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*PUBLISHED MEETING ABSTRACTS**

Oral Presentations

A1.1

A novel approach for measurement of retinal oxygen extraction based on laser speckle flowgraphy and retinal oximetry

Viktoria PAI[1], Patrick JANKU[1], Theresa LINDNER[1], Liudmyla PYLYPENKO[1], Anton HOMMER[1,2], Leopold SCHMETTERER[1,3,4,5,6,7,8], Gerhard GARHÖFER[1,*], Doreen SCHMIDL[1]

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Background: Retinal oxygen saturation difference between arteries and veins is altered in several ocular and systemic diseases. A reduction in arteriovenous oxygen difference suggests a reduction in retinal oxygen extraction. However, this can only be confirmed by taking retinal blood flow into account. Measurement techniques for the assessment of retinal oxygen extraction are highly warranted but are limited because most of them are time-consuming and they are usually based on experimental setups. Hence, the aim of this study is to assess retinal oxygen extraction based on measurement of retinal blood flow with the commercially available laser speckle flowgraphy and measurement of retinal oxygen saturation with the commercially available retinal oximetry.

Methods: Ten young healthy subjects participated in the present study. In each vessel around the optic disc retinal blood flow and retinal oxygen saturation were measured at the same position. The corrected arterial ($cO_{2,CRA}$) and venous ($cO_{2,CRV}$) oxygen content was estimated from the parameters evaluated and the difference between these two determined parameters ($cO_{2,DIFF}$) was assessed. Retinal oxygen extraction was calculated using mean vessel flow rate (Q_{MV}) and $cO_{2,DIFF}$. It is known that retinal oxygen saturation decreases during systemic hyperoxia. Therefore, this novel approach for measurement of retinal oxygen extraction was validated by a baseline measurement and a measurement during inhalation of 100% oxygen.

Results: Q_{MV} significantly decreased during 100% oxygen breathing (−23 ± 10%, $p < 0.001$). While $cO_{2,CRA}$ only slightly increased by 1 ± 2% ($p = 0.093$), $cO_{2,CRV}$ increased by 9 ± 14% ($p = 0.050$). Consequently, $cO_{2,DIFF}$ decreased by 10 ± 20% ($p = 0.087$). This led to a pronounced decrease in retinal oxygen extraction by −31 ± 18% during hyperoxia ($p < 0.001$).

Discussion: During systemic hyperoxia, induced by breathing 100% oxygen, a significant decrease in total retinal blood flow and retinal oxygen extraction was observed, which is consistent with previous findings in the literature and physiologically expected. Therefore, the presented approach for measurement of retinal oxygen extraction using commercially available devices appears to be feasible. Studies using this method in larger patient cohorts should be conducted in the future.

Keywords: laser speckle flowgraphy – retinal oxygen extraction – hyperoxia

A1.2

Effect of orally administered, low-dose dronabinol on ocular hemodynamics in healthy subjects

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Background: It is hypothesized that besides its potential of lowering intraocular pressure (IOP) and neuroprotective effects, tetrahydrocannabinol (THC) may also improve ocular hemodynamics. In this study we investigated whether single orally administered dronabinol, a synthetic THC derivate, alters optic nerve head blood flow (ONHBF) and its autoregulation in healthy subjects. In addition, we investigated

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the effect of dronabinol on retinal blood flow and retinal oxygen metabolism.

Methods: The study was conducted in a randomized, double-masked, placebo-controlled, two-way crossover design. Measurements were taken on two study days before and 1 hour after drug intake. Participants received capsules containing 5 mg dronabinol on one study day and placebo on the other. ONHBF was assessed at rest and during isometric exercise using laser Doppler flowmetry. The isometric exercise was done for 6 minutes to increase mean arterial blood pressure (MAP). Ocular perfusion pressure (OPP) was calculated as $2/3 \times MAP - IOP$. We used a custom-built Doppler optical coherence tomography system to quantify total retinal blood flow (TRBF) and a commercially available Dynamic Vessel Analyzer to measure oxygen saturation of major retinal vessels. Based on these values, retinal oxygen extraction was calculated.

Results: A total of 12 female and 12 male healthy subjects (mean 26 ± 4 years) finished the study according to the protocol. The intake of dronabinol or placebo had no effect on IOP, MAP or OPP. During the study days, dronabinol was well tolerated and no cannabinoid-related psychoactive effects were reported. Dronabinol induced a significant increase in ONHBF at rest by 9.5 ± 8.1% ($p < 0.001$). Isometric exercise did not alter the ONHBF autoregulatory response. As for retinal blood flow, dronabinol resulted in a significant increase in TRBF from 38.9 ± 6.1 to 40.7 ± 6.7 µl/min ($p < 0.001$), which was accompanied by a significant increase in retinal venous oxygen content (from 0.129 ± 0.008 to 0.132 ± 0.009 ml O_2/ml; $p = 0.02$). Retinal oxygen extraction remained stable (2.2 ± 0.4 *vs*. 2.2 ± 0.4 µl O_2/min; $p = 0.29$), as no change in retinal arterial oxygen content occurred ($p = 0.12$). Placebo had no effect on retinal arterial or venous oxygen content, retinal oxygen extraction, or TRBF ($p > 0.10$ each).

Discussion: Our data show that low-dose dronabinol increases ONHBF and TRBF in healthy subjects without altering the autoregulatory response of ONHBF or causing psychoactive side effects. Retinal oxygen extraction, IOP and OPP remained unchanged. Further studies are needed to investigate whether this drug may be a candidate for improving perfusion in patients with ocular vascular disease or glaucoma.

Acknowledgements: This study was funded by the Austrian Science Fund FWF (project KLI 340).

Keywords: dronabinol – cannabinoids – hemodynamics – optic nerve head blood flow – retinal oxygen extraction – retinal blood flow – laser Doppler flowmetry – Doppler optical coherence tomography – randomized controlled clinical trial

A1.3

Attitudes of patients with opioid use disorder towards synthetic cannabinoids

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Background: There is limited data on the awareness and use of synthetic cannabinoids (SCs) in high-risk populations in Serbia, despite SCs becoming more and more common at the illicit drug market. This pilot study aimed to examine the awareness and prevalence of use of SCs in patients with an opioid-use disorder and to identify patient characteristics and other factors associated with SC use.

Methods: This cross-sectional study was conducted at the Clinic for Psychiatry, Clinical Center Vojvodina, Serbia, the largest tertiary health care institution in this region of the country. All patients hospitalized due to the treatment of opioid dependence during November and December 2017 were included, and filled out an anonymous questionnaire specifically developed for the purpose of this study.

Results: Out of 64 patients (median age 36.4 years), one third (32.8%) reported using SCs. Socio-demographic characteristics of the subjects were not associated with SC use. There were differences in the most common sources of information reported between the SC users and non-users. The majority of SC users (76.0%) were informed about SCs through friends, compared with just 26.0% of non-users ($p < 0.001$). Nearly all study participants (93.8%) were daily tobacco users. The share of respondents reporting alcohol and marihuana use was significantly higher among the SC users (52.0% *vs.* 20.9%, $p = 0.011$ and 15.6% *vs.* 12.5%, $p = 0.015$), respectively. A higher proportion of SC users used multiple psychoactive substances (38.1% *vs.* 16.3%), and this difference was statistically significant ($p = 0.047$). The most commonly reported adverse effect of SCs among users included dry mouth (81.0%), troubles to think clearly (52.4%) and panic attacks (52.4%).

Discussion: Understanding the awareness and use of SCs among high-risk drug users, as well as associated factors can help to improve substance-use disorder treatment in our setting. Educational activities targeting public are urgently needed to raise awareness on SCs, considering that social contacts are the main sources of information on SCs for this vulnerable population. Users of SCs have also reported using other psychoactive substances more often, and this calls for a holistic approach addressing multiple factors to improve substance-use treatment in our setting.

Acknowledgements: This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant no. 451-03-68/2022-14/200114).

Keywords: synthetic cannabinoids – opioid use disorder – attitudes

A1.4

Suicidality and drug self-poisoning during the COVID-19 pandemic

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Background: Suicide is conscious and deliberate attempt at taking one's own life. Due to the increase in suicide rates, suicide is considered a significant health and social problem. The uncertainties during the coronavirus pandemic led to new and/or exacerbation of existing psychiatric problems among which are also suicide attempts. Such stress will assumably lead to an increase of suicide rates during the coronavirus pandemic. Our goal was to determine a frequency of suicide behavior among patients that were treated at the Clinic for Psychiatry at the Clinical Centre of Vojvodina and to determinine psychopathological and sociodemographic characteristics of this cohort.

Methods: A total of 112 patients' anamnestic data that contained information about a suicide attempt in 2020 were analysed. The data were statistically analyzed in JASP 0.14.1 and Microsoft Excel 2016.
Results: The analysis of collected data showed that suicide attempts were more frequent among female patients, 11–24 and 35–44 years old. We found no statistically significant correlation between suicidality and COVID-19 motivation for suicide attempt. The most frequent method used for attempting suicide was drug intoxication. The frequency of suicide attempts was higher in the second half of the year. Some patients attempted suicide more than once in 2020. Most of the patients have psychiatric comorbidities.
Discussion: During the coronavirus pandemic, an early diagnosis of psychiatric illness is of great importance. It is also significant for vulnerable groups to stay socially engaged in order to prevent as many as possible suicide attempts.
Acknowledgements: The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant no. 451-03-68/2022-14/200114).
Keywords: drug self-poisoning – suicidality – COVID-19 pandemic

A1.5

Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation–contraction coupling

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Background: The adaptor protein STAC3 was discovered to be an essential protein for skeletal muscle excitation–contraction (EC) coupling and exerts three distinct functions: (i) it facilitates membrane expression of $Ca_V1.1$; (ii) it is crucial for $Ca_V1.1$ function as the voltage sensor of EC coupling; (iii) lastly, it is essential for the conformational coupling between $Ca_V1.1$ and the ryanodine receptor 1 (RyR1). Previously, two distinct interactions between STAC3 and $Ca_V1.1$ were identified: the one between the SH3-1 domain of STAC3 and the II–III intracellular loop of $Ca_V1.1$, and the one between the C1-linker region of STAC3 and the proximal C-terminus of $Ca_V1.1$.
Methods: To determine which interaction is important for each function, two STAC3 fragments, each containing the domain responsible for one interaction, were reconstituted in a double $Ca_V1.1$/STAC3 knockout skeletal muscle cell line. With electrophysiological recordings using the fluorescent calcium indicator Fluo-4, both calcium currents and the calcium release from the SR could be measured simultaneously. Charge movement was measured using the non-conductive $Ca_V1.1$ channel.
Results: Electrophysiological recordings revealed that the STAC3 C1-linker fragment expression rescued $Ca_V1.1$ charge movement and calcium currents. However, the calcium release from the sarcoplasmic reticulum was severely reduced. Conversely, reconstitution of only the STAC3-SH3s domains did not rescue any function. Simultaneous reconstitution of both fragments also did not fully rescue EC coupling, suggesting that the isolated SH3 domains interact with low affinity. To increase the local concentration, we linked the SH3 domains to the Ca_V ß1a subunit. This fragment alone rescued minimal EC coupling, but no calcium currents. However, when co-expressed with the other STAC3 fragment, full currents and EC coupling were reconstituted.
Discussion: These results demonstrate that the C1-linker/C-terminus interaction is responsible for STAC3-targeting to the EC-coupling machinery and $Ca_V1.1$ functional expression, while the low-affinity SH3s/II–III loop interaction merely enhances EC coupling.
Acknowledgements: This study was supported by the Austrian Science Fund FWF (grant ZFP337760).
Keywords: excitation–contraction coupling – STAC3 – voltage-gated calcium channels – $Ca_V1.1$ channels

A1.6

Selective activation of the κ-opioid receptor as an effective strategy for treatment of chronic pain

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Background: Adequate treatment of chronic pain remains an unmet clinical need at the beginning of the third millennium. Chronic pain is still poorly managed because of the lack of efficacious therapies, high side-effect burden or abuse liability of the present pharmacotherapies. Drugs targeting the μ-opioid receptor (MOR) are very effective analgesics. With continued use, opioid safety is dramatically reduced because of the side effects of physical dependence and addiction, promoting development of opioid-use disorders and overdose deaths. The κ-opioid receptor (KOR) has a central role in modulating neurotransmission in central and peripheral neuronal circuits that subserve pain and other behavioral responses. Among alternative treatment strategies, the KOR is viewed as a promising strategy for pain therapeutics without the deleterious adverse effects of the MOR. In this study, we report and compare the antinociceptive effects of structurally distinct and selective KOR agonists in a mouse model of chronic inflammatory pain. The investigated KOR agonists include the prototypical KOR ligand U50,488, the clinically used antipruritic drugs nalfurafine and difelikephalin, the two diphenethylamines HS665 and HS666, and the peptide–small molecule conjugate DNCP-β-NalA(1).
Methods: Chronic inflammatory pain was induced in mice by injection of complete Freund's adjuvant (CFA) to the dorsal side of the right hindpaw. Nociceptive behavior was assessed 72 hours post-CFA by measuring paw withdrawal latencies to thermal stimulation using the Hargreaves test. Locomotor activity was determined using the rotarod and open-field tests. The elevated plus maze (EPM) test was used for anxiety-like behaviors. The experimental drugs were administered subcutaneously.
Results: Behavioral studies showed that all KOR agonists effectively inhibited pain responses of mice with CFA-induced inflammatory hyperalgesia. Subcutaneous drug administration produced a significant increase in paw withdrawal latencies to thermal stimulation in a time- and dose-dependent manner. However, characteristic differences were observed in the time course of the antinociceptive effects. Nalfurafine and DNCP-β-NalA(1) had a rapid but short duration (up to one hour) of the antinociceptive effect, whereas a long duration of action (4–7 hours) was measured for the other KOR agonists. We also show that the attenuation in pain-related behavior was antagonized by the selective KOR antagonist nor-binaltorphimine, demonstrating a KOR-dependent mechanism of action. In the rotarod test, no sedation or motor incoordination was caused by HS665, HS666 or DNCP-β-NalA(1), whereas locomotor dysfunction was produced by U50,488. No significant effects on spontaneous

locomotor activity were observed after acute and chronic administration in mice with HS665 and HS666. In the EPM test, HS665 and HS666 did induce anxiety-like behavior in mice.

