Caloric restriction improves in vivo cortical function in aged mice

N. Asavanapummas1, N. Fröhlich1, C. Lerdkrai1, E. Zirdum, O. Garaschuk
1Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tübingen, Germany

Aging is accompanied by an increasing risk of developing cognitive impairment, including learning and memory deficits. Caloric restriction (CR) is known to extend the life expectancy, delay the onset of aging-related disorders and to enhance the cognitive function. Here we monitored the animal’s body mass and characterized the functional in vivo properties of cortical neurons during normal aging or under CR. During aging of ad libitum fed mice we observed an increased body weight by 23.1% associated with a mortality of 33.3%. In vivo two-photon Ca2+ imaging revealed a significant aging-mediated increase in the frequency of spontaneous Ca2+ transients in layer 2/3 neurons of the frontal/motor and the primary visual cortex. Interestingly, the aging-related hyperactivity developed earlier in the frontal/motor compared to the visual cortex. Moreover, aging-related increase in neuronal hyperactivity was accompanied by an impairment of visual processing, i.e. a significant decrease in the orientation and to a lesser extent in the direction selectivity. In aged (15-month-old) mice the two-choice visual discrimination test and the object recognition test revealed a significant reduction of (i) both pattern detection and discrimination abilities as well as (ii) the ability to remember the familiar object, selective deficits in cognition and memory performance. A reduction of the daily food intake by 30% for 12 months (CR) led to a decrease of the body weight by 16.1% and to a decline of the mortality (6.4%). CR counteracted the hyperactivity of cortical neurons and partially ameliorated the deficits in visual processing by causing a significant improvement of the orientation selectivity. Consistently, CR significantly improved both pattern detection and discrimination abilities of aged mice as well as their memory performance in the novel object recognition test to the levels observed in young 3-month-old mice. Taken together, normal aging is accompanied by (i) an increased body weight, (ii) region-specific development of the hyperactivity of cortical neurons, (iii) impairment of visual processing as well as (iv) the aging-related deterioration of cognition. CR significantly reduces neuronal hyperactivity and ameliorates the observed deficits in cognitive abilities of aged mice.

Effects of activin A on hippocampal network activity

M. Dahlmanns1, J. K. Dahlmanns1, F. Zheng1, C. Alzheimer1
1University Hospital Erlangen, University Hospital Erlangen, Erlangen, Germany

In addition to its established role in neurodevelopment and neuroprotection, the TGF-β family member activin A is increasingly recognized as a modulator of synaptic transmission, cognitive functions and affective behavior. Here, we explored how activin affects oscillations and spike routing within hippocampal networks using slice and culture preparations. Transverse hippocampal slices (400 µm thick) were prepared from wt mice and mice expressing a dominant-negative mutant of activin receptor IB (dnActRIB), which disrupts activin signaling. Hippocampal oscillations were induced by the cholinergic agonist carbachol (25 µM) and monitored by means of field potential recordings in CA1 stratum oriens and CA3 stratum radiatum. Superfusion of slices with carbachol induced robust oscillations in the low theta range in both regions of wt slices, whereas no such drug effect was observed in dnActRIB slices. Only at a higher carbachol concentration (37.5 µM), theta oscillations emerged also in the mutant preparation, suggesting that endogenous activin signaling serves to promote cholinergically driven theta activity. The impact of activin on spike routing was explored in dissociated primary hippocampal cultures, in which network activity was monitored with the calcium fluorescent dye Fluo-4/AM. Based on the calcium responses of electrically-activated neurons, spiking was detected and the functional network was reconstructed. In cultures incubated with activin A (25ng/ml for 48 h) we found no alterations in the synchrony of the cells. An analysis of the functional network properties however showed, that activin A although not changing the overall connectivity degree, had a number of significant effects on parameters of signal transmission and routing: an increase in network strength, a decline in the characteristic path length, and an increase in network assortativity. When computationally removing individual cells from the network, activin A-treated networks displayed a lower vulnerability than control networks, reflecting an increased reliability of information transfer. Our study is the first to interrogate the impact of activin on functional network connectivity. We found that disruption of activin signaling impairs the appearance of theta oscillations, whereas a rise in activin, as observed during behaviourally relevant stimuli, fosters a number of features that are expected to augment the performance of the network.
mPFC was illuminated with yellow light on half of the trials. In each testing session light delivery was temporally restricted to one of the three phases of the task in order to examine the contribution of the mPFC to different task components. In mice, A. Böttcher, O. Garaschuk, N. Mojtahedi Eberhard Karls University of Tübingen, Institute of Neurophysiology, Tübingen, Germany eGFP control animals had no significant effect on behavior. The results of this experiment suggest that pyramidal neurons in

