfused hearts. Single ventricular myocytes were isolated and field-stimulated to measure effects on sarcomere shortening and Ca^{2+} transients. Furthermore, L-type Ca^{2+} channel current, action potentials and non-selective ion currents were analyzed electrophysiologically.
Results: AngII (100 nM) reduced left ventricular pressure (LVP) within 2 min to 64%, +dP/dt to 50% and −dP/dt to 55% of control in TRPC3(+/−) hearts, while even producing a positive inotropic effect in wild-type (WT) hearts. Simultaneously, ECG recordings demonstrated AngII-induced episodes of acute arrhythmogenicity in all TRPC3(+/−) hearts (n = 6), whereas rhythm of WT hearts (n = 6) remained unaffected. The AngII-induced impairment of cardiac functions in TRPC3(+/−) hearts was partially reversed by Pyr3 (30 μM). The amplitude of Ca^{2+} transient was significantly higher ($p < 0.05$; n = 60) in myocytes from TRPC3(+/−) mice ($[Ca^{2+}]$ F/F_0 0.354 ± 0.024) as compared to WT ($[Ca^{2+}]$ F/F_0 0.262 ± 0.021). Also, the time constant (τ) of Ca^{2+} decline was different between WT (0.196 ± 0.009 ms; n = 61) and TRPC3(+/−) (0.170 ± 0.008; n = 67; $p < 0.05$). Sarcomere shortening showed no significant difference between the two groups (3.80 ± 0.69% vs. 3.52 ± 0.65%; n = 10) whereas the SR-loading estimated from rapid application of caffeine (20 mM) revealed an increased SR loading of up to 40% in TRPC3(+/−) myocytes as compared to WT ($p < 0.05$; n = 43). The time constant of Ca^{2+} decline during caffeine challenge was also significantly changed ($p < 0.05$) in TRPC3(+/−) myocytes (3.04 ± 0.44 ms; n = 11) as compared to WT cells (1.65 ± 0.158 ms; n = 16). Importantly, AngII (100 nM) induced a rise in diastolic Ca^{2+} levels, which was accompanied by irregular contractions in TRPC3(+/−) but not in WT myocytes. The rise in the diastolic Ca^{2+} levels was significantly suppressed by Pyr3 (10 μM; n = 16), SEA 0400 (1 μM; n = 14) and KN-93 (1 μM; n = 12). Electrophysiological characterization of L-type voltage-gated Ca^{2+} currents and action potentials revealed that baseline electrophysiological parameters were not affected by TRPC3 overexpression, while AngII induced a transient prolongation of action potential duration only in TRPC3(+/−) myocytes. This TRPC3-dependent response was associated with a higher incidence of delayed afterdepolarizations.
Conclusions: Our results demonstrate that AngII modulation of cardiac functions is strictly dependent on TRPC3 expression and suggest a key role of TRPC channels in AngII-mediated arrhythmogenicity.
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A84

Axonal transport of botulinum toxin A from periphery to CNS in sensory and motor nerves

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Background: Botulinum toxin A (BTX-A) has been approved for treatment of movement disorders and migraine. The widely assumed peripheral mechanism of action has been questioned by recent studies which demonstrated an axonal transport in the facial nerve and within central nerves. Findings in our laboratory suggested a central antinociceptive activity following axonal transport in the sciatic nerve.
Methods: To characterize the axonal transport of BTX-A, the toxin's enzymatic activity in the CNS was assessed using immunofluorescent detection of its cleaved substrate synaptosomal-associated protein 25 (SNAP-25) following injections into the rat whisker pad and hind-paw, intramuscular injection into the gastrocnemius and intraneural injections into the sciatic nerve. Intraneural and intraganglionic colchicine was employed to block axonal transport. To investigate the significance of axonal transport for antinociceptive activity of BTX-A, we assessed the effect of peripheral and intraganglionic injections of low dose BTX-A in orofacial formalin-induced pain in rats.
Results: Following whisker pad BTX-A injection, cleaved SNAP-25 was observed in the dorsal horn of the medulla. Cleaved SNAP-25 following subcutaneous, intramuscular and intraneural toxin injection in rat hind limbs was observed in corresponding segments of ipsilateral dorsal and ventral horn. Central SNAP-25 cleavage following BTX-A injection into the sciatic nerve was prevented by colchicine. In the ventral horn, BTX-A protease was localized within cholinergic neurons. Facial and intraganglionic injections of BTX-A prevented orofacial pain dependent on axonal transport in the trigeminal sensory nerve.
Conclusions: Our results suggest that the axonal transport of BTX-A in different sensory and motor nerves commonly occurs after peripheral administration. Axonal transport in sensory neurons followed by central enzymatic activity is involved in botulinum toxin's antinociceptive effects. The possible functional role of axonal transport in motor neurons remains to be examined.
Acknowledgements: The antibody to cleaved SNAP-25 was a kind gift from Ornella Rossetto (University of Padua, Italy). The study was funded by the Croatian Ministry of Science, Education and Sport and the Deutscher Akademischer Austausch Dienst.