Discussion: We show that selective activation of the KOR by different structural classes of KOR agonists, peptide and small molecules, effectively attenuates thermal sensitivity in mice with CFA-induced chronic inflammatory pain, with a favorable benefit / side effect ratio regarding CNS-mediated KOR side effects. Thus, targeting the KOR represents a promising strategy for an improved and safer treatment of chronic pain states.

Acknowledgements: The study was supported by the Austrian Science Fund FWF (grants no. I4697 and P32109).

Keywords: κ-opioid receptors – chronic pain – antinociception – sedation – anxiety

A1.7

Impact of a moderate decrease in the abundance of P-glycoprotein at the blood–brain barrier on the brain distribution of model P-glycoprotein substrates

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Background: P-glycoprotein (P-gp / ABCB1) is an efflux transporter which is abundantly expressed in the luminal (blood-facing) membrane of brain capillary endothelial cells forming the blood–brain barrier (BBB). Experiments in P-gp knockout mice (*Abcb1a/b*$^{-/-}$) revealed pronounced increases in the brain distribution of various P-gp substrates (*e.g.* ivermectin, vinblastine, digoxin, loperamide, verapamil) in absence of P-gp activity. However, a complete absence of P-gp activity is unlikely to occur under different pathophysiological conditions (*e.g.* in neurodegenerative diseases), in which the abundance of P-gp was shown to decline only moderately. The aim of this work was to compare the effect of a moderate decrease in P-gp abundance with a complete absence of P-gp on the brain distribution of radiolabelled model P-gp substrates by means of positron emission tomography (PET) imaging. To this end, we used wild-type, heterozygous (*Abcb1a/b*$^{+/-}$) and homozygous (*Abcb1a/b*$^{-/-}$) *Abcb1a/b* knockout mice as models with controlled levels of cerebral P-gp abundance.

Methods: Wild-type, *Abcb1a/b*$^{+/-}$ and *Abcb1a/b*$^{-/-}$ mice underwent PET scans after intravenous injection of (*R*)-[^{11}C]verapamil, [^{11}C]*N*-desmethyl-loperamide or [^{11}C]metoclopramide. After the PET scan, brains were collected to quantify cerebral P-gp abundance with immunohistochemistry. Brain uptake of all three P-gp substrates was expressed as the area under the brain concentration–time curve (AUC_{brain}).

Results: Wild-type, *Abcb1a/b*$^{+/-}$ and *Abcb1a/b*$^{-/-}$ mice had normal, intermediate and no cerebral P-gp abundance, respectively. All three radiotracers had markedly increased AUC_{brain} values in *Abcb1a/b*$^{-/-}$ mice *vs.* wild-type mice (2.5- to 5.7-fold, $p < 0.0001$). However, in *Abcb1a/b*$^{+/-}$ mice AUC_{brain} values were only significantly increased over wild-type mice for [^{11}C]metoclopramide (1.46-fold, $p < 0.001$), but not for (*R*)-[^{11}C]verapamil and [^{11}C]*N*-desmethyl-loperamide.

Discussion: The effect of a moderate decline (−50%) in cerebral P-gp abundance as it is expected to occur in neurodegenerative diseases was markedly less pronounced than complete absence of P-gp activity and appeared to be substrate-dependent. Brain distribution of the avid P-gp substrates (*R*)-[^{11}C]verapamil and [^{11}C]*N*-desmethyl-loperamide was unchanged in heterozygous *Abcb1a/b*$^{+/-}$ mice. This may be related to the high transport capacity of P-gp for these compounds, which can effectively restrict their brain distribution even under conditions of partially decreased P-gp abundance. On the other hand, the brain distribution of the weak P-gp substrate [^{11}C]metoclopramide was significantly increased which suggests a better sensitivity of this radiotracer to detect disease-related changes in P-gp abundance / activity.

Acknowledgements: This research was funded by the Austrian Research Promotion Agency FFG (882717 PETABC) and the Austrian Science Fund FWF (I4470-B EPIFLUX).

Keywords: P-glycoprotein – blood–brain barrier – brain distribution

A1.8

Unraveling the tumor-supressive role of STAT3β in acute myeloid leukemia

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Background: One major mediator of cytokine signaling is the signal transducer and activator of transcription 3 (STAT3). In acute myeloid leukemia (AML) patients, STAT3 is frequently found constitutively activated, which is associated with poorer overall survival. Apart from this, STAT3 exists in two distinct isoforms generated by alternative splicing, the full-length transcript STAT3α and the C-terminally truncated isoform STAT3β. While STAT3 in general is considered as an oncogenic driver in malignant diseases, STAT3β gained attention as a favorable prognostic marker and was shown to regulate gene transcription also in a STAT3α-independent manner. Analysis of AML patient samples revealed that a high *STAT3β/α* mRNA ratio is a favorable prognostic marker correlating with disease outcome. However, the underlying molecular mechanism remained elusive. In this study we provide novel insights into the STAT3 isoform-specific impact on AML development.

Methods: We use a combination of *in vivo* models and *in vitro* assays coupled to next-generation sequencing approaches. Briefly, hematopoietic stem cells were isolated from fetal livers of wild-type and STAT3β-deficient mice, and were retrovirally transduced with the human fusion-oncogene MLL-AF9. Transformed cells were used to study the STAT3 isoform-specific impact on different cellular mechanisms *in vitro*. Moreover, the cells were transplanted into immunocompromised animals to explore leukemic potential.

Results: Lack of STAT3β in murine AML blasts led to accelerated disease progression and poorer overall survival in immunocompromised mice despite of any proliferation advantage *in vitro*. Flow-cytometry-based analysis of infiltrated blasts revealed that leukemic cells lacking STAT3β are less committed to the myeloid lineage and less responsive to differentiation stimulus. By performing transcriptome analysis of leukemic cells isolated from organs of diseased animals we identified a strong enrichment of genes involved in interferon signaling in absence of STAT3β.
Discussion: Understanding the tumor-suppressive role of STAT3β is crucial to discover novel therapeutic targets in AML. We aim to validate STAT3β-specific targets that could serve as novel therapeutic targets. Especially patients with lower STAT3β levels and poor prognosis could benefit from these findings.
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Keywords: acute myeloid leukemia – STAT3 – tumor suppressor – MLL-AF9

A1.9

Neuronal pathomechanisms of anti-AMPA receptor autoimmune encephalitis

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Background: Anti-AMPA receptor encephalitis patients mostly suffer from limbic encephalitis, including memory loss, psychiatric symptoms and, in approximately a third of the cases, epilepsy. The pathomechanisms leading to these symptoms are incompletely to poorly understood. A body of evidence exists demonstrating that the antibodies lead to internalization of the AMPA receptor as a primary effect, thereby leading to reduced AMPA receptor-mediated excitatory receptor currents. While such reduction of AMPA receptor-mediated neurotransmission can be readily reconciled with memory deficits, the pathogenesis underlying epilepsy in these patients is difficult to understand, considering the compromised neuronal excitation. From previous work on cultured neuronal networks derived from rat, compensatory reductions of inhibitory GABAergic signalling together with enhanced intrinsic (*i.e.* independent of synaptic activity) excitability of the neurons were proposed as possible underlying pathomechanisms. In this study we set out to investigate this possibility by exploring the *in vitro* effects of an anti-AMPA receptor sample (which was derived from a patient who had been tested at the Division of Neuropathology and Neurochemistry) on rat hippocampal neurons.
Methods: Patch-clamp electrophysiology was performed on primary rat hippocampal neurons (co-cultured with glial cells) that had been pretreated with the autoimmune sample or control samples for 72 hours. Current-clamp recordings were conducted to measure neuronal membrane voltage and spontaneous or evoked electrical activities, and voltage-clamp experiments served to investigate ionotropic receptor currents either induced by exogenous application of ligands (*e.g.* AMPA or muscimol, a $GABA_A$ receptor agonist) or as spontaneously occurring synaptic events.
Results: Our electrophysiological recordings confirm a reduction of neuronal responses to AMPA and of AMPA-elicited receptor currents, as well as of spontaneously occurring excitatory postsynaptic events in anti-AMPA receptor antibody-treated neurons compared to control neurons. Conversely, muscimol-elicited currents remained unaltered, and a reduction of spontaneously occurring inhibitory postsynaptic events was also not observed. However, the intrinsic neuronal excitability, which was probed by depolarizing current injections of increasing amplitude from a resting voltage set to −80 mV by experimental hyperpolarization, was considerably augmented in anti-AMPA receptor antibody-treated neurons. This effect showed up prominently as an elicitation of high discharge frequencies of action potentials at relatively moderate levels of depolarizing currents (30 to 120 pA). Unprovoked discharge activity was readily observed in neurons treated with control samples, but neurons treated with the anti-AMPA receptor autoimmune sample lacked spontaneous activity in most cases, though discharge activity could always be elicited by current injection. However, it was repeatedly observed that neurons suddenly switched to seizure-like discharge activity during the recordings. Such events were not seen in the control neurons.
Discussion: A previous report has proposed homeostatic reduction of $GABA_A$ receptor-mediated inhibitory neurotransmission as a potential cause of epilepsy in anti-AMPA receptor encephalitis patients. While seizure-like activity was also found to arise spontaneously in this study of primary neuronal cultures treated with our anti-AMPA receptor autoimmune sample, a decrease of $GABA_A$ receptor-mediated signalling could not be confirmed as a seizure activity-precipitating mechanism. Instead, potentiation of the intrinsic excitability at relatively moderate depolarization levels appeared to render neurons prone to discharge in abnormally elevated discharge patterns.
Keywords: AMPA receptor – autoimmune encephalitis – epilepsy – electrophysiology – hippocampus

A1.10

The human A749G *CACNA1D* ($Ca_V1.3$) variant alters channel gating and causes a phenotype in mice similar to the human neurodevelopmental disorder

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Background: *De novo* missense variants of *CACNA1D*, encoding the pore-forming α1 subunit of $Ca_V1.3$ L-type Ca^{2+} channels (LTCCs), are associated with treatment-resistant hypertension and a neurodevelopmental syndrome that can also manifest with endocrine features (hyperaldosteronism, hyperinsulinemic hypoglycemia). Patch-clamp recordings revealed mutation-induced alterations in channel gating that are predicted to promote $Ca_V1.3$ channel activity

at subthreshold potentials. However, to date definite proof of their disease-causing nature is still missing.

Methods: To study the pathophysiological consequences of such gating-modifying *CACNA1D* variants, we have introduced the A749G variant found in a patient with autism spectrum disorder and intellectual disability into C57BL/6N mice ($Ca_V1.3^{AG}$ mouse line). We have characterized $Ca_V1.3^{AG}$ mice using behavioural, neuroanatomical and electrophysiological methods, and conducted an *in vivo* pharmacological rescue experiment using the LTCC inhibitor isradipine (extended-release formulation).

Results: $Ca_V1.3^{AG}$ mutant mice are viable, reproduce and appear overall healthy except for a delayed gain of body weight (more pronounced in homozygous mutants). Patch-clamp recordings in cultured mouse chromaffin cells from heterozygous mutants confirmed altered native LTCC Ca^{2+} currents resembling gating changes observed upon heterologous expression of A749G-containing $Ca_V1.3$ channels. While plasma aldosterone levels were elevated only in adult female mutants, blood glucose levels at baseline and after an i.p. glucose challenge were significantly decreased in adult homozygous males only, indicating a sexual dimorphism. Mutants of both sexes displayed increased locomotion induced by handling and/or a novel environment in a gene-dose-dependent manner. Further behavioral analysis of adult male mice identified an anxiety-like behavior in the light-dark box, absent marble-burying behavior (homozygous mice only), altered grooming and rearing patterns and a social deficit (3-chamber test). Gross neuroanatomy was unaltered, whereas dendritic spine morphology of CA1 pyramidal neurons in the dorsal hippocampus in Golgi-Cox-stained brain sections of adult $Ca_V1.3^{AG}$ mice was altered (see poster by Nikonishyna *et al.* [1] for details). Electrophysiological recordings in acute brain slices revealed increased cellular excitability in striatal medium spiny neurons and medial dopaminergic substantia nigra neurons projecting to the dorsomedial striatum in heterozygous mutants. Finally, oral pretreatment over 2 days with 0.3 or 1–2 mg/day isradipine (doses based on a separate pharmacokinetic study) resulted in therapeutically relevant plasma levels but did not rescue the hyperlocomotive phenotype in female or male mice, respectively.

Discussion: Here, we provide the first direct proof for the pathogenicity of a gating-modifying *CACNA1D* missense variant and demonstrate that our construct-valid disease mouse model can be used to study disease-underlying mechanisms as well as therapeutic interventions.

Acknowledgements: Support for this study was obtained from the Austrian Science Fund FWF (grants P35722 to J.S., P35087 to N.J.O., and FG18-B to N.S.), the Jubilee Fund of the Innsbruck universities (to N.J.O. and E.P.), the Erika Cremer Habilitation Fellowship of the University of Innsbruck (to N.J.O.), the German Research Foundation DFG (project 431549029/SFB1541 to J.R.) and the Telethon Foundation (grant GGP15110 to E.C.).