Endogenous neuronal activity is a hallmark of the developing brain. In rodents, a handful of such activities were described in different subregions on attention and waiting impulsivity

B 01-8 Influence of chemogenetic G-protein coupled receptor modulation of medial prefrontal cortex subregions on attention and waiting impulsivity

B. van der Veen, P. Steele-Perkins, K. Kilienzö, S. Schulz, M. Jendryka, A. Kęccek, W. Nissen, B. Kätzel
1University of Ulm, Institute of Applied Physiology, Ulm, Germany
2Boehringer Ingelheim Pharma GmbH, Discovery Research, Biberach an der Riss, Germany

Introduction: A lack of proper attention and impulse control, have been found in many psychiatric disorders, such as ADHD, substance abuse and bipolar disorder. The medial prefrontal cortex (mPFC), has been implicated in attentional control and various forms of impulsivity, e.g. waiting impulsivity, stopping impulsivity, delayed and probabilistic discounting. Lesion studies in rats have implicated the anterior cingulate cortex (ACC) and infralimbic cortex (IL) in impulse control, and the ACC and the prefrontal cortex (PFC) in sustained attention and the extend of lesions and adaptation effects evoked by them are difficult to delineate. Therefore, we sought to clarify the specific role of the distinct subregions of the PFC in attention and waiting impulsivity in intact animals.

Methods: We used a chemogenetic approach to selectively activate or inhibit G-protein-cascades on excitatory cells by Designer-Receptors Expressed in Diverse Expressed (DREADDs), specifically expressed excitatory cells infected mPFC subregions of mice. The mice were trained and tested on the five choice serial reaction time task (5CSRTT). Sustained attention and impulse control were assessed on this task by presenting challenges, such as decreased stimulus duration or an increased intertrial interval, respectively. During those challenges, DREADDs are activated by application of different doses of CNO in a Latin-square design, counteraffine for targeted subregion and DREADD/control vector. We also used arterial spin labelling (ASL) to assess the CNO/DREADD-induced activity changes in vivo.

Results: Using ASL imaging before and after application of CNO (2 mg/kg) we found increased blood flow in the transfected PFC subregion when activating Gs signaling, but not in an untransfected region, proving the capability of DREADDs to activate excitatory cells in vivo. In the 5CSRTT, we found that activating Gi signaling in the ACC by application of CNO decreases premature responding when impulse control is challenged but activation of Gi signaling in the IL does not show such effect. Activating Gs signaling in the ACC increased premature responding and decreased sustained attention.

Conclusion: Decreasing activity of excitatory neurons in the ACC but not IL can improve impulse control when challenged.

B 01-9 Delayed matching to position working memory in mice relies on N-methyl D-aspartate receptors in prefrontal pyramidal cells