A85

A TRPC3 blocker, Pyr3, prevents stent-induced arterial remodeling

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Background: TRPC-mediated Ca^{2+} entry has been implicated in the control of smooth muscle proliferation and might represent a pivotal mechanism underlying in-stent restenosis. As we have observed significant expression of TRPC3 in human smooth muscle from coronary arteries as well as from aorta, we tested the efficiency of a recently discovered TRPC3-selective Ca^{2+} entry blocker, Pyr3 (10 μM) to prevent vascular smooth muscle proliferation and stent implantation-induced hyperplasia of human aorta.

Methods and results: The effect of Pyr3 on proliferation was measured by detection of BrDU incorporation and PCNA expression in human coronary smooth muscle and microvascular endothelium, which displays significantly smaller expression levels of TRPC as compared to smooth muscle. Pyr3 inhibited smooth muscle proliferation with an IC_{50} of about 3 μM but lacked detectable effects on endothelial proliferation. Measurements of ATP-induced Ca^{2+} signals revealed that Pyr3 suppressed agonist-induced Ca^{2+} entry more effectively in vascular smooth muscle as compared to endothelial cells. Inhibitory effects of Pyr3 on stent implantation-induced arterial injury were tested using a novel *in vitro* model of in-stent hyperplasia in human arteries based on organ-typical culture of human aortic constructs. Pyr3 (10 μM) effectively prevented increases in tissue levels of proliferation markers (PCNA and Ki67) at 2 weeks after stent implantation into human aortae. Similarly, proliferation markers were significantly suppressed when implanting a Pyr3-releasing stent prototype as compared to a bare metal stent control.
Conclusions: Our results suggest TRPC3 as a potential target for pharmacological control of smooth muscle proliferation. Selective inhibition of TRPC Ca^{2+} entry channels in vascular smooth muscle is suggested as a promising strategy for in-stent restenosis prevention.

A86

Consumption of serum lipid-reducing drugs in Serbia compared with Scandinavian countries: a population-based study, 2004–2010

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Background: Serbia is one of the leading countries in the world with respect to mortality from cardiovascular diseases. Drugs that reduce serum lipids belong to the group of drugs that can significantly lower complications of cardiovascular diseases and their utilization in Serbia deserves special attention. The aim of this study was to measure the consumption of serum lipid reducing drugs in Serbia from 2004 to 2010, to compare these data with those from Scandinavian countries, and to compare the consumption of lipid-lowering drugs and the rate of mortality from cardiovascular diseases in these countries.
Methods: A population-based study was undertaken to analyse lipid-lowering drug consumption using the Anatomical Therapeutic Chemical/Defined Daily Dose methodology. Cause-specific mortality rates were obtained from the WHOSIS annual report for the year 2009.
Results: In 2010, a total of 966 DDD/1000 inhabitants/day (DID) of all drugs was used in Serbia, of which 38.9% belonged to drugs for cardiovascular diseases. While in Scandinavian countries 17.0–24.8% of drugs for cardiovascular diseases belonged to lipid-lowering drugs, in Serbia it was substantially lower (3%). In 2004 in Serbia, 1.50 DID of statins were used. In 2008, this value was 32.5 DID. In every investigated country, simvastatin made up more than 50% of the consumption of statins. After simvastatin, the next most frequently used statin was atorvastatin, with 5.52, 11.0, 11.2 and 24.8 DID, in Serbia, Denmark, Finland and Norway, respectively. In 2004 Serbia had the highest mortality rate for cardiovascular diseases among the investigated countries with 762/100.000 inhabitants and Norway has the lowest rate with 158/100.000 inhabitants.
Conclusions: The use of lipid-lowering drugs is 6–8 times lower in Serbia as compared to Scandinavian countries but there is an evident rise in lipid lowering drugs consumption in Serbia during the investigated years.
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Neuropeptide Y modulates fear and fear extinction in distinct nuclei of the amygdala