Keywords: $Ca_V1.3$ channels – *CACNA1D* – L-type Ca^{2+} channels – voltage-gated calcium channels – neurodevelopmental disorders

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Poster Presentations

A2.1

Exploring simvastatin bioaccumulation and biotransformation in probiotic bacteria for enhanced insight into drug–microbiota interactions

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Background: The considerable challenge faced in clinical practice is the variability in drug response among individuals, primarily resulting from differences in drug pharmacokinetics. The factors contributing to these differences between individuals are sometimes challenging to attribute solely to genetic factors. It is suggested that these variations may be influenced, at least in part, by the effects of the intestinal environment. Within the intestinal lumen, the presence of microbiota can alter the absorption and pharmacokinetic profile of numerous drugs [1,2,3]. The potential involvement of gut microbiota in the response to simvastatin, which exhibits significant interindividual variations, has received insufficient attention so far. To gain a deeper understanding of the underlying mechanisms and their impact on clinical outcomes in patients receiving simvastatin therapy, our study aimed to investigate the bioaccumulation and biotransformation of simvastatin in probiotic bacteria under *in vitro* conditions.

Methods: Simvastatin samples with probiotic bacteria were incubated under anaerobic conditions at 37 °C for 24 hours. Extracellular and intracellular samples were collected at predetermined time intervals and prepared for analysis with liquid chromatography–mass spectrometry (LC–MS). Simvastatin concentrations were measured using LC–MS/MS. Potential biotransformation pathways were explored using a bioinformatics approach in conjunction with experimental assays.

Results: During the incubation, simvastatin was transported into bacterial cells, resulting in increased drug bioaccumulation over time. A decrease in total drug levels during the incubation period suggests partial biotransformation of the drug by bacterial enzymes. Bioinformatics analysis indicated that the lactone ring of simvastatin was most susceptible to metabolic changes, with ester hydrolysis followed by hydroxylation being the most likely reactions.

Discussion: Our findings reveal that the bioaccumulation and biotransformation of simvastatin by intestinal bacteria may underlie the altered bioavailability and therapeutic effects of the drug. Since our study focused on selected bacterial strains *in vitro*, further comprehensive research is necessary to fully understand the complex interactions between drugs and the microbiota in influencing the overall clinical response to simvastatin. Such insights could lead to novel approaches for personalized lipid-lowering therapy.

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Scientific and Technological Development of Vojvodina (grant no. 142-451-3179/2022).
Keywords: gut microbiota – drug metabolism – drug pharmacokinetics

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A2.2

Dahi-derived probiotic *Enterococcus munditii* QAUEM2808 ameliorates stress-induced depression-like behaviour and tryptophan metabolism

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Background: A growing number of findings link the gut microbiota with psychiatric disorders due to bidirectional gut–brain communication. Several preclinical and clinical studies reported significant changes in gut microbiome composition and function in depression. This emerging link suggests that gut microbiome modulation by probiotics may have a translational potential in the treatment of depression. Mechanistically, changes in tryptophan metabolism and immune modulation could underly the central effects of probiotics. The current project is designed to evaluate the role of the probiotic *Enterococcus munditii* QAUEM2808, isolated from Dahi, an indigenous fermented milk product of Southern Asia, in a mouse model of stress-induced of depression-like behaviour.
Methods: To induce depression-like behaviour, BALB/c mice were subjected to unpredictable chronic mild stress (UCMS) [1]. Concomitantly, *E. munditii* QAUEM2808 was administered via drinking water for 28 days at 109 CFU/ml. Depression-like behaviour and anxiety were evaluated by a behavioural test battery including the forced swimming test, open field test, splash test and sucrose preference test. Fecal pellets, serum and brain tissue were collected at the end of the experiment in order to determine gut microbiome composition by 16S rDNA sequencing and molecular analysis.
Results: *E. munditii* QAUEM2808 was able to ameliorate stress-induced depression-like behaviour and anxiety as exemplified by a reversal of stress-induced decrease in sucrose preference. Molecular analysis revealed probiotic-induced expression changes of genes involved in tryptophan and serotonin metabolism (*e.g.* central *Slc6a4*, *Tph2*) in the hippocampus. Similarly, microbiome analysis indicated changes in bacterial taxa involved in tryptophan metabolism (*e.g. Clostridia UCG 014*) in the probiotic-fed groups.
Discussion: In conclusion, *E. munditii* QAUEM2808 has the potential to ameliorate stress-induced depression-like behaviour and changes in tryptophan metabolism could underly the observed behavioural effects.
Keywords: gut–brain axis – microbiome – depression-like behaviour – hypothalamus

Reference:

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A2.3

The effect of intranasal neuropeptide Y on high-fat diet-induced anhedonia

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Background: Western diet contains a high percentage of saturated fatty acids and is dense in energy, which leads to overeating and the development of diet-induced obesity. There is growing evidence about the role of obesity in the development of mental health disorders such as major depression. Metabolic disturbances implicated in the development of depressive mood due to diet-induced obesity include dysregulation of the hypothalamic–pituitary–adrenal axis, release of proinflammatory cytokines, leptin and insulin resistance. In addition, both patients with mild depressive disorder and mouse models of high-fat diet (HFD)-induced depression show lower expression of neuropeptide Y (NPY), a peptide with antidepressant, anxiolytic and orexigenic properties. Therefore, the aim of this study was to evaluate the effect of intranasal NPY on HFD-induced anhedonia and its potential as a new therapeutic approach.
Methods: In this study, we fed 48 male mice a HFD containing 48 kJ% of fat or a control diet containing 12 kJ% of fat for a period of 8 weeks. Mice were housed in cages of two under standard laboratory conditions. Subsequently, the mice were placed in single cages in order to perform the sucrose preference test (SPT) to evaluate anhedonia. On day 2 and day 4 of SPT either NPY or sterile distilled water was applied intranasally at a dose of 100 µg per mouse. Mice were sacrificed 3 hours after the second NPY application. The same experiment was repeated in Y_2 receptor knockout mice.
Results: HFD reduced NPY gene expression and protein levels in the hypothalamus. Intranasal NPY reduced sucrose preference in the HFD group 3 hours after application, which is indicative of anhedonia. This effect was accompanied by lower food intake, greater weight loss and increased corticosterone levels in plasma. Sucrose preference, food intake and body weight remained unchanged in the experiment with Y_2 knockout mice.
Discussion: Our results are contrary to current knowledge about NPY as an orexigenic and antidepressant peptide. The fact that NPY inhibited food intake and induced anhedonia in the SPT may be due to the sedative effect of NPY, which occurs at higher doses. Another possible explanation might be a desensitization to NPY by HFD. Since no effect was seen in the Y_2 knockout group, the Y_2 receptor may also play a role in our observations.
Acknowledgements: The authors would like to thank Ingrid Liebmann for technical assistance.
Keywords: depression-like behaviour – hypothalamus – sucrose preference – neuropeptide Y

A2.4

A prospective, randomized, double-blind, placebo-controlled trial to investigate the effects of dextroamphetamine or zolpidem on attention and reaction time in healthy males

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Background: Driving and handling of machinery under the influence of medicines may be associated with a significant risk. However, legally acceptable intakes regarding the use of medicines are not defined and a subjective assessment by an authorized physician determines the individual physical and mental suitability. Thus, in contrast to established legal limits that exist for alcohol consumption, there is a gap in the standardization of tests and in the potentially acceptable concentration of medicines in the body to support physician's discrimination of the status of handling ability. This study has been designed to investigate (i) whether the changes in psychomotoric performance can be objectively measured in healthy people after intake of model drugs that are known to impair or enhance subject's concentration, and (ii) whether changes in psychomotoric performance are related to drug concentration in the body in order to establish minimally acceptable plasma concentrations.

Methods: In this randomized and double-blind phase I study, the effect of single doses of the investigational drug was analyzed in 60 healthy males allocated to one of 3 parallel groups: 20 subjects received dextroamphetamine (30 mg), zolpidem (5 mg) or placebo. Differences in reaction time from baseline were assessed with a computer test (Psytest; continuous attention, reaction time and working memory) at 3 h and 8 h after intake of the medication. Concentrations of the biologically active substances in blood plasma and in body sweat were measured. The subjective drug effect was assessed with the Drug Effect Questionnaire (DEQ) and the Barratt Impulsiveness Scale (BIS-15) questionnaire.

Results: Four out of 48 participants reported tachycardia and/or palpitations as adverse reactions. In this subgroup, mean arterial pressure (MAP) increased from 92 ± 5 mmHg (mean ± SD) at pre-dose to 106 ± 10 mmHg and pulse rate from 70 ± 21 bpm to 75 ± 28 bpm at three hours after dosing, respectively. This was paralleled by an increase in average error rate in the Psytest® from 2.5 ± 2.1 to 2.8 ± 2.1 and a decrease in omission rate from 8.3 ± 6.2 to 5.5 ± 3.9 omissions per test, respectively.

Discussion: Development of standardized methods to characterize psychomotoric effects of medicines may help to establish guidelines for handling of machinery and concomitant use of drugs. An acceptable upper limit of drug concentration that does not affect individual's physical or mental performance may be defined by using appropriate standardized tests during drug development.

Keywords: cognition – psychomotoric performance – reaction time – attention – Psytest

A2.5

Neuronal activation patterns following interoceptive stimulation: role of high trait anxiety

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Background: Emotions are influenced by one's internal state of bodily arousal via interoception. It is reported that interoception is altered in anxiety disorders which are the most prevalent psychiatric disorders with approximately 25% of the population being affected during their lifetime. For example, the subjective and physiological responses to CO_2 inhalation are elevated in subjects with high trait anxiety compared to those with normal anxiety [1]. Yet, although altered interoception is increasingly recognized as an important component of anxiety-related disorders, its underlying neural mechanisms remain insufficiently understood. In the present study, we aimed to elucidate whether differences in trait anxiety levels determine the engagement of the anxiety network in response to CO_2 challenge.

Methods: Mice selectively bred for high (HAB) and normal (NAB) anxiety-related behavior of both sexes were habituated to the test arena and exposed for 10 minutes to either CO_2-enriched (10%) or synthetic atmospheric air on the next day. Locomotor activity and anxiety-related parameters were analyzed during the test period. Using immunohistochemistry, neuronal activation patterns were assessed by mapping the expression of the immediate early genes c-Fos and Zif268 in the cortex, hypothalamus and amygdala of HABs and NABs.

Results: Relative to chamber or air control conditions, CO_2 reduced locomotor activity and increased anxiety-related parameters in the test arena. These behavioral effects were associated with altered expression of c-Fos and/or Zif268 in the central and basolateral amygdala, key brain areas of the anxiety neurocircuitry. HAB mice displayed behavioral hyperresponsivity to the test challenges and increased neuronal activation of hypothalamic nuclei including the paraventricular hypothalamus. Furthermore, we obtained the first evidence that neuronal activation of the insula and hypothalamus, along the rostro-caudal axis, differed between HAB and NAB mice. Sex differences in behavior and neuronal activation patterns were also revealed.

Discussion: Here, we demonstrate that the CO_2-induced anxiogenic effects in mice were associated with altered neuronal activation of the amygdala, particularly of the central amygdala, the major output nucleus that plays a pivotal role in promoting anxiety-related behavioral responses. These data support the translational value of the paradigm in assessing negative valence. The observed greater effect of CO_2 on behavioral responses in the high anxiety HAB mice is in line with human studies showing that anxious individuals are hypersensitive to CO_2. Altered engagement of the hypothalamic stress centers and the insula is suggested to represent neuronal correlates of behavioral hypersensitivity to CO_2 of anxiety-prone subjects. Detailed characterization of affected neurons is ongoing.

Acknowledgements: The study was supported by the Austrian Science Fund FWF (grant FG 18-B).

Keywords: anxiety – hypercapnia – interoception – mouse model

Reference:

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A2.6

The influence of antacids on the permeability of gliclazide – PAMPA model of the gastrointestinal mucosa

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Background: Gliclazide is a sulfonylurea derivative that is used for the treatment of diabetes melitus type 2. Since it causes gastrointestinal side effects such as gastroesophageal reflux, patients often use it along with antacids. In case of a failure of antidiabetic therapy, drug–drug interactions should always be suspected but patients generally do not inform physicians about the concomitant use of antacids. The aim of the study was to examine the effect of antacids on the permeability of gliclazide *in vitro* and to gain a better insight into the mechanisms responsible for the observed effects.

Methods: The permeability of gliclazide alone and in the presence of antacids (sodium bicarbonate, calcium carbonate, aluminium hydroxide, hydrotalcite and calcium carbonate / magnesium carbonate) was investigated using the parallel artificial membrane permeability assay (PAMPA), in a set of four media (three buffers pH 1.2, pH 4.5, pH 6.8 and in water). After a six-hour incubation period, the concentrations of gliclazide were measured by the HPLC method, and permeability coefficients were determined. The pH values of all groups were tested in order to determine how much the antacids changed the pH of the medium.

Results: At pH 1.2, groups with calcium carbonate, hydrotalcite and the combination calcium carbonate / magnesium carbonate showed significantly better permeability of gliclazide than the control group. At pH 4.5, calcium carbonate and the combination calcium carbonate / magnesium carbonate significantly increased the permeability of gliclazide, while sodium bicarbonate and aluminium hydroxide reduced permeability. All groups with antacids at pH 6.8 showed reduced permeability of gliclazide in comparison to the control group.

Discussion: Considering the results, it can be concluded that antacids significantly decrease, but also increase the permeability of gliclazide at different pH values, which may consequently affect the bioavailability of gliclazide. The tested pH values of all groups suggested that the permeability of gliclazide is largely influenced by the degree of its ionization which depends on the change in pH of the environment by antacids. Nevertheless, also other mechanisms may be involved such as complex and salt formation.