K. Kilienzö, B. van der Veen, J. Teutsch, S. Schulz, B. Kätzel
1Ulmer University, Applied Physiology, Ulm, Germany

Cognitive dysfunction remains an untreated medical need in the treatment of psychiatric and neurological illnesses. These dysfunctions include impairments in attention, cognitive flexibility and memory (working and long-term) and are associated with pathological molecular signaling in the medial prefrontal cortex (mPFC). Working memory is the ability to selectively encode, actively maintain specific information in the mind, and later use it to achieve behavioural goals. Our study focuses on the role of N-methyl D-aspartate receptors (NMDARs) in working memory deficits. Using a mouse model of selective ablation of NMDARs in glutamatergic pyramidal neurons of the mPFC, we examined different components of visuo-spatial working memory with a newly established operant, delayed matching to position (DMPfT), task. Targeted knock-down of mPFC was illuminated with yellow light on half of the trials. In each testing session light delivery was temporally restricted to one of the three phases of the task in order to examine the contribution of the mPFC to different task components. In mice expressing the neural silencer ArchT, light application significantly inhibited the majority of putative pyramidal cells. Furthermore, mPFC inhibition in any of the three phases of the task impaired SWM performance while the same treatment in eGFP control animals had no significant effect on behavior. The results of this experiment suggest that pyramidal neurons in the mPFC are involved in the encoding, maintenance, and retrieval of spatial information. Motivated by recent findings of increased interactions between the mPFC and the ventral medial area (VTA) during SWM, a follow-up experiment was conducted, in which we examined whether mPFC projections to the VTA contribute to different aspects of SWM. In order to accomplish projection-specific inhibition of VTA-projecting mPFC neurons, the feasibility of two different optogenetic approaches was tested in head-restrained mice. Based on the results, a terminal inhibition strategy was chosen for reducing prefrontal inputs to the VTA during SWM. By again restricting optogenetic inhibition to different task phases, we found impaired task performance when disrupting mPFC inputs to the VTA during the delay phase, suggesting that this pathway is critical for maintaining, but not encoding or retrieving spatial information.

B 01-6 Amplitudes and propagation of cortical spreading depolarization (CSD) in adult rats are influenced by Calcitonin gene-related peptide (CGRP)

F. Gimeno-Ferre, F. Richter, R. Bauer, A. Lehmenkühler, H. - G. Schalb, Eberhard Karls University of Tübingen, Institute of Physiology, Tübingen, Germany

It is known from the literature that CGRP plays an important role in mice and antagonize CGRP is an effective pre-treatment against migraine pain. CGRP is able to increase neuronal excitability. Further it has been shown that a CSD - that is known to be the correlate of the migraine aura - can release CGRP in rat neocortical slices. Whether CGRP enhances brain’s susceptibility for CSD or influence CSD itself in vivo has not been investigated yet. To test this, we applied CGRP at different concentrations topically to a restricted part of the cortical surface and compared the electrocorticographic (ECoG) response in the untreated and the treated part of the cortex.

In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSDs were recorded in cerebral cortex with two pairs of glass micropipettes (distance 5-6 nm) at depths of 400 and 1200 µm in two areas of the cortex, separated by a wall. In addition, in the treated area CSD-related changes in extracellular potassium concentrations ([K+]c)

In all rats tested, a pulse of KCl elicited a single propagating CSD. The topical application of CGRP to the brain surface reduced the amplitudes of CSD in the treated area (10-4 M to 60 % of controls, 10-5 M to 70 % of controls, untreated to 85-90 % of controls) and slowed the propagation velocity (10-4 M from 3.0 to 2.6 cm/s; 10-5 M from 2.4 to 2.2 cm/s). Rarely spontaneous CSD were observed originating from the CGRP-treated area. In a few other rats, CGRP induced focal ictal activity after 2-2.5 hours of application that did not spread into the untreated cortex. Focal ictal activity occurred at intervals of 11-15 minutes and was accompanied by transient increases in [K+]c. However, so far neither the ignition of CSDs nor the induction of focal ictal activity showed a dose-dependency to CGRP.

Our results identify the neuropeptide CGRP as a candidate that could interfere with CSD by changing neuronal excitability.
GluN1 (NMDAR) expression in excitatory cells of the mPFC in adult mice elicited an impairment in DMTP working memory. Surprisingly, the impairment was not observed in the standard, delayed non-matching to position (DNMTP), T-maze alternation task, suggesting that the cognitive correlates assessed by the two tasks are dissoicable. Furthermore, there were no impairments attributable to the knock-down in tests of attention, impulsivity, novelty-induced locomotor hyperactivity, anhedonia, nesting, sociability and anxiety. These results elucidate the multifaceted nature of working memory assessment and suggest that NMDAR-hypofunction in prefrontal excitatory cells may underlie working memory deficits in psychiatric disorders.