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Background: Fear and anxiety are integrated in the amygdaloid nuclei and involve the interplay of the amygdala with various other brain areas. Neuropeptides play a critical role in regulating these processes. Neuropeptide Y (NPY) is highly expressed in limbic brain areas, including the amygdala. Depending on the receptor subtypes involved (Y_1, Y_2 or Y_4), NPY has different, in part opposing effects on anxiety, fear and depression-related behaviors.
Methods: We combined site-specific deletion of NPY receptors and locally restricted over-expression of NPY receptor subtype-selective ligands with behavioral analysis to elucidate the contribution of the individual receptor subtypes in the modulation of emotional behavior.
Results: In Pavlovian fear conditioning, NPY knock-out (KO) mice display a dramatically accelerated acquisition of conditioned fear while fear extinction was impaired. Interestingly this phenotpye was only reproduced in mice lacking both the Y_1 and the Y_2 receptor. In Y_1 single KO mice acquisition was moderately faster while fear extinction was delayed. Deletion of NPY and in particular of Y_2 receptors resulted also in a generalization of cued as well as context fear. Local over-expression of NPY by an rAAV vector in the basolateral amygdala delayed the acquisition and facilitated the extinction of fear, both in WT and NPY KO mice, emphasizing the crucial role of this area in NPY-mediated fear acquisition and extinction. On the other hand, deletion of Y_2 receptors in the central amygdala resulted in an increased expression and delayed extinction of conditioned fear, while there was no change in fear acquisition.
Conclusions: Taken together, our data demonstrate that NPY delays acquisition and reduces expression of conditioned fear whereas it promotes fear extinction. Both Y_1 and Y_2 receptors are involved in these processes. Y_1 receptors in the basolateral amygdala are modulating the acquisition and extinction of fear while Y_2 receptors in the central amygdala are preferentially inhibiting the expression but facilitating the extinction of learned fear. Furthermore, Y_2 receptors are crucially involved in the discrimination of fear-related stimuli.
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Plasma nitrite concentrations decrease after hyperoxia-induced oxidative stress in healthy humans

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Background: We measured plasma nitrite, the biochemical marker of endothelial nitric oxide ($^{\bullet}$NO) synthesis, before and after hyperoxia, in order to test the hypothesis that hyperoxia-induced vasoconstriction is a consequence of reduced bioavailability of $^{\bullet}$NO due to elevated oxidative stress.
Methods: Ten healthy males breathed 100% normobaric O_2 for 30 min between the 15th and 45th min of the 1 h study protocol. Plasma nitrite and malondialdehyde (MDA), arterial stiffness (indicated by augmentation index, AIx) and arterial oxygen ($P_{tc}O_2$) pressure were measured in the 1st, 15th, 45th and 60th minute of the study.
Results: Breathing of normobaric 100% oxygen during 30 min caused an increase of $P_{tc}O_2$ (from 75 ± 2 to 412 ± 25 mm Hg), AIx (from −63 ± 4 to −51 ± 3%) and MDA (from 152 ± 13 to 218 ± 15 nmol/L) and a decrease in plasma nitrite (from 918 ± 58 to 773 ± 55 nmol/L). During the 15-min recovery phase the plasma nitrite, AIx and MDA values remained altered.
Conclusions: This study suggests that the underlying mechanism of hyperoxia-induced vasoconstriction may result from reduced $^{\bullet}$NO bioavailability due to elevated and sustained oxidative stress.