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Keywords: drug absorption – drug interactions – gliclazide – antacids – parallel artificial membrane permeability assay (PAMPA)

A2.7

Influence of carob extract on body mass and markers of adipose tissue function in rats

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Background: Carob (*Ceratonia siliqua* L.) has emerged in recent years as a novel potential lipid-lowering plant-based treatment. Carob is a Mediterranean and Middle Eastern evergreen tree or shrub belonging to the Caesalpinioideae subfamily of the Fabaceae (Leguminosae) family. Over time its edible fruit pods became well-known worldwide, while carob has been recognized for its numerous health benefits, particularly its anti-inflammatory, antioxidant, body mass and lipid-lowering effects in animals and humans.

Methods: Adult Wistar albino rats, weighed between 225 and 275 grams, were obtained from the Military Medical Academy (Belgrade, Serbia), randomly selected and confined in the vivarium of the Novi Sad Faculty of Medicine, Department of Pharmacology, Toxicology, and Clinical Pharmacology for the course of the experiment. Standard laboratory conditions included a temperature range of 23 to 25°C, a relative humidity of 55 ± 1.5%, a 12/12-hour dark–light cycle, and unlimited access to pellet (cholesterol-enriched) food and water. The Ethics Committee for the Protection of Laboratory Animal Welfare of the University of Novi Sad provided approval for this study.

Results: Weight gain of all animals fed a cholesterol-rich diet for four weeks was significantly higher than that of control animals fed a standard diet. Weight gain was reduced by treatment with carob extract or simvastatin plus carob extract. Average liver mass of animals given cholesterol-enriched food and treated with saline, carob extract, simvastatin, and a combination of simvastatin and carob extract was significantly higher compared to the control group that was fed a stadard diet. Average liver weight of rats treated with carob extract and the combination of carob extract and simvastatin was substantially reduced related to other groups. The concentration of leptin in the serum of animals fed cholesterol-enriched food and given saline was significantly higher than in the standard-diet group and in the groups of animals given carob extract, simvastatin, or a combination of carob extract and simvastatin. The concentration of adiponectin in animals fed a cholesterol-rich diet was significantly higher than in control animals fed the standard-diet. Adiponectin concentrations in animals treated with carob extract either alone or in combination with simvastatin were lower than concentrations found in animals treated with simvastatin alone.

Discussion: In this research, we showed the potential effect of carob on body weight of animals that received high-calorie food, as well as a reduction of liver mass in animals that were treated with carob. In addition, carob showed a beneficial effect on markers of adipose tissue function.

Acknowledgements: This research was supported by the Provincial Secretariat for Higher Education and Scientific Research of Vojvodina (project no. 142-451-2574/2021-01).

Keywords: lipid-lowering treatment – carob extract – body mass – leptin – adiponectin

A2.8

Drug consumption in the treatment of obstructive lung diseases in the Republic of Serbia in the period from 2011 to 2020

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Background: Obstructive lung diseases include chronic obstructive pulmonary disease (COPD) and asthma. It has been shown that in the treatment of this pathology certain groups of drugs such as methylxanthines can cause numerous side effects. The aim of this study was to analyze the consumption of drugs used in the therapy of obstructive lung diseases in the Republic of Serbia in the period from 2011 to 2020, as well as to examine the relationship between the price of individual preparations and their consumption.
Methods: The consumption of drugs was monitored by the internationally accepted ATC/DDD methodology, as well as by the use of the DU 90% method. The total amount of consumed drugs was expressed as the number of defined daily doses per 1000 inhabitants per day (DDD / 1000 inhabitants / day). The relationship between drug consumption and price was examined by linear regression at the level of statistical significance of $p < 0.05$.
Results: In our research, a statistically significant correlation between the increase in the consumption of certain medicines and the decrease in their price was proven (salbutamol, formoterol, montelukast, fenoterol, ipratropium bromide). However, within subgroup R03, methylxanthines showed high consumption. Their consumption in the Republic of Serbia was 4 to 12 times higher than in Finland, Croatia and Norway.
Discussion: Such a large consumption of aminophylline in Serbia can be explained by the low price of this drug per DDD, which was between 0.15€ and 0.16€. The high consumption of methylxanthines should be replaced by some of the drugs with a better profile of side effects such as β_2 receptor agonists. The current situation is unfavorable and it is necessary to change the attitude and awareness of doctors and patients. However, the fact is that despite the large consumption of methylxanthines, the consumption of fixed combinations of beta agonists and corticosteroids, as well as beta agonists and anticholinergics, is increasing year by year.
Keywords: drug consumption – methylxanthines – asthma – chronic obstructive pulmonary disease

A2.9

Consumption of drugs in the therapy of hypothyroidism in the Republic of Serbia in the period from 2009 to 2020

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Background: Hypothyroidism is a chronic disease characterized by triiodothyronine and thyroxine deficiency. A third of the world's population lives in areas that are deficient in iodine. The number of recorded cases of hypothyroidism is also increasing among the population of iodine-sufficient countries. Achieving a euthyroid state was made possible by oral administration of levothyroxine. The average dose for an adult is 1.6 µg/kg of body weight per day. The aim of this study was analysis of the use of drugs in the treatment of hypothyroidism in the Republic of Serbia in the period from 2009 to 2020 and comparison of the obtained results with the consumption of the same group of drugs in Finland and Croatia.
Methods: Data on drug consumption in the period from 2009 to 2020, as well as drug prices, were obtained from the official website of the Agency for Medicines and Medical Devices of the Republic of Serbia, for Croatia from the official website of the Agency for Medicines and Medical Products, and for Finland from the official website of the Finnish Medicines Agency. The consumption of drugs was monitored by the internationally accepted ATC/DDD methodology. The total amount of consumed drugs was expressed as the number of defined daily doses per 1000 inhabitants per day (DDD / 1000 inhabitants / day).
Results: A trend of increasing consumption of levothyroxine was observed in all countries in the period from 2009 to 2020. In the Republic of Serbia, levothyroxine consumption was four times higher in 2020 compared to 2009. In Croatia a similar trend in the consumption of levothyroxine was observed, while in Finland, the consumption of this drug in the same period is not characterized by a drastic, but a more gradual increase.
Discussion: The use of levothyroxine is widespread in all the countries studied. In Serbia, in 2020, compared to 2009, it almost quadrupled. Croatia records a similar trend, while in Finland a slight increase was observed, with a higher rate of drug use in 2009. A difference in the prices of this medicine was noticed in the Serbia and the other two investigated countries. Also, a clear difference was observed in the costs of applying levothyroxine on an annual basis in Finland compared to Serbia and Croatia.
Keywords: drug consumption – hypothyroidism – levothyroxine

A2.10

The pathogenic, autism-linked *de novo* variant A749G in $Ca_V1.3$ Ca^{2+} channels affects neuronal morphology *in vitro* and *in vivo*

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Background: Due to activation at subthreshold potentials, in brain, voltage-gated L-type $Ca_V1.3$ calcium channels (*CACNA1D*) contribute to pacemaking activity in neurons and regulate synapse maturation and dendritic refinement. Functional studies from our group revealed that *de novo* missense mutations in the $Ca_V1.3$ α1 subunit, such as variants A749G and S652L, identified in patients with neurodevelopmental disorder, induce prominent gain-of-function gating changes [1]. However, whether these pathogenic mutations influence dendritic and synaptic structure remains unclear.

Methods: Here, we investigated the effect of the autism-causing $Ca_V1.3$ variant A749G on neuronal morphology in transfected hippocampal cultures and Golgi-Cox-stained CA1 hippocampal neurons from heterozygous A749G knock-in mice ($Ca_V1.3^{A749G}$).
Results: In cultured neurons the A749G mutation introduced into the either HA-tagged or untagged $Ca_V1.3$ long splice variant induced a significant increase in dendritic spine length, fiber length and spine area. Cumulative frequency distribution of spine shape factor was significantly shifted to lower values compared to wild-type (WT) indicating an elongation of the entire spine population. Consequently, the percentage of thin and filopodia-like spines was increased in neurons transfected with mutant channels. Our anti-HA live-cell staining did not reveal major differences between WT and mutant channels surface expression. This indicates that the mutation-induced spine elongation does not result from altered channel membrane targeting. In Golgi-Cox-stained brains of 3- to 3.5-months-old heterozygous $Ca_V1.3^{A749G}$ mice we observed a significant increase of branching in proximal dendritic regions of CA1 hippocampal neurons compared to WT. Moreover, dendritic spine analysis revealed an increase in the proportion and density of stubby spines and a decrease in the proportion and density of thin spines in basal and apical dendrites. Additionally, neuronal soma size was significantly reduced in CA1 hippocampal neurons from $Ca_V1.3^{A749G}$ mice.
Discussion: In this study, we demonstrated that the pathogenic, autism-linked mutation A749G in $Ca_V1.3$ affects neuronal morphology both *in vitro* and *in vivo*. Differences in mutation-induced effects on dendritic spines observed between hippocampal cultures and mouse brains can be explained by: (i) differences in $Ca_V1.3$ expression levels leading to distinct contributions of $Ca_V1.3$ to intraspine Ca^{2+} levels and therefore to spine stability; and (ii) differences in the levels of neuronal development represented by two systems. Additionally, a more stable neuronal network is developed in intact mouse brain compared to embryonic neurons differentiated *in vitro*. Our data strongly suggest that $Ca_V1.3$ is required for normal neuronal morphology in hippocampal neurons. The changes induced by the pathogenic A749G $Ca_V1.3$ variant may contribute to the neurodevelopmental pathology in affected patients.
Acknowledgements: This study was supported by the Austrian Science Fund FWF (grants P35722, P35087, DOC-30/CavX) and the University of Innsbruck.
Keywords: voltage-gated calcium channels – $Ca_V1.3$ channels – gain-of-function mutations – dendritic spine morphology – dendritic arborization – neurodevelopmental disorders
Reference:
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A2.11

Protection of intellectual property of drugs – Impact on the dispensing of antidiabetics in Serbia

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Background: Diabetes mellitus type 2 (T2DM) is one of the most common non-communicable diseases and metabolic disorders characterized by chronic hyperglycemia. Drug patenting is the process of obtaining exclusive rights to manufacture and sell a new drug on the market. After the patent expires, other pharmaceutical companies can begin manufacturing that drug. The aim of this study was to investigate whether the intellectual property protection of original drugs has an impact on the consumption of antidiabetic drugs in Serbia, and whether the consumption trend changed after the patent expiration and the emergence of a greater number of parallel antidiabetic drugs.
Methods: The data for our study were taken from publications of the Agency for Medicines and Medical Devices of Serbia (ALIMS) for a period of 15 years (2006–2020). Data on the number of registered antidiabetic drugs and their generics were taken from the "Drugs in circulation" handbook on drugs and their use, which is published every year.
Results: Looking at the total consumption of all antidiabetic drugs for each year of the observed period, we can see that the lowest number of drugs from this group was consumed in 2010 (5,930,496 packages), while the highest consumption was recorded in 2019 when a total of 11,349,247 packages for the treatment of T2DM were consumed. In the observed fifteen-year period, metformin was the most-used antidiabetic drug. The total annual expenditure on antidiabetic drugs shows a rising trend, so that in 2020, over 2 billion Serbian dinar (more than 17 million €) more was spent compared to 2006.
Discussion: After the patent expiration and the emergence of a greater number of generic drugs, the consumption of some antidiabetic drugs has changed in Serbia. A greater number of generics to an antidiabetic drug increases its availability and consumption.
Acknowledgements: This work was supported by the Provincial Secretariat for Higher Education and Scientific Research of Vojvodina (project no. 142-451-2574/2021-01/01).
Keywords: drug utilization – antidiabetic drugs – generic drugs

A2.12

Microglia profiling in a model of high trait anxiety

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Background: Neuroinflammation with altered microglial function is proposed to contribute to the pathology and outcome of anxiety disorders in specific patient subgroups. We have previously shown that a mouse model of high trait anxiety (HAB) displays increased microglial density in key regions of anxiety circuits, including the hippocampus, compared to normal-anxiety (NAB) controls. Microglia, however, can transit from a neuroprotective to a pro-inflammatory state. These functions of microglia are tightly linked with their intricate shape. In order to get insight into the functional states of increased microglia densities in anxiety-prone subjects, we investigated morphological and genetic characteristics of microglia in HAB *vs.* NAB mice.