**B 01-10**

**Dopamine Neurons Drive Fear Extinction Learning by Signaling the Omission of Expected Aversive Outcomes**

X. I. Salinas-Hernández, P. Vogel, R. Kalisch, T. Sigurdsson, S. Duvernoy

Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt am Main, Germany

University Medical Center of the Johannes Gutenberg University, Deutsches Resilienz Zentrum, Mainz, Germany

Extinction of fear responses is critical for adaptive behavior and deficits in this form of learning are hallmark of anxiety disorders. However, the neuronal mechanisms that initiate fear extinction learning are largely unknown. Associative learning theories propose new learnings initiated when outcomes violate our expectations. Such violations are thought to cause "prediction error signals" (PE) that will initiate the neural processes that ultimately lead to behavioral changes. During fear extinction, the absence of an aversive unconditional stimulus (US) is an unexpected event and likely generates a PE signal that initiates extinction learning. More specifically, the omission of the aversive US can be conceptualized as a better-than-expected outcome. It is well-established that the activity of midbrain dopamine (DA) neurons represents the degree to which outcomes are better or worse than expected. We therefore hypothesized that DA neurons could drive fear extinction learning by signaling the omission of an expected aversive outcome. To address this, we recorded the single-unit spiking activity of ventral tegmental area DA neurons in mice that were trained in a fear conditioning protocol. Analysis of neuronal firing rates during the US omission revealed that a subpopulation of putative DA neurons were significantly excited by the omission of the US during extinction. This DA signal occurred at the beginning of the extinction when the US omission is unexpected, and correlated strongly with extinction learning. To further confirm that DA neurons signal the unexpected omission of the US, we measured the activity-dependent calcium signals selectively in DA neurons using fiber photometry. Consistent with the above results, we observed a significant increase in calcium signal in the time of the US omission as compared to baseline. Next, we asked whether this signal is necessary for fear extinction. To this end, we performed temporally-specific optogenetic inhibition of DA neurons at the time of the US omission and found that such a manipulation impaired fear extinction learning. Conversely, optogenetic excitation of DA neurons during the US omission accelerated extinction. Together, these results identify a prediction error-like neuronal signal that is necessary to initiate extinction learning and reveal a crucial role of DA neurons in this form of learning. Currently, we are investigating which neural circuitry receives the DA-PE signal to drive extinction learning.

**B 01-11**

**Regulation of locomotion and reward seeking behaviour by the ventral tegmental area inputs onto the lateral hypothalamus**

V. Myutyul'kova, T. Korokovskiy

Max Planck Institute for Metabolism Research, Neural Circuits and Behavior Group, Cologne, Germany

University of Cologne, Institute of Vegetative Physiology, Medical Faculty, Cologne, Germany

To ensure the survival and reproduction animals must adapt their behaviour to acquire rewards and avoid punishments. Rewarding stimuli induce pleasurable feelings and promote approach and consummatory behaviours, eventually leading to behavioural reinforcement. The ventral tegmental area (VTA) and the lateral hypothalamus (LH) are densely interconnected structures, which are critical for reward-seeking behaviour. While the LH-VTA pathway has been recently reported to modulate the approach towards diverse rewarding stimuli, the role of a feedback connection has not yet been investigated. Here we investigated the role of dopaminergic and GABAergic projections from the ventral tegmental area onto the lateral hypothalamus in reward seeking behaviour of mice. In order to examine the activity of the LHVTA-projecting dopaminergic and GABAergic VTA cells affects behaviour we optogenetically manipulated these pathways in several behavioral assays. We found that optogenetic stimulation of VTA-LH dopaminergic projections facilitates locomotion during spontaneous exploration, increasing the average running speed and total distance traveled. It also has an inhibitory effect on feeding in food-deprived mice. Moreover, activation of these projections promotes real-time place preference, which indicates their role in reward processing. Optogenetic stimulation of GABAergic VTA-LH projections, on the contrary, decreases the locomotory activity of mice and induces food consumption in ad libitum fed mice. In conclusion, optogenetic stimulation of two complementary VTA-LH pathways exhibits opposite effects on reward-seeking behaviour and locomotion in mice, possibly by targeting distinct populations of cells within the LH.