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Peptidomics screening for the discovery of uterotonic plant peptides

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Background: Drug discovery from natural products is still one of the biggest sources of novel lead compounds. In particular, plant cyclotides, disulfide-rich peptides comprising three conserved disulfide bonds in a knotted arrangement, known as cyclic cystine knot motif, and a head-to-tail cyclization, have been extensively investigated over the last four decades for their use as scaffolds in drug development. However, their distribution among flowering plants still remains limited to few species of the families of *Rubiaceae* (coffee), *Violaceae* (violet), *Cucurbitaceae* (cucurbit), *Fabaceae* (bean) and recently *Solanaceae* (potato family), but it is very likely that cyclotides are more widely distributed since their predicted number in *Rubiaceae* alone is ~50.000. Additionally, the pharmacological validation of plants used in traditional medicines may trigger the discovery of novel uterotonic compounds [1].
Methods and Results: Based on the use of plants in traditional Nigerian medicine during pregnancy and childbirth, we analyzed several plants from different families to evaluate their uterotonic properties at cellular level and to identify cyclotides as active molecules. Using a MALDI-TOF/TOF-based screening protocol we were able to identify many novel cyclotide-containing species which was confirmed by manual *de novo* sequencing and automated database identification. The aqueous extracts and semipure peptide fractions have been tested further in a collagen gel contractility assay model and showed varying ability to induce contractions in human myometrial smooth muscle cells.
Conclusions: In conclusion, our results underpin earlier suggestions that cyclotides are one of the largest peptide classes within plants, covering a large chemical space based on their high sequence diversity. The evaluation of contractile properties of plants used in traditional medicines offers new starting points for the discovery and development of peptide-based uterotonic drug leads.
Acknowledgements: This work is funded by the Austrian Science Fund (FWF, grant P22889) and an "Ernst-Mach" Scholarship from the Austrian Agency for International Mobility and Cooperation in Education, Science and Research (OeAD) (A.A.).
Reference
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A90

Antiepileptic activity and subtype-selective action of flupirtine at $GABA_A$ receptors

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Background: Flupirtine is used as analgesic drug with muscle-relaxant properties. In addition, it has been suggested to possess antiepileptic properties. Recently, flupirtine has been revealed to simultaneously act at K_V7 channels and $GABA_A$ receptors. Here, antiepileptic activity and underlying mechanisms of action of flupirtine were investigated.
Methods: We used the patch clamp technique and primary cultures of hippocampal neurons or transfected tsA cells to investigate effects of flupirtine.
Results: In hippocampal neurons, flupirtine reduced seizure-like activity with no effect at 1 to 3 µM, and maximal effects at 10 to 30 µM; it enhanced currents through K_V7 channels with EC_{50} values at 6 µM. Flupirtine (30 µM) modulated GABA-induced currents in hippocampal neurons by reducing EC_{50} values for GABA threefold and maximal current amplitudes by 15%. Hence, flupirtine acted as an uncompetitive antagonist. Flupirtine did not alter rise time, decay time, or amplitudes of miniature inhibitory postsynaptic currents (mIPSCs), but enhanced the bicuculline-sensitive tonic current. When phasic GABAergic inhibition was blocked by penicillin G (5 mM), flupirtine enhanced maximal amplitudes of GABA-evoked currents by 43%, but hardly affected EC_{50} values. As these results suggested that flupirtine was able to differentiate between different $GABA_A$ receptor subtypes, its effects on recombinant $GABA_A$ receptors were investigated in tsA cells. With α1β2γ2 receptors, flupirtine reduced EC_{50} values for GABA threefold and maximal current amplitudes by 25%; with α1β2 receptors, it reduced EC_{50} values for GABA twofold, but reduced maximal current amplitudes by 35%.
Conclusions: These results indicate that flupirtine (i) exerts antiepileptic activity, (ii) modulates tonic, but not phasic, GABAergic inhibition and blocks K_V7 channels in hippocampal neurons, and (iii) affects $GABA_A$ receptors in a subunit-dependent manner.
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Activation of kappa opioid receptors reduces seizure activity in a dose-dependent way