Methods: Anxiety-related behaviours of HAB and NAB mice were assessed in the light/dark test. Microglia were visualized by using immunohistochemistry for ionized calcium-binding adapter molecule 1 (Iba1). Microglial morphology was assessed using the MICMAC automated analysis aided with MATLAB programing. Gene profiling was performed by single-cell RNA sequencing of FACS-sorted whole brain microglia.
Results: Using the automatized MICMAC analysis we now replicated the observed increased microglial density in the hippocampus, previously assessed using manual counting. Additionally, we observed that the prevalence of amoeboid, 'activated' microglial morphology was significantly higher in HAB as compared with AB. The expression of phagocytic cell surface markers including CX3CR1, CR3, and TREM2 was also altered in HAB as compared to NAB mice of both sexes. Interestingly, sex differences in pathways of microglial expression, microglial morphology as well as synaptic pruning were revealed in the brain of HABs as compared to NABs. Finally, we demonstrate that reducing microglia activation either systemically or locally in the hippocampus by administration of the anti-inflammatory drug minocycline attenuated the enhanced anxiety in HABs.
Discussion: The increased abundance of amoeboid microglia together with the altered expression of the cell surface markers CX3CR1, CR3, and TREM2 in HABs may be indicative of enhanced phagocytosis, activation and/or altered tissue integrity in the hippocampus of HABs. These changes in the hippocampal microenvironment are suggested to contribute to the neuronal dysfunctions and altered neurogenesis previously reported in HABs and putatively supporting their anxious phenotype. Furthermore, we provide proof of concept that reducing microglia activation in the hippocampus is paralleled by attenuation of the high anxiety in HABs. Thus, the activated microglia system could represent an interesting pharmacological target for the therapy of hyperanxious individuals with an altered neuroinflammatory system.
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Keywords: anxiety – microglia – phagocytosis – neuroinflammation

A2.13

Novel oxymorphone analogues, as bifunctional μ/δ opioid receptor agonists, produce antinociception in mice without the risks of antinociceptive tolerance and physical dependence

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Background: Opioids are highly effective painkillers for the treatment of moderate-to-severe pain. Chronic use of opioids is associated with analgesic tolerance, physical dependence and addictive potential. Opioids mediate their pharmacological effects via activation of opioid receptors, μ (MOR), δ (DOR) and κ (KOR). The MOR is the primary target for the therapeutic analgesic effect, but also for severe side effects. Currently, alternative chemical and pharmacological strategies are explored to mitigate the deleterious effects of MOR agonists and to limit abuse and misuse, amongst which are multifunctional ligands. Bifunctional opioid ligands gained a particular interest over the recent years. In this study, we present the *in vitro* and *in vivo* pharmacology of two novel oxymorphone analogues that emerge as bifunctional MOR/DOR agonists.
Methods: Radioligand binding assays were performed with rat (MOR and DOR) and guinea-pig brain (KOR) membranes. [^{35}S]GTPγS binding assays were performed with membranes from Chinese hamster ovary (CHO) cells stably expressing the human MOR, DOR or KOR. Antinociception after s.c. administration was assessed in pain models of acute nociception (tail-flick test) and inflammatory pain (formalin test) in mice. Antinociceptive tolerance was measured in mice treated daily over a 5-day period, with tail-flick latencies measured on days 1 and 5. Physical dependence was determined using naloxone-precipitated withdrawal.
Results: Radioligand binding studies showed the new oxymorphone derivatives to display very high affinities (picomolar to subnanomolar range) to the rodent MOR, DOR and KOR. In the [^{35}S]GTPγS functional assays, they were very potent and full agonists to the human MOR and DOR, and partial agonists to the human KOR. *In vivo*, both oxymorphone analogues displayed dose-dependent antinociceptive efficacy in experimental models of acute nociception and inflammatory pain after s.c. administration to mice. Their antinociceptive effect was reversed by selective antagonists of MOR (β-funaltrexamine) and DOR (naltrindole), but not by a KOR antagonist (nor-binaltorphimine), demonstrating the involvement of MOR and DOR to the antinociceptive action. Chronic s.c. drug treatment of mice did not cause antinociceptive tolerance and withdrawal syndrome.
Discussion: We show that targeted structural modifications on the oxymorphone scaffold resulted in significant alterations in opioid activity by influencing the pharmacological properties of the ligands. Oxymorphone, a potent and selective MOR agonist, was converted into bifunctional MOR/DOR agonist ligands. The dual activation of the MOR and DOR by the novel oxymorphone analogues produces effective antinociception without the CNS-mediated risks of antinociceptive tolerance and physical dependence. These findings pave the way to new pain therapeutics with limited side effects on both acute and chronic use.
Acknowledgements: This study was supported by the Austrian Science Fund FWF (grants no. I4697 and TRP19-B18) and the University of Innsbruck.
Keywords: opioid receptors – bifunctional opioid ligands – pain – analgesia – oxymorphone

A2.14

The role of a basement membrane fragment on neutrophil function

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Background: During acute inflammation in the lung, neutrophil granulocytes form the first line of immune defense. Despite their beneficial role, uncontrolled and excessive recruitment and consequently activation may further contribute to tissue damage. Proteolytic degradation of the basement membrane, a specialized compartment of the extracellular matrix, is a common feature in various acute and chronic lung diseases and can lead to the generation of novel, bioactive fragments. These so-called matrikines have functions distinct to their parent molecule and their function on neutrophils is until now mostly unknown.

Methods: We hypothesize that pentastatin (PS)-1, a matrikine derived from type IV collagen α5, modulates neutrophil function by acting as a damage-associated molecular pattern (DAMP), thus contributing to continuous inflammatory response within the lung.
Results: To investigate the effect of PS-1 on neutrophils, functional assays *in vitro* were carried out via flow cytometry such as determination of shape change, chemotaxis and apoptosis assays. To look further into involved pathways, western blot analysis was performed.
Discussion: PS-1-concentrations ranging from 3–50 µg/ml induced neutrophil shape change and migration, indicating activation of the cells upon treatment. At lower concentrations (3–12 µg/ml) of PS-1 an increased pro-survival effect was observed, whereas the highest concentration exerted a toxic effect upon neutrophils. By inhibiting integrin αLβ2, αMβ2, αXβ2 receptors, migration was decreased, suggesting them as cognate receptors. Treatment with PS-1 induced ERK1/2 phosphorylation as determined by western blot analysis. Using a MEK1/2 inhibitor (U0126, 20 µM), reduced migration of neutrophils towards lower concentrations of PS-1 was observed, indicating an involvement of the ERK/MAPK pathway. We could demonstrate that PS-1 leads to neutrophil activation. Future experiments will identify further downstream signaling partners and upstream receptors.
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Keywords: neutrophil granulocytes – basement membrane – matrikines – extracellular matrix

A2.15

Blood–brain barrier – Peptide shuttles for drug delivery into the CNS

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Background: Blood–brain barrier (BBB) permeability is needed for new therapeutics with targets in the central nervous system. Nevertheless, most molecules (proteins in general and over 98% of small molecules) fail to meet this requirement due to the tightly regulated transport processes into and out of the brain [1]. To overcome this limitation, the 'Trojan Horse' approach exploits endogenous receptor-mediated transcytosis, which can shuttle bioactive cargos across the BBB. A recent approach in the field are peptide BBB shuttles but intrinsic proteolytic instability of peptides, resulting in short half-life *in vivo*, remains an important challenge in the development of peptide therapeutics. Especially natural peptides promise solutions for this problem [2], as they can be used as scaffolds for molecular grafting to increase the stability of peptide therapeutics [3].
Methods: A series of peptide probes was designed using the molecular grafting method. As starting point, established peptides BBB shuttle motifs were selected and the sequences were incorporated into the proteolytically stabilized cyclic sunflower trypsin inhibitor 1 (SFTI-1) scaffold. We chose three different linear sequences (COG1410, peptide 22 and MiniAp-4) that are transported via different mechanisms, and designed novel hybrid molecules for bioactivity evaluation. To screen the probes, an endothelial cell monolayer transport assay (human cerebral endothelial cell line hCMEC/D3) was established using liquid chromatography–mass spectrography (LC–MS) for quantification of transport.
Results: The cell monolayer of hCMEC/D3 cells was validated by showing increasing tight junction protein expression until day 6 after seeding cells into the insert and stable expression until day 9. Lucifer-yellow permeability measurements showed that apparent permeability (Papp) values were ~ 2×10^{-6} from day 5 on, which is considered as low permeability in the literature. As control substances, atenolol (negative control), quinidine (positive control) and a reference peptide shuttle were tested in the assay and quantified using LC–MS. Papp values of 1×10^{-6} for atenolol, 8×10^{-6} for quinidine and 1.7×10^{-6} for peptide 22 were obtained.
Discussion: Integrity of the cell monolayer was validated and functionality of the assay set-up was confirmed by testing the reference substances atenolol and quinidine. We were not able to confirm the transport of peptide 22, which was published to be transported across the BBB. Next, we will screen the probes for transport across the BBB in this model and measure serum stability to demonstrate that a nature-derived peptide scaffold increases the stability of an incorporated linear peptide, while still mediating transcytosis. This work provides proof of concept for the design of novel stabilized peptide BBB shuttles.
Acknowledgements: The study was supported by the Austrian Science Fund FWF (grant ZK-81B).
Keywords: blood–brain barrier – drug transport – peptides
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A2.16

Anti-obesity effects of bile acids

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Background: By activating nuclear and membrane receptors like farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), bile acids (BAs) act as steroid signalling molecules that orchestrate postprandial metabolism. Changes in the composition and the size of the BA pool are associated with altered BA signalling and increased susceptibility to obesity.
Methods: Detailed and comprehensive search of articles indexed in PubMed from 1999 to 2023, using key words: bile acid, FXR, TGR5, obesity, microbiota.
Results: Primary BAs produced by hepatocytes are metabolized into secondary BAs by intestinal microbiota. A dysregulated microbiota–

bile acid axis is associated with metabolic alterations. Anti-obesity effects of TGR5 activation by BAs are cell-type-specific and involve improvement of mitochondrial function, increase in thermogenesis, decrease in inflammation and improved glucose and lipid homeostasis. Secondary BAs stimulate the production of glucagon-like peptide-1 (GLP-1) via TGR5 expressed in entero-endocrine L-cells. Circulating GLP-1 activates the GLP-1 receptor in the vagal afferent neurons in the intestine to regulate feeding behaviour and energy and glucose metabolism. Intestinal FXR activation promotes the production of fibroblast growth factor 19 (FGF19) which signals to the brain to regulate metabolic homeostasis. Serum bile acids, GLP-1 and FGF19 levels are increased following bariatric surgery, and this quickly improves insulin sensitivity and glycemic control in obese patients. Since both BAs and their receptors are found in the brain, they can be considered centrally-acting neurosteroids controlling satiety. By activating the sympathetic nervous system, BAs promote negative energy balance.

Discussion: The strategy of the modulation of BA signalling, using BAs and BA receptor-modulating agents, is a potent therapeutic approach for the treatment of obesity and other components of the metabolic syndrome.

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Keywords: farnesoid X receptor (FXR) – fibroblast growth factor 19 (FGF19) – Takeda G protein-coupled receptor 5 (TGR5) – Glukagon-like peptide 1 (GLP-1)

A2.17

Bile acids alter clindamycin permeation through the skin: *in vitro* permeability study

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Background: Acne vulgaris affects approximately 9.4 % of the global population, regardless of age, gender, race and skin type. It has been estimated that 11 million prescriptions per year are aimed at treating this dermatosis. Clindamycin-based topical preparations are well tolerated and widely applied in the treatment of mild-to-moderate acne. However, poor bioavailability, restricted drug penetration depth and rising antimicrobial resistance considerably limit their therapeutic efficacy. Penetration enhancement represents a promising and rational strategy to overcome the drawbacks of conventional topical formulations.

Methods: We carried out the skin parallel artificial membrane permeability assay (skin-PAMPA) to examine the skin permeability of clindamycin hydrochloride, alone and in combination with cholic acid (CA) or deoxycholic acid (DCA). Bile acids were used in submicellar concentrations. The measurements were conducted at two relevant pH values (5.5 and 6.5). After the incubation period, clindamycin hydrochloride concentrations in both compartments were determined spectrophotometrically and apparent permeability coefficients (P_{app}) were calculated.

Results: Bile acids altered the skin-PAMPA membrane permeability of clindamycin hydrochloride in a concentration-dependent manner. Both CA and DCA at the highest studied concentration of 100 µM increased the permeability of clindamycin hydrochloride *in vitro*. This effect was more pronounced in the case of CA and at a higher studied pH value of 6.5, which is characteristic of most dermatological indications treated with topical clindamycin preparations.

Discussion: The results of our study suggest that CA is a more promising penetration enhancer for clindamycin hydrochloride in a solution formulation, compared to DCA. Notably, the increase in the drug permeability was more prominent at pH 6.5, typical for inflammatory skin diseases, including acne. This could be explained by clindamycin / cholic acid complex's higher stability than the clindamycin / deoxycholic acid complex, as revealed by molecular mechanics calculations.

Acknowledgements: This research was supported by the Provincial Secretariat for Higher Education and Science, Autonomous Province of Vojvodina (project no. 142-451-3176/2022-02).

Keywords: clindamycin – bile acids – skin – transdermal application – drug permeability

A2.18

Characterising the 'hold-and-pull' mechanism of the creatine transporter (CRT1) occlusion through an *in silico* approach

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Background: In the human body, energy buffering during periods of high ATP consumption is in part achieved through the creatine–phosphocreatine system, which requires creatine to be uptaken from the blood by the membrane-embedded creatine transporter (CRT1). Malfunction of CRT1 is associated with creatine transporter deficiency, for which there is only limited treatment. However, thus far no empirically-solved structure of CRT1 has emerged, impairing our understanding of the structure–function relationship of this protein.

Methods: Here we use a homology modelling approach to generate a theoretical structure of CRT1, and confirm our predictions through experimental validation.

Results: We show that a key residue at the substrate binding site, C144, is not present in a charged state, contrary to previous proposals. We then dock creatine into the substrate binding site, and show that the mechanism of CRT1 occlusion follows the 'hold-and-pull' mechanism of the serotonin transporter, with a key interaction chain of Y148–creatine–Na^+ essential to the process of occlusion.

Discussion: To the best of our knowledge, our study represents the first set of molecular dynamics simulations aimed at exploring the structure–function relationship of CRT1. Given the clinical importance of CRT1, our insights could provide an important launching pad for further studies aimed at developing CRT1 ligands.

Acknowledgements: This work was supported by the Austrian Science Fund FWF (grant P33263).