We gratefully acknowledge funding by the ERC Consolidator Grant 2017 (HyPFeedNet, TK) and the DFG (23386668/GK1960, VA).
significantly suppressed, indicating successful NMDAR dysfunction at these synapses. In contrast, mossy fiber LTP in CA3 neurons during blood-brain barrier-dysfunction-induced epileptogenesis

B 02-2
Increased theta-resonance behavior in CA1 pyramidal neurons during blood-brain barrier-dysfunction-induced epilepsy

J. K. Lapp

1 Albert Einstein College of Medicine, New York, US
2 Marine Biological Laboratory / Grass Foundation, Grass Laboratory, Woods Hole, US
3 Leipzig University, Medical Faculty / Carl-Ludwig-Institute of Physiology, Leipzig, Germany

Brain insults like stroke, traumatic injury or infections often lead to blood-brain barrier-dysfunction (BBBD) that frequently results into epileptogenesis. Epilepsy patients do not only suffer from seizures but also from cognitive comorbidities such as learning or memory impairment, which might be associated with changes in brain network activity. To more deeply understand the process of epileptogenesis and altered network activity we were electrophysiological studying the BBBD-disrupted rat hippocampus following a cortical phototoxotonic. We have previously shown that BBBD leads not only to early seizures in two-thirds of animals but also to an associated power shift from gamma (30-100 Hz) towards theta (4-8 Hz) oscillations in vivo hippocampal activity (Lippmann et al., 2017). However, the underlying mechanisms remain still undefined.

It has been proposed that hippocampal CA1 pyramidal neurons contribute to population θ-activity through intrinsic subthreshold oscillations and resonance (increased voltage response) at θ-frequencies. While all pyramidal neurons display θ-resonance at hyperpolarized potentials (<-70 mV, INaP mediated), the expression of resonance at depolarized potentials (D-Res, >-65 mV, INaP mediated) depends on the Ih/Ihmax ratio and is observed only in 30% of neurons. The remaining 70% of cells can express subthreshold resonance if the Ih/Ihmax ratio is modulated. To elucidate the mechanisms contributing to seizures and θ-increase, we are characterizing intrinsic properties of CA1 pyramidal neurons in vitro one week after BBBD-induction. When analyzing sub- and suprathreshold voltage responses to oscillatory and squared current stimuli we find that the fraction of D-Res neurons increases from 30 to 50 % in BBBD compared to sham animals. Whereas resonance properties did not differ between groups at subthreshold potential, the perisynaptic excitability was significantly increased in D-Res neurons after BBBD. D-Res neurons revealed a more hyperpolarized action potential threshold together with a slight increase in input resistance and maximal impedance. The higher number of resonant-neurons together with the increased excitability is the higher the transmission of in vivo-oscillations in BBBD-treated animals. Our data prove evidence that BBBD-induced changes in intrinsic excitability of CA1 pyramidal neurons contribute to epileptogenesis and power increase in θ-oscillations that may be associated with cognitive comorbidities.

B 02-3
Increase of neuronal activity by 4-Aminopyridine improves sensory-motor dysfunction in a mouse model of SMA

J. K. Slime Longang1, J. Pagazitla2, E. Fletcher2, B. Blanco Redondod, J. Buetneret2, G. Mentis3, C. M. Simon1

1 University of Leipzig, Carl-Ludwig-Institute for Physiology, Leipzig, Germany
2 Columbia University, Center for Motor Neuron Biology and Disease, New York, Germany
3 Columbia University, Dept. of Pathology and Cell Biology and Neurology, New York, Germany
4 University of Leipzig, Rudolf-Schoenheimer Institute for Biochemistry, Leipzig, Germany