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Background: Neuropsychiatric disorders are one of the main challenges of human medicine with epilepsy as one of the most frequent. Temporal lobe epilepsy represents the most common type of epilepsies and is often accompanied by marked neuronal

degeneration. One main factor that causes neural loss is the excitotoxicity of glutamate, which is copiously released during seizures and hypoxia accompanying seizures. It was also shown that the deletion of prodynorphin in mice and low expression in humans is associated with increased epilepsy vulnerability. Dynorphin targets opioid receptors and in particular the κ opioid receptor (KOP). The KOP receptors in the hippocampal formation are located in very strategically points for the control of glutamate release and, most importantly, they are not altered under epileptic conditions. Still, the functional background of these neuroprotective effects is not fully understood. The aim of this study was to investigate the influence of KOP agonists on EEG patterns of epileptic mice.

Methods: Kainic acid (KA; 1 nmol in 50 nL saline) was injected unilaterally into the dorsal hippocampus, causing acute and delayed behavioral and EEG effects. Four-channel EEG traces were recorded from ipsi- and controlateral hippocampi and motor cortices applying depth and surface electrodes, respectively. The KOP-specific agonist U-50488H was dissolved in saline (adjusted to pH 7.4) and applied i.p.

Results: Sharp waves and paroxysmal discharges in the ipsilateral hippocampus were recorded about 14 days after KA injection. Paroxysmal discharges were accompanied by behavioral arrest and stereotypic behavior like head nodding. Application of KOP agonists blocked paroxysmal discharges up to 2 hours in a dose-dependent manner, which was comparable to the effect of 2.5 mg/kg diazepam. Moreover, animals treated with U-50488H were awake.

Conclusions: Data collected so far demonstrate the anticonvulsant action of KOP agonists in the chronic phase of epilepsy, suggesting that KOP agonists may represent potential drug targets for novel anti-epileptics.

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Late breaking (not pubishled) abstracts:

L1

Title to be announced

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Abstract not published

L2

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Abstract not published

L3

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Abstract not published

L4

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Abstract not published

L5

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L6

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L7

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L8

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Abstract not published

L9

The role of pharmacokinetic/pharmacodynamic parameters in the optimization of antibiotic dosing

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Abstract not published

Background: In the context of increasing bacterial resistance it is necessary to review the current dosage regimen of antibiotics and to apply the pharmacokinetic/pharmacodynamic approach in determining the optimal dosage regimen. The three most important

pharmacokinetic/pharmacodynamic (PK/PD) parameters are the relationship of C_{max}/MIC, AUC/MIC and $t_{>MIC}$. Their use in the correlation with the mode of bacteria killing and the postantibiotic effect (PAE) is the basis for determining dosage regimens of both old and new antibiotics.

Methods: Using these PK/PD parameters we analyzed the suitability of the recommended dosage regimen of cefachlor in oral form. For this reason, the pharmacokinetic and pharmacodynamic parameters as well as the PK/PD values for critical-sensitive bacteria were determined for cefachlor 750 mg capsules.

Results: The results obtained after the analysis of the application of cefachlor indicated a disharmony of the recommended dosage regime and the PK/PD parameters. For the sensitive Gram-positive (G+) and Gram-negative (G–) strains the most important parameter for antibiotics with time-dependent killing of the bacteria, $t_{>MIC}$ ranged from 37% to 43% (depending on the MIC, PK/PD breakpoint of 1 mg/L), while AUC/MIC ranged from 14.6 to 29.2 (PK/PD breakpoint of 1.17 mg/L). The relatively short duration of PAE, especially in G– bacteria (max. 0.9 h), is another potential reason for the inefficiency of the recommended therapeutic dose of cefachlor (500 mg/8 h).

Conclusions: The harmonization of the antimicrobial dosage regimen of antibiotics with their PK/PD parameters is important for the improvement of therapeutic efficacy and thereby combating the development of resistant strains of bacteria.

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Author Index (Numbers refer to abstract no.)