Keywords: molecular dynamics simulations – creatine transporter – CRT1 – protonation

A2.19

Artificial intelligence in automated image analysis for drug screening

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Background: With technologies' steady advance, high-content screening based on completely automated microscopes generating an endless stream of data has become the method of choice to screen compound efficacies, not only in companies but universities as well. Hand in hand with these capabilities, research questions have evolved accordingly, reaching from multiplexed 200+ colour images, 3D scans of whole mice to chasing waves of Ca^{2+} ions across organelle membranes. The answer, more often than not, is thought to be found in artificial intelligence (AI)-based image analysis, a trend supported by imaging companies and independent analysis service providers alike. However, many researchers are yet unaware of what lies behind these 'educated' programs and what they really could allow us to do.

Methods: Common image analysis questions have been evaluated with or without AI tools to provide a critical comparison of adaptive, algorithm-based and AI-based image analysis results. To this extent various image acquisition techniques have been employed to generate the raw data, such as fluorescent microscopy, histological slide scanners and light tomography. The automated analysis programs were generated in the two open-source environments FIJI and Python.

Results: Our setting as a research unit of the Medical University of Graz grants us access to samples generated as part of the clinical routine and samples arising from basic scientific research. For samples arising from clinical routine work, AI-based analysis offered specificity and adaptability to context-specific interpretation, which we were not able to attain from conventional mathematical models. However, the limited sample size of most fundamental research projects, as well as changing parameters / setup configuration and the lack of a possible ground truth to generate a training set in the first place led to unstable performance or made the approach inapplicable in the first place. A more common use for AI in smaller-size experiments was found in data curation like image segmentation, de-noising and other image preparation steps profiting from a universally applicable base and specific training to each individual imaging system.

Discussion: Whilst AI has become more readily implemented in everyday imaging applications, especially de-noising, analysis programs solely relaying on it are few and far between. Reasons can be found in intrinsic heterogeneity of generated data, which necessitates extensive training and/or frequently changing experimental parameters. However, if training sets are non-limiting and the sampling procedure is standardized, these programs tend to surpass even trained personell, highlighting their potency in the clinical setting of digital pathology. Nevertheless, the cumbersomeness of manual feature extraction and lacking documentation about happenings in the backline are problems which need to be improved upon in the future.

Acknowledgements: The authors would like to thank the Core Facility Imaging from the Centre for Medical Research, Graz, for their support.

Keywords: artificial intelligence – high-content screening – image analysis

A2.20

The role of glutamate metabolism in neuronal excitotoxicity

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Background: Glutamate excitotoxicity is a cell death mechanism triggered by accumulation of glutamate in the extracellular space. It is involved in a variety of brain pathologies including traumatic brain injury (TBI) and epilepsy. Hence, strategies leading to neuroprotection from the toxic effects of excess glutamate are needed. The TCA-cycle enzyme 2-oxoglutarate dehydrogenase complex (OGDHC) acts as a branching point enabling both glutamate synthesis and its consumption, and may thus provide a promising target for neuroprotection. OGDHC activity can be modulated experimentally by succinyl phosphonate (SP; inhibitor) and thiamine (TH; co-factor promoting OGDHC activity). Hence, we used these compounds to address the potential role of OGDHC activity in glutamate excitotoxicity.

Methods: We performed propidium iodide-based viability assays on primary hippocampal neurons (co-cultured with glial cells) exposed to excitotoxic stimulation (exposure to Mg^{2+}-free solution). Before this, neurons were pre-incubated for 36–48 hours in presence of either 1 mM TH or 200 µM SP. The percentage of dead neurons was determined after 6 hours in Mg^{2+}-free solution. Furthermore, to gain insight into possible mechanisms of action, the glutamate content of synaptic vesicles was determined via sucrose-shock release using whole-cell patch-clamp electrophysiology. Perforated patch-clamp recordings were performed to test the neuronal response to glutamate as well as to probe NMDA receptor-mediated and AMPA receptor-mediated membrane currents. Additionally, viability was also assayed in SP- and TH-pretreated neurons after exposure to exogenously applied glutamate (30 µM, 1.5 hours).

Results: Viability assays performed after induction of excitotoxicity in Mg^{2+}-free solution indicated that promotion of OGDHC activity by 1 mM TH had a neuroprotective effect, while its inhibition with 200 µM SP enhanced cell death. However, glutamatergic current induced in neurons by sucrose shock was increased by TH and decreased by SP. Electrophysiological experiments demonstrated altered voltage responses to exogenously applied glutamate, which were augmented in SP-treated neurons and reduced in TH-treated neurons. In line with this observation, the neuroprotective effect of TH and the neurotoxic effect of SP could also be demonstrated when application of glutamate was used as the excitotoxic stimulus. Measurement of glutamate receptor currents using increasing concentrations of AMPA and NMDA provided evidence of changes of NDMA receptor-mediated signaling (*i.e.* potentiation by SP and reduction by TH), whereas AMPA receptor mediated signaling remained unaltered.

Discussion: Our data demonstrate that interfering with OGDHC activity alters glutamate-dependent excitotoxicity. The decrease by TH or increase by SP of excitotoxicity was not matched by corresponding changes of the vesicular content of glutamate. Hence, these effects could not be explained by synaptic release. Instead, we identified changes in NMDA-receptor signaling that we currently

interpret as secondary (possibly mGlu-receptor-dependent) effects arising in the course of altered metabolism of glutamate.

Acknowledgements: This work was funded by the Austrian Science Fund FWF (grants P33799 and P36145).

Keywords: 2-oxoglutarate dehydrogenase complex (OGDHC) – glutamate – excitotoxicity

A2.21

Uncovering new NK-cell checkpoints in the context of triple-negative breast cancer

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Background: Breast cancer is the most common cancer diagnosed in women worldwide. Screening programs for early detection of primary tumours are widely implemented and have improved the outcome of breast-cancer patients. However, metastasis to distant organs is the main cause of death of these patients, which underlines the need for novel therapeutic approaches. Natural killer (NK) cells are able to kill metastasizing cells and limit distant metastasis. However, their function is often suppressed by the cancer environment, for example through the activation of immune checkpoints. These immune checkpoints are key negative regulators of cytotoxic lymphocytes and are important targets for restoring their activity. Furthermore, immune checkpoint inhibitors started a new era of immune therapy. Although inhibition of the checkpoint pathway programmed cell death protein 1 (PD-1) / programmed cell death protein ligand 1 (PD-L1) was a breakthrough in many cancer types, its success in breast cancer remains limited. The goal of this project is to identify changes in the NK-cell receptor repertoire in the course of metastatic triple-negative breast cancer (TNBC).

Methods: We used a previously established mouse model for TNBC metastasis to obtain blood from metastasis-bearing mice at the timepoint of euthanization. Blood NK cells were subjected to single-cell RNA sequencing using the 10x Genomics technique, and to mass spectrometry to identify subpopulations with exhausted signatures. As breast cancer is known to modify NK cells, we expect to uncover new checkpoints, which, when inhibited, unleash NK-cell responses against metastasizing cells. Identified targets were validated *in vitro* and *in vivo* and blocking antibodies of these targets were used in the course of the metastasis mouse model, which should limit metastasis formation.

Results: An *in vitro* system for the co-cultivation of NK cells with TNBC cells was established to mimic the changes in NK-cell surface receptors in the course of TNBC. After co-cultivation of a human NK cell line and primary murine NK cells with the respective TNBC cell line, both human and murine NK cells showed decreased cytotoxicity. Co-cultivation of primary human NK cells with a TNBC cell line resulted in an altered surface receptor expression, which was also shown in TNBC patients.

Discussion: These results show that our co-cultivation system can be used as an *in vitro* tool to validate changes in NK-cell surface receptor expression.

Keywords: NK cells – triple-negative breast cancer – immune checkpoints

A2.22

TRPM7 ion channel and kinase drives AKT signaling and immune-cell activation

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Background: Ion channels of the TRP family are crucial for cellular homeostasis. TRPM7 is a unique member as ion-channel-coupled protein kinase. Besides its fundamental ability to transduce divalent cations such as Mg^{2+} and Ca^{2+}, it drives cellular signaling by a constitutively active intracellular kinase domain. In previous work we have demonstrated a role of TRPM7 kinase in T-cell signaling, promoting Th17 differentiation and gut immunity. In a murine model, TRPM7 kinase facilitated induction of acute graft-versus-host disease. SMAD2 thereby serves as TGF-β-dependent cellular substrate of TRPM7 kinase, driving proinflammatory signals and Th17 differentiation.

Methods: We here investigated TRPM7-dependent activation pathways of human immune cells and related cell lines, by a combinatorial approach of flow cytometry, western blot, imaging and *in vitro* assays.

Results: We have identified the AKT signaling hub downstream of TRPM7 kinase, facilitating activation of neutrophils and T cells. In human and murine neutrophils, we pinpointed this to AKT/mTOR-mediated induction of oxidative burst and directed cell migration [1]. In T cells, TRPM7-dependent Mg^{2+} conductance is crucial for cellular survival and proliferation, and the protein facilitates T-cell-receptor-mediated Ca^{2+} flux. We could show that TRPM7 is required for T-cell activation, involving induction of AKT-dependent signaling pathways. *In vitro*, we identified a direct interaction of TRPM7 kinase phosphorylating AKT protein, confirming previous hypotheses.

Discussion: Our data suggest TRPM7 kinase as potential target in inflammatory and/or malignant diseases, due to its interconnection with AKT signaling and pro-inflammatory cellular responses.

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Keywords: ion channels – immunity – AKT signalling – TRPM7 kinase

Reference:

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A2.23

Aqueous extracts from tobacco cigarette smoke reversibly inhibit isolated soluble guanylyl cyclase *in vitro*

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Background: Nitric oxide (NO) and its target soluble guanylyl cyclase (sGC) play a central role in vessel homeostasis by initiating vasodilation via the NO/cGMP signaling pathway. In that regard sGC has become an important target in the therapy of cardiovascular diseases. Thus, several compounds have been developed to pharmacologically modulate the enzyme. In addition, inhibitors of this enzyme are often necessary for research purposes. However, the available inhibitors have certain issues. The most prominent one, ODQ (1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one), generally lacks specificity, as it oxidizes the central iron atom in the heme moiety of sGC, but also of other heme-containing proteins. Others, like carboxy-PTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), inhibit sGC by scavenging of NO. However, this has the disadvantage that NO is also not available for other processes in the vascular system. During studies of the effects of aqueous tobacco cigarette smoke extracts (CSE) on isolated sGC, we found a concentration-dependent decrease in enzyme activity. Thus, we further investigated the characteristics of this inhibitory effect.
Methods: CSE were obtained by passing tobacco cigarette smoke through buffer using a glass impinger and a volume-controlled rodent ventilator. The activity of purified bovine lung sGC was measurement as conversion of [α-^{32}P]GTP to [^{32}P]cGMP under stimulation with an NO donor (DEA/NO) in the absence or presence of the extracts. Electrochemical determination of NO was performed using a Clark-type electrode.
Results: The presence of CSE greatly deceased NO-stimulated sGC activity. The maximal enzyme activity was lowered to roughly 13%. This effect was concentration-dependent and surprisingly fully reversible by simple dilution. In addition, the inhibitory effect on maximal enzyme activity was not attributable to scavenging of NO.
Discussion: Due to its key role in the regulation of vascular tone, several pharmacological compounds that interact with sGC have emerged. Activators, but also inhibitors of this enzyme are of great value, the latter especially for research purposes. The findings of the current study demonstrate a concentration-dependent inhibitory effect of CSE on isolated sGC. Unlike other sGC inhibitors, the observed inhibition of CSE was fully reversible. Therefore, it seems unlikely that oxidation of the heme moiety causes enzyme inhibition as is the case with ODQ. For the same reason, other permanent chemical modifications (*e.g.* nitrosation of cysteine residues) can be ruled out. Another potential inhibitory pathway is scavenging of NO. However, measurements of NO in the presence of CSE revealed, that detectable scavenging only occurs at low NO concentrations (<1 µM). Hence, it can hardly explain the inhibitory effect of CSE on fully stimulated sGC (>1 µM NO). It is thus assumed that CSE contains a compound or compound mixture that directly interacts with sGC in an inhibitory manner. Work is under way to further investigate this issue, which could pave the way for the development of a new sGC inhibitor.
Acknowledgements: This work was supported by the Austrian Science Fund FWF (grant no. P32922).

Keywords: tobacco cigarette smoke – nitric oxide synthase – soluble guanylate cyclase – guanylate cyclase inhibitors

A2.24

The role of JAK-STAT signaling in neutrophilic airway inflammation

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Background: Neutrophilic inflammation is a common feature of chronic inflammatory respiratory diseases and is associated with corticosteroid resistance. The IL-23–T_h17 axis is a key contributor to airway neutrophilia. IL-23 from antigen-presenting cells (APCs) binds to its receptors (on naïve T cells), associated with TYK2 and JAK2 leading to T_h17 polarization. IL-17, a T_h17 cytokine, binds to its receptors on structural and immune cells, which results in neutrophil activation. Despite clinical reports suggesting the changes in the IL-23 level in the serum of patients with neutrophilic inflammation, the direct effects of the cytokines are unclear. We aim to study the potential role of JAKs and cytokines involved in the IL-23–T_h17 axis.
Methods: For *in vitro* studies, functional assays like migration, reactive oxygen species (ROS), CD11b were performed on neutrophils. Neutrophils were pretreated with IL-17, IL-23 and vehicle (plus buffer) and stimulated with C5A to study ROS production and CD11b expression. Migration assays were performed with IL-17A and IL-23 as stimulants as well as pretreatments. Whole blood stainings were performed to study TYK2 and JAK2 expression in the various immune cell populations in the whole blood of healthy non-allergic and allergic donors using flow cytometry. For *in vivo* studies, mice were intranasally injected with IL-23 for 3 days and the infiltration of the immune cell population in bronchoalveolar lavage (BAL) and blood was measured using flow cytometry.
Results: IL-17 and IL-23 pretreatments increased ROS. IL-17A increased the migration of neutrophils and enhanced IL-8-stimulated chemotaxis. IL-17A pretreatment significantly increased CD11b in neutrophils. Non-allergic donors showed significantly higher expression of TYK2 and JAK2 compared to allergic donors. In the *in vivo* studies, we observed increased neutrophil count in the BAL fluid with the intranasal treatment of IL-23.
Discussion: Our results indicate that IL-17 and IL-23 might be potential players in neutrophilic lung inflammation, affecting neutrophil migration and ROS production. TYK2 and JAK2 are differentially expressed in immune cell populations from allergic donors compared to healthy controls. From our preliminary data, we conclude that IL-17 and IL-23 directly affect neutrophil functions. In further experiments we plan to evaluate the neutrophil function and JAK-STAT expressions in samples from patients with COPD and non-allergic asthma.
Keywords: neutrophilic inflammation – corticoid-steroid resistance – JAK-STAT signaling

A2.25

In silico analysis of molecular descriptors for quercetin analogues: a way to improve blood–brain barrier permeation

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Background: Numerous studies suggest the neuroprotective effects of quercetin. A recent structure/activity analysis confirmed that quercetin can significantly affect the activity of inositol phosphate multikinase (IPMK), which produces phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which is very important in patients with Huntington's disease. Despite its beneficial effects, the therapeutic use of quercetin is limited due to its poor aqueous solubility, low oral bioavailability and low permeation through the brain–blood barrier (BBB). The aim of this study was to identify quercetin analogues with improved BBB permeation and possessing high binding affinities towards IPMK.