Dysfunction of neuronal circuits are important determinants in neurodegenerative diseases. Spinal muscular atrophy (SMA) — caused by deficiency in the ubiquitously expressed SMN protein — is characterized by loss of central synapses, neuromuscular junction (NMJ) deterioration, motor neuron death and skeletal muscle atrophy. SMA vulnerable motor neurons exhibit a reduced firing frequency as a response to impaired premotor synapses early in the disease process in mice, suggesting a pharmacological increase of neuronal activity could be a therapeutic strategy. The FDA approved 4-aminopyridine (4-AP) increases neuronal activity by block of voltage activated K+ channels. When an increase of neuronal activity could be beneficial to the SMA phenotype in vertebrates, we investigated daily 4-AP injected SMA animals at P30 (P30-3). Extracellular recordings from i) ventral root (VR) following ii) dorsal root stimulation (DR) and iii) quantification (Q) of maximum amplitude from the same groups shown in (A). (E) The first (black) and fifth (red) VR responses following stimulation of the homonymous ii DR at 10Hz. (F) Percentage change in the amplitude of the homonymous responses for the first five stimuli relative to the first response from the same experimental groups shown in (B).
Slc1a3P290R/+ mice suffer from epilepsy, ataxia, and cerebellar atrophy and thus closely resemble the human disease. EAAT1 is the major glutamate transporter in cerebellar Bergmann glial cells [7], and microscopic analysis of cerebellar cortices from Slc1a3P290R/+ mice indicated that Bergmann glial cells undergo apoptosis during the second week of life. We hypothesized that ER-located enzymes PDI and PERK relating to the Huntingtin-mediated ER-stress in SH-SYSY cells Chorea Huntington is an inherited autosomal neurodegenerative disorder caused by expanded CAG repeats (PolyQ > 36) in the huntingtin gene. Mutant huntingtin (mHTT) expression results in cytoplasmic aggregation and severe endoplasmic reticulum (ER) stress. Neuronal cells respond by activating a self-rescue program, the unfolded protein response (UPR) to regrow in a physiological manner. However, prolonged ER stress finally leads to apolotic cell death and is thereby the major cause for the neurodegenerative progression of this disease. Of note, the protein disulfide isomerase A1 (PDI) is an important activator of the PERK pathway, which is one of the three branches (PERK, IRE1, ATF6) of the UPR. The inhibition of PDI in SH-SYSY cells has not been characterized so far. Using an inducible lentiviral knockdown system, we compared the consequences of inhibition of PDI and PERK after mHTT aggregation for ER-Stress level, proliferation and apoptosis in SH-SYSY and HEK293T cells. MHTT caused an activation of all branches of the UPR and reduced proliferation in both cell lines as measured by luciferase-assays, Western blot and proliferation assays. The length of the PolyQ chain influenced the negative effects of mHTT-Aggregation since a Q120 construct induced more ER-Stress even during the first days of expression than a Q74 construct and reflects thereby the situation in vivo. We compared the knockdown of PDI with the ensuing downregulation of PERK activity to a knockdown of PERK itself. The PDI knockdown improved proliferation and cell viability of mHTT expressing cells whereas PERK knockdown showed less proliferation and decreased viability. In summary these results suggest that inhibition of PDI instead of PERK itself can potentially act as a clinical target for future therapy approaches.

B 02-7
Modulation of cortico-striatal GABAergic inhibition by short-term deep brain stimulation in a phenotypic animal model of dystonia

M. Zwar1, D. Franz2, M. Rohde1, V. Neubert1, M. Paap1, S. Perl1, C. Niemann1, F. Plochsties1, D. Timmermann1, A. Richter1, R. Köhling1

1University Medical Center Rostock, Oscar Langendorff Institute of Physiology, Rostock, Germany
2University of Leipzig, Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, Leipzig, Germany
3University of Rostock, Institute of Applied Microelectronics and Computer Engineering, Rostock, Germany

Dystonia is the third most common movement disorder affecting around three million people worldwide. The loss of striatal GABAergic transmission has been suggested as a possible pathophysiological basis of dystonia. Previous work in d(1/2z) hamsters, a unique phenotypic model for idiopathic paroxysmal dystonia, identified a reduced number and density of parvalbumin-positive GABAergic interneurons in all striatal subregions. The deficit of striatal GABAergic interneurons is accompanied by an overactivity of inhibitory medium spiny neurons (MSN) and further reduces basal ganglia output. An effective, but not fully understood treatment is deep brain stimulation (DBS), which improves dystonic symptoms. To test this hypothesis, bipolar DBS-electrodes were bilaterally implanted in the entopeduncular nucleus (the homolog of the substantia nigra pars reticulata) of (d1/2z) and control hamsters. After short-term bilateral DBS (3 h, 130 Hz, 50 µA) 48 hours prior to sacrifice, we analyzed the effect of GABAergic inhibition on excitatory synapses of striatal MSN by recording field excitatory postsynaptic potential (fEPSP) in cortico-striatal slices of control and DBS conditions, and in the presence of the AMPA-receptor blocker gabazine. In patch clamp measurements the passive membrane properties of striatal MSN were investigated. Furthermore, the action potential characteristics, i.e. threshold, burst patterns, and frequency, were analyzed. Comparisons were made between healthy and dystonic hamsters as well as stimulated and sham-stimulated animals.

B 02-8
Mutant Huntington accumulation in the nucleus affects nucleolar function in Huntington’s disease mouse models and patients at early stages

A. Sönmez1, S. Speith1, 2, R. Mustafa1, 2, K. Kojer3, C. Litke1, J. Koch3, M. Orth1, B. Liss1, R. Parlato1, 2

1Dund University, Institute of Applied Physiology, Ulm, Germany
2Heidelberg University, Institute of Anatomy and Cell Biology, Heidelberg, Germany
3Ulm University, Department of Neurology, Ulm, Germany

Question
Reduced ribosomal RNA (rRNA) synthesis is beneficial against cellular stress conditions by limiting energy consumption. On the other hand, mutant proteins and RNAs interfere with RNA Polymerase I activity and impair rRNA synthesis in the nucleolus. Hence, we aimed to map in the stage- and cell-specific link between nucleolar function and Huntington’s disease (HD) progression, an inherited neurodegenerative movement disorder characterized by the accumulation of mutant Huntington (mHtt) protein aggregates in the nucleus and increased cellular stress.

Methods
Striatal and muscle tissues from a knock-in mouse model carrying the human mHtt allele, as well as human HD patient tissue, were processed for in situ hybridization with specific nucleolar markers to allow an imaging and comprehensive evaluation of various nucleolar parameters.

Results
We showed that rRNA synthesis and nucleolar integrity were affected in both mouse models as well as in HD patients. In particular reduced rRNA synthesis correlated with the presence of mHtt nuclear inclusions. More precisely, rRNA synthesis was altered in mouse striatal neurons but not in the muscle tissue.

Conclusions
Our results indicated that mHtt nuclear inclusions interfere with the activity and integrity of the nucleolus in a stage- and cell-specific fashion. Thus, determination of nucleolar pathology in skeletal muscle biopsies from HD patients could offer a marker for disease progression, and also for assessing the beneficial effects of novel therapeutic approaches to slow down HD progression.

B 02-9
Enhanced KCa2 function following CK2 inhibition in vivo in a model of temporal lobe epilepsy

F. Schulze, S. Müller, X. Guí, S. Lukas, B. Hannes, R. Till, M. Marco, T. Kirschstein, R. Köhling

Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

Pharmacotherapy in epilepsy patients acts against the occurrence of seizures, but do not modify the course of the disease, i.e. they do not have anti-epileptogenic effects. In our previous study, we observed that in vivo casein kinase 2 (CK2) inhibition with 4,5,6,7-tetrabromotriazole (TBB) prevented the emergence of epileptic activity in the acute epilepsy slice model. Therefore, we now tested the effect of TBB in the in vivo pilocarpine-induced status epilepticus (SE) model of temporal lobe epilepsy. Thus, we applied TBB four times starting on day 3 prior to SE. We found that TBB pretreatment delayed onset of seizures after pilocarpine and slowed down disease progression during epileptogenesis. As a potential mechanism of the anti-epileptogenic effect, we observed a reduced proportion of burst firing neurons in the CA1 area in addition. Western blot analyses confirmed that CA1 tissue from TBB-pretreated epileptic animals contained significantly less CK2 than TBB-pretreated controls. On the transcriptional level, TBB pretreatment led to differential gene expression changes of CK2β, but also of NCN1 and HCN3 proteins. Therefore, we next repeated our electrophysiological experiments in the presence of the HCN channel blocker ZD7288. Under these conditions, pretreatment with TBB rescued the afterhyperpolarizing potential as well as spike frequency adaptation in epileptic animals, both of which are prominent functions of KCa2 channels. These data indicate that TBB pretreatment prior to status epilepticus slows down disease progression during epileptogenesis involving increased KCa2 function, probably due to a persistently decreased CK2 protein expression.
People with diabetes mellitus (DM) commonly develop olfactory dysfunctions. However, clinical results are controversial and preclinical studies are scarce. There are only two reports demonstrating that streptozocin (STZ)-induced diabetic (SD) male rats exhibit a reduction in odor discrimination and decreased olfactory sensitivity, as measured in the buried food pellet and the novel object discrimination tests, respectively. The aim of this work was to explore whether SD female rats present impaired olfactory function. This is especially important since several structures related with olfaction exhibit sexual dimorphism. To induce hyperglycemia, STZ (50mg/kg) was injected i.p. in two consecutive days to female young-adult Wistar rats; controls were injected with vehicle (Citrate Buffer 0.1M, pH 4.5). Ten days after STZ or Buffer administration animals were submitted to retronasal conditioned odor aversion (CAO) learning, in which an aversive stimulus that provokes visceral malaise (LICI 2% BW, i.p.) is paired with the consumption of an odorant diluted in water. We used two different odorants: Isoamyl Acetate (IA), a food odorant that activates the main olfactory system; and 2-Heptanone (2-Heq), a pheromone that activates the accessory olfactory system. To establish the olfactory perception threshold, we used six different decreasing concentrations (Sx10<10<Sx10<17> of IA or 2-Heq in consecutive days. We calculated an index of preference (IP) computing the volume of IA or 2-Heq solution consumed x 100 / the total volume consumed (AI solution + water). This index allowed us to know whether the animals that were properly conditioned perceived the odorant at decreasing concentrations. A Student t-test was utilized to compare results between experimental groups. Our results show that all experimental subjects (euglycemic and hyperglycemic) avoided odorant consumption (AI/2-Heq) after CAO training. Moreover, odor avoidance decreased when using less concentrated solutions. Notably, hyperglycemic females showed a reduced capacity to recognize IA. No statistical changes were observed in olfactory discrimination using 2-Heq, indicating a specific hyposmia. Our data are in line with clinical observations in people with DM and in SD male rats. These results also suggest that STZ treatment (which provokes oxidative stress in the Central Nervous System) affects differentially the main and accessory olfactory systems.

**Cav2.3 channels trigger selective dopaminergic neuron loss in Parkinson’s disease**

J. Benkert¹, S. Hess¹, S. Roy¹, N. Wiederspohn¹, J. Duda¹, H. Hollmén¹, S. Mueller¹, C. Poetschke¹, R. Pán̄at², J. Strissinger², T. Schneidere², T. Frank³, P. Kloppenburg³, B. Liss³

¹Universität Ulm, Institute of Applied Physiology, Ulm, Germany
²University of Cologne, Institute for Zoology, Biocenter, CECAD, Cologne, Germany
³University Medicine Göttingen, Department of Neurology, Göttingen, Germany

Selective degeneration of dopaminergic (DA) neurons in the Substantia nigra (SN) causes the motor symptoms of Parkinson’s disease (PD), while neighboring DA neurons in the ventral tegmental