Methods: The 3D crystal structure of human IPMK in a complex with quercetin was retrieved from the Protein Data Bank, and the ZINC database was used for screening of ligand structures using the structural similarity search method, which resulted in the identification of 34 quercetin analogues. Docking studies were performed using the Molegro Virtual Docker (MVD) software. Molecular descriptors relevant to membrane permeability for quercetin analogues were predicted using the VolSurf+ software. Permeation through BBB was also predicted using the SwissADME web tool, while the interactions of quercetin and its analogues with P-glycoprotein (P-gp) were predicted using the PgpRules Server. The values of size/shape and physicochemical molecular descriptors for each compound were calculated using the VolSurf+ software and principal component analysis (PCA) was performed.

Results: Binding energies of all tested compounds at the IPMK active site, *i.e.* the potential energies of the formed ligand–receptor complexes, were in the range from −91.827 kcal/mol for geraldol to −72.415 kcal/mol for 3,5-dihydroxy-2-(4-phenyl)chromen-4-one. Quercetin had a binding energy of −82.233 kcal/mol at the IPMK active site, and 19 compounds exerted higher affinity towards the IPMK active site. The calculated values of the logarithm of the blood–brain barrier distribution (LgBB) were lower than −0.5 for all quercetin analogues (range: −3.311 to −1.263), indicating poor brain permeation. Similarly to logP values, 27 analogues had higher values of LgBB than quercetin, with compound 33 (quercetin 3,4′-dimethyl ether) having the highest LgBB value. The 'BOILED-Egg' method also showed that none of the analyzed compounds can pass through the BBB, but the majority of them can be passively absorbed in the intestines. Besides, only 2 compounds, quercetin 3,4′-dimethyl ether and 3-*O*-methylquercetin, were shown to be substrates for P-gp. The results of PCA suggested that intrinsic solubility and logP have the dominant influence on the ability of quercetin analogues to permeate through the BBB. The exceptions from the rule were compounds 27 and 33, which are special, since they are the only two quercetin analogues that are substrates for P-gp.

Discussion: Using several *in silico* methods, we showed that none of 34 analyzed quercetin analogues have sufficient BBB permeability. However, the application of the PCA statistical method in this study proved to be significantly beneficial in the analysis of data regarding the relationship between molecular descriptors and their properties, enabling the synthesis of new compounds with desired properties reflected in favourable values of molecular descriptors.

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Keywords: quercetin analogues – blood–brain barrier – molecular docking – *in silico* analysis

A2.26

Synthetic HDL nanodiscs as a drug candidate in pulmonary inflammation

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Background: High-density lipoproteins (HDLs) are anti-inflammatory lipoprotein particles whose function and composition are critically altered in disease. Therefore, it is reasonable to replace or even increase the loss of HDL function in inflammation. Apolipoprotein A-I (ApoA-I) is the most abundant protein of HDL and is primarily responsible for its well-documented immunomodulatory effects. Artificially synthesized 18–37-amino-acid-long peptides that mimic the activity of full-length ApoA-I are being evaluated in clinical trials for atherosclerosis (*e.g.* NCT04216342). Due to the ease of production and highly specific nature, peptidomimetics are naturally preferred as drug candidates. We aim to re-purpose novel HDL-mimetic nanoparticles, targeting inflammation in the lung.

Methods: We prepared differentially lipidated ApoA-I-mimetic nanoparticles using the NanoAssemblr™ platform and confirmed the size and morphology of the nanodiscs using transmission electron microscopy and native gel electrophoresis. Cholesterol efflux capacity of nanoparticles was measured using macrophages labelled with [^{3}H]cholesterol. Anti-inflammatory effects on human granulocytes were evaluated with chemotaxis assay and flow cytometry. *In vivo* experiments were performed with IL-5 transgenic and C57BL/6 mice.

Results: Upon testing the functionality, we found that the nanodiscs potently mobilised cholesterol *in vitro* and *in vivo*. Moreover, the nanodiscs significantly suppressed monocyte activation and neutrophil integrin activation, and down-modulated the human eosinophil migration response to eotaxin and prostaglandin D_2. Synthetic HDL nanodiscs also reduced eosinophil transmigration in response to eotaxin in IL-5 transgenic mice. All these effects were dependent on the lipidation status of the nanoparticles.

Discussion: ApoA-I-mimetic peptide-based nanodiscs have therapeutic potential in hyper-inflammatory and hyper-eosinophilic diseases such as sepsis and asthma, where immune cell migration and activation is a critical factor for disease exacerbation.

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Keywords: high-density lipoproteins (HDL) – ApoA-I-mimetic nanodisks – pulmonary inflammation

A2.27

How do missense mutations of a highly conserved glycine residue in the human GABA transporter 1 trigger epilepsy?

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Background: The human γ-aminobutyric acid (GABA) transporter 1 (hGAT-1) belongs to the solute carrier 6 (SLC6) gene family. It mediates neurotransmission by rapidly clearing GABA from the synapse into neurons and astrocytes. Copious point mutations in hGAT-1 have been associated with developmental delay, myoclonic-atonic and generalized epilepsies, autism and intellectual disability. Many of these mutants are known to impair protein folding, causing their retention in the endoplasmic reticulum (ER) and precluding their proper delivery to the cell surface. Folding defects can be corrected by treatment with chemical and pharmacological chaperones. Our aim is to probe the molecular features of two novel hGAT-1 disease variants in HEK 293 cells (*in vitro*), as well as in primary neuronal cultures and *Drosophila melanogaster* (*in vivo*).
Methods: The cellular localization and expression distribution of the wild-type hGAT-1 and two epilepsy variants thereof, G443D and G443V (created by site-directed mutagenesis), were investigated in transiently transfected HEK 293 cells. Their deglycosylation profiles were studied by immunoblotting using endoglycosidase H (Endo H). Confocal microscopy was utilized to examine the subcellular localization of the individual GATs, tagged with yellow fluorescent proteins (YFP) at their amino-terminal domains. Cyan fluorescent protein (CFP)-tagged calnexin was employed as an ER marker, while trypan blue delineated plasma membrane compartments. Functional consequences of the above mutations were examined by radiotracer GABA uptake assays, to determine the Michaelis–Menten kinetic parameters (K_m and V_{max}), and ultimately the potential of various small molecules to rescue the variants by pharmacochaperoning.
Results: Deglycosylation experiments revealed distinct expression patterns for the wild-type hGAT-1 and the two G443 variants. In short, ER-resident proteins are core-glycosylated (and sensitive to Endo H) whereas proteins located at the plasma membrane are mature-glycosylated (and hence resistant to Endo H). Accordingly, two protein bands were observed for the wild-type hGAT-1. The G443V variant presented only core-glycosylated species, contrasting to G443D, which also exhibited some mature-glycosylated bands. Confocal microscopy substantiated these findings: the mutants accumulated in the cell interior, co-localized with the ER-resident chaperone calnexin. Wild-type hGAT-1, on the other hand, was correctly targeted to the plasma membrane, overlapping with the trypan blue staining. We thus infer that both variants happen to be partially-to-fully misfolded and trapped in the ER compartment. Additional studies are underway to elucidate the functional consequences of both G443V and G443D mutations on GABA uptake.
Discussion: Upon dysfunction of plasmalemmal GATs, presynaptic GABA pools decline, thus perturbing the ensuing phasic neurotransmission. Concomitantly, there is a rise in extrasynaptic GABA levels, which in turn impact extrasynaptic $GABA_A$ and $GABA_B$ receptors to induce tonic inhibition. Since most of the reported pathogenic hGAT-1 variants are loss-of-function/misfolded transporters, their clinical manifestations are severe. Small molecules, such as the chemical chaperone 4-phenylbutyrate (4-PBA), liothyronine and the specific hGAT-1 blocker tiagabine, can be beneficial in mending the folding and activity of such variants. We plan to extrapolate our ongoing experiments to hippocampal neurons, and further translate them to *in vivo* studies in *Drosophila melanogaster*. Collectively, these data ought to impart mechanistic acumens crucial for developing effective therapeutic options for hGAT-1-linked syndromes.
Acknowledgements: This work was supported by the Austrian Science Fund FWF (project no. P36574-B27) to S.S.
Keywords: human GABA transporter 1 (hGAT-1) – γ-aminobutyric acid (GABA) – epilepsy – protein folding – pharmacochaperoning

A2.28

TRPC1 impact on calcium release mechanisms from the endoplasmic reticulum via interactions with inositol trisphosphate receptors

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Background: Transient receptor potential canonical proteins (TRPC1–7) are a group of calcium-permeable cation channels that exhibit a significant presence in the brain. Among the seven isoforms, TRPC1 stands out for its role in hippocampal physiology and pathophysiology via the regulation of cellular calcium homeostasis. A peculiar feature of TRPC1 is its targeting to the endoplasmic reticulum (ER) membrane, where it can potentially physically associate with other proteins involved in calcium release mechanisms. These interactions might result in functional implications and the engagement of TRPC1 could potentially affect the extent of calcium signaling within the cell, influencing various cellular processes. Based on the intracellular localization of TRPC1, we hypothesize that the channel has the ability to engage with the primary calcium efflux channel in the ER membrane, namely inositol trisphosphate receptors (IP_3R) and, consequently, impact the release of calcium.
Methods: To investigate a potential interaction between TRPC1 and IP_3R, we utilized fluorescence resonance energy transfer (FRET) and Ca^{2+} imaging techniques as well as total internal reflection fluorescence (TIRF) microscopy in transiently transfected HEK 293 cells.
Results: We confirmed via TIRF microscopy that TRPC1 channels target to the ER membrane. FRET experiments, using D1ER as a calcium sensor, showed a significantly lower calcium efflux via IP_3R after application of carbachol (CCh) when TRPC1 was present as opposed to control cells without TRPC1. When introducing the TRPC1 pore dead mutant D582K instead, calcium release levels were restored to match those observed in control cells. Ca^{2+} imaging measurements, using R-GECO as a cytosolic calcium sensor, validated these findings. We detected significantly lower cytosolic calcium levels after perfusion with CCh in cells expressing TRPC1 compared to control cells. Furthermore, cells transfected with D582K displayed increases of cytosolic calcium levels similar to the control.
Discussion: Due to the association of TPC1 with neurodegenerative disorders, we have a vast interest in gaining a deeper understanding of the role of TRPC1 in cellular calcium regulation and the function it plays in the ER. Our findings strongly suggest that TRPC1 engages in regulating IP_3R since its presence led to alterations in calcium release mechanisms. On one hand, we hypothesize that TRPC1 acts as a channel regulator for IP_3R, restricting its function in calcium release. On the other hand, we propose an alternative speculation that TRPC1 serves as a calcium leak channel in the ER since its presence led us to observe a reduced calcium release via IP_3R. This could be attributed to the fact that calcium stores in the ER were already partially depleted through the actions of TRPC1.

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Keywords: TRPC channels – TRPC1 – calcium dynamics – inositol trisphosphate receptors (IP_3R)

A2.29

Exploring the impact of STAT3-targeting on NK-cell-mediated killing in acute myeloid leukemia

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Background: Signal transducer and activator of transcription 3 (STAT3) is an integral component of the Janus kinase (JAK)-STAT pathway, which plays a critical role in cancer development. In the context of cancer, STAT3 primarily functions as a facilitator of tumor growth, promoting cell proliferation, suppressing apoptosis, facilitating angiogenesis, promoting metastasis, and aiding tumor cells in evading immune detection by natural killer (NK) cells. NK cells are innate lymphocytes capable of killing transformed cells. Recently, NK-cell-based therapies gained attention in the treatment of hematopoietic cancers, especially in acute myeloid leukemia (AML). The interaction between AML and NK cells is tightly regulated by surface ligands capable of either triggering or inhibiting the lysis of target cells. In most cancer types, blocking STAT3 signaling results in increased immune cell activation in the tumor environment.
Methods: We aim to investigate the interaction between NK cells and AML in the absence of STAT3, using AML knockout cell lines achieved through CRISPR-Cas9 technology. Our ongoing research involves analysing the impact of pharmacological STAT3 inhibition in AML on the NK-cell surveillance using flow cytometry, qPCR and western blot.
Results: Our findings demonstrate that the lack of STAT3 in human AML cell lines leads to diminished surveillance by primary human NK cells. Our data so far revealed a decrease in ICAM-1 and CD48 expression in AML cells lacking STAT3, which both contribute to immunological synapse formation. In the STAT3 knockout AML cell lines, we observed less conjugate formation between AML and NK cells compared to the wild-type AML cell line counterparts.
Discussion: As a result, we propose that the diminished killing of STAT3 knockout AML cells is likely attributable to disrupted synapse formation and the subsequent lack of NK-cell activation. Our novel finding reveals the unforeseen influence of STAT3 on the vulnerability of AML cells to NK-cell-induced elimination, potentially improving the treatment possibilities for patients.
Keywords: STAT3 – NK cells – acute myeloic leukemia

A2.30

Pharmacological inhibition of endolysosomal Ca^{2+} channels in immune cells and related (ultra)structural and physiological implications

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Background: Pharmacological modifications of endolysosomal Ca^{2+} channels have led to a better understanding of the mechanisms of immune diseases. Targeting endolysosomal Ca^{2+} channels could potentially impact diseases such as anaphylaxis or viral infections. Only recently it was shown that endolysosomal two-pore channels (TPCs) play a major role in the interaction of endolysosomes and the endoplasmic reticulum (ER), thus maintaining interorganellar Ca^{2+} homeostasis. If TPCs were impaired, an increased anaphylactic reaction was observed in mice [1,2]. In addition, TPCs also play an important role, both in virus entry and during endolysosomal processes and potential endolysosomal virus escape. However, the underlying (ultra)structural alterations are still pending.
Methods: To shed some more light on this topic, we have implemented confocal laser scanning microscopy (CLSM) techniques as well as 2D, 3D and analytical transmission electron microscopy (TEM) methods. This range of methods should help to investigate the (ultra)structure of rat basophilic leukemia cells (RBL-1) treated with or without the plant alkaloid and potent TPC inhibitor tetrandrine as well as with the novel tetrandrine analogue SG-094.
Results: Our CLSM investigations showed that endocytosis and lysosomal activity as well as co-localization between lysosomes and ER in RBL-1 cells decreased significantly after pharmacological TPC inhibition. The 2D-TEM investigations showed that ER and endolysosomes formed direct interorganellar contact sites at the biomembrane level. Finally, 3D-TEM tomography revealed the full extent of the large contact envelopes between the two organelles. In comparison, ER–endolysosomal contact sites decreased significantly in cells treated with tetrandrine and SG-094, further supporting the hypothesis that TPC function is essential for interorganellar Ca^{2+} exchange.
Discussion: It is our goal to gain a better understanding of the role of TPCs in the crosstalk between ER and endolysosomes at an (ultra)structural level. The correlation of our results with analytical EM and molecular biological methods as well as the implementation of correlative light and electron microscopy (CLEM) methods could be crucial to clarify whether TPCs are indeed promising pharmacological targets for the treatment of several diseases such as allergic hypersensitivity or viral infections.
Acknowledgements: This work is part of the MED Call Impetus Project KOMIPHA (no. I-18-22 to P. S.).
Keywords: two-pore channels – calcium – endoplasmic reticulum – endolysosomes – ultrastructure
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A2.31

ERC1 increases membrane and functional expression of the voltage sensor of excitation–contraction-coupling $Ca_V1.1$

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Background: ERC1, a member of the family of CAST/ELKS scaffold proteins, is responsible for supporting the structure of presynaptic active zones. ERC1 directly interacts with the Ca_V β subunit of voltage-gated Ca^{2+} channels (VGCC) through the guanylate kinase-like (GK) domain [1]. Importantly, this interaction affects VGCC activity, as ERC1 deletion leads to reduced calcium influx at inhibitory synapses in the hippocampus, the calyx of Held, rod photoreceptors, and pancreatic β cells [2,3,4]. Here, we hypothesized that ERC1, which is endogenously expressed in skeletal muscle, might also influence the membrane and functional expression of $Ca_V1.1$, as well as voltage-induced Ca^{2+} release from the sarcoplasmic reticulum.
Methods: We investigated the effect of ERC1 overexpression or deletion on $Ca_V1.1$ and the ryanodine receptor 1 (RyR1) membrane and functional expression in different cell types (skeletal muscle C2C12 wild-type and ERC1 knockout and HEK-TetOn-STAC3 cells), utilizing immunocytochemistry and electrophysiology analyses.
Results: First, we examined the impact of ERC1 overexpression on $Ca_V1.1$ and RyR1 levels in skeletal muscle C2C12 cells. Whereas $Ca_V1.1$ cluster intensity was enhanced by 15.6%, RyR1 expression remained unchanged. Additionally, we generated an ERC1 knockout C2C12 cell line (clone C3) with CRISPR/Cas9, in which we analysed the effect of ERC1 deletion or reconstitution on $Ca_V1.1$ and RyR1 expression levels. Similarly to the overexpression experiments, ERC1 enhanced $Ca_V1.1$ membrane expression by 15.5% while the RyR1 expression remained unaltered. In addition, to analyse the effect of ERC1 on $Ca_V1.1$ functional expression, we performed patch-clamp experiments in HEK cells and found that ERC1 increased $Ca_V1.1$ current density by 44%.
Discussion: Our results demonstrate that ERC1 increases the number of $Ca_V1.1$ channels in the membrane of skeletal muscle cells and $Ca_V1.1$ current density in HEK cells. In order to investigate the effect on endogenous $Ca_V1.1$, we are currently analysing the $Ca_V1.1$ currents and excitation–contraction coupling in muscle cells upon ERC1 overexpresssion.
Acknowledgements: The study was supported by the Austrian Science Fund FWF (grant P33776 and DocFunds DOC30/178).
Keywords: ERC1 – $Ca_V1.1$ channels – voltage-gated calcium channels – skeletal muscle
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A2.32

Mutation-induced changes in channel gating parameters increase sensitivity of pathogenic $Ca_V1.3$ L-type Ca^{2+} channel variants towards the Ca^{2+} channel blocker isradipine

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Background: Pathogenic *de novo* missense variants in the pore-forming α1 subunit (*CACNA1D*) of $Ca_V1.3$ voltage-gated Ca^{2+} channels cause a neurodevelopmental disorder with or without endocrine symptoms. We have shown that these variants enhance channel function and that some of them display higher sensitivity for the dihydropyridine (DHP) L-type Ca^{2+} channel blocker isradipine. Currently licensed DHPs may therefore provide a potential off-label treatment option in affected patients. To elucidate the mechanism leading to the higher isradipine sensitivity of disease variants, we tested if this can be solely explained by the enhanced voltage-dependent inactivation introduced by these variants as predicted by the modulated receptor hypothesis (MRH) [1].
Methods: We expressed wild-type (WT) $Ca_V1.3$ as well as variants A749T and L271H together with β2a and α2-δ1 subunits in HEK 293T cells and measured isradipine sensitivity using 50-ms pulses (0.1 Hz) from various holding potentials (HP; −89 mV to −27 mV) to vary the fraction of inactivated channels.
Results: Both A749T ($V_{0.5,act}$: −35.3 mV) and L271H ($V_{0.5,act}$: −41.7 mV) variants caused significant shifts in steady-state inactivation (SSI) towards negative potentials compared to WT ($V_{0.5,act}$: −16.5 mV). At −89 mV HP, A749T showed 1.4-fold higher sensitivity to isradipine (IC_{50}: 93.3 nM) compared to WT (IC_{50}: 134.9 nM) and 2.4-fold higher potency at −54 mV HP (IC_{50}: 17.3 nM) compared to WT (IC_{50}: 42.9 nM). This is consistent with voltage-dependent inhibition favored by a higher degree of inactivation of A749T channels at a given HP compared to WT. Accordingly, at HPs selected to stabilize similar SSI of 10% and 20%, respectively, for WT and A749T, IC_{50} values were no longer significantly different between A749T ($IC_{50(10\%)}$: 7.00 nM; $IC_{50(20\%)}$: 3.27 nM) and WT ($IC_{50(10\%)}$: 5.50 nM; $IC_{50(20\%)}$: 3.24 nM). These data suggest that enhanced voltage-dependent inactivation alone can explain the increase in isradipine sensitivity of this variant. We also observed a voltage-dependent five-fold increase of the isradipine sensitivity for variant L271H in comparison to WT at −89 mV HP but with a significantly lower IC_{50} at a predicted 10% SSI ($IC_{50(10\%)}$: 1.80 nM) compared to WT and A749T.
Discussion: WT, A749T and L271H channels are inhibited by isradipine in a strongly voltage-dependent manner. SSI at more negative voltages of the A749T variant accounts for its higher isradipine sensitivity, because more inactivation occurs at a given voltage compared to WT. The voltage-dependent increase of the isradipine sensitivity of WT and A749T channels can be quantitatively predicted from the modulated receptor hypothesis (MRH) by assuming affinities of 134.9 nM for resting and 0.70 nM for inactivated channels. We currently investigate whether the higher sensitivity of variant L271H to isradipine at depolarized voltages is explained by its larger inactivation during our recording protocol. Our data

encourage ongoing efforts for the symptomatic treatment of affected individuals with isradipine.

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Keywords: voltage-gated calcium channels – $Ca_V1.3$ channels – *CANAC1D* – gain-of-function mutations

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A2.33

The balance between STAT3 isoforms as essential feature in acute myeloid leukemia

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Background: Dysregulation of the JAK/STAT pathway and overexpression of the signal transducer and activator of transcription 3 (STAT3) is frequently found in hematologic malignancies. Constitutively activated STAT3 is associated with significantly poorer outcomes. On account of this, STAT3 became an attractive therapeutic target. However, until now these drugs have not yielded the intended effects. This phenomenon might be related to the expression ratio of the two alternatively spliced isoforms: the full-length isoform STAT3α and the C-terminally truncated STAT3β. Recently, STAT3β was shown to act as a tumor suppressor in acute myeloid leukemia (AML). In line, the *STAT3β/α* mRNA ratio in leukemic blasts of patients with bad prognosis was significantly lower than in those with good prognosis.In the light of these previous findings, the pharmacological induction of a higher STAT3β/α ratio could be a novel therapeutic option in AML.

Methods: To examine the efficiency of candidate drugs in affecting the STAT3 isoform ratio in leukemic cells, we perform *in vitro* assays such as real-time quantitative PCR and western blot. To check their migration behavior *in vitro* we use Transwell Migration Assays. In addition, we plan to test the anti-leukemic drug activity *in vivo*, using an MLL-AF9-induced mouse model and xenograft models.

Results: We observed that STAT3 isoform expression on protein and mRNA levels can be influenced by drug treatment in different human and murine AML cell lines. Therefore, we want to further analyze the underlying mechanism of these drugs influencing STAT3 isoform expression. Interestingly, we could identify the common antimalaria drug atovaquone as a potentially attractive candidate. The effect of an increased STAT3β/α ratio is paralleled by the anti-leukemic activity of atovaquone. Furthermore, we plan to address the anti-leukemic effect of atovaquone *in vivo*. Other antimalaria drugs and a structure analogue of atovaquone had no impact on the expression of the two alternatively spliced STAT3 isoforms.

Discussion: We have demonstrated a novel property of the antimalaria drug atovaquone. It positively affects the STAT3β/α ratio in AML cell lines, which could be potentially of therapeutic relevance in AML.

Keywords: acute myeloid leukemia – STAT3 – atovaquone

A2.34

Electrophysiological characterization of cardiac organoids

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Background: The number one cause of human fetal death are defects in heart development. Because the embryonic heart is inaccessible and no chamber-specific *in vitro* models exist, determining the causes of disease is difficult. Therefore, a human cardiac organoid (cardioid) platform was recently established recapitulating the development of all major embryonic heart compartments, including right and left ventricles, atria, outflow tract, and atrioventricular canal [1,2]. Here, we characterize the electrophysiological properties of these cardioids.

Methods: The following methods were used in this study: (i) multi-electrode arrays for the estimation of action-potential duration of 3D cardioids determined by extracellular field potential recordings; (ii) single-cell patch-clamp experiments to perform spontaneous action-potential recordings and subsequent parameter analysis; and (iii) optical calcium signaling experiments employing genetically encoded GCaMP6f reporter lines to optically visualize calcium transients.

Results: We revealed embryonic electrophysiological properties of cardioids. Single-cell patch-clamp experiments demonstrated high homogeneity of the cardiomyocyte cell populations, and action-potential durations matched those of humans. Multi-electrode-array measurements of 3D cardioids showed RT intervals, an indicator for action-potential duration, that were similar to the patch-clamp data. Finally, calcium signaling experiments further validated the cardioid platform by showing calcium-transient durations closely matching patch-clamp and multi-electrode-array experiments.

Discussion: In this study we characterized the electrophysiological traits of cardioids and confirmed their embryonic-like identity. Low upstroke velocities and a lack of chamber-specific action-potential characteristics in atrial cardiomyocytes further support the embryonic-like character. The high overlap of experimental data between the three different techniques used in this study underlines the robustness of the cardioid system, especially because multiple different biological replicates were used. Preliminary pharmacological experiments (data not shown) revealed responsiveness to multiple known ion-channel blockers, however complete pharmacological profiling is still outstanding. Future attempts at metabolic maturation of cardioids should yield cardioids more closely resembling the adult-like state, thereby generating a platform suitable for disease modeling (*e.g.* Brugada syndrome, Long-QT syndrome) and precision pharmacology. Moreover, the possibility to fuse cardioids and work with a multi-chambered system to evaluate chamber-specific pharmacological effects has the potential to revolutionize preclinical cardiac safety pharmacology.

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Keywords: cardiac electrophysiology – organoids – patch clamp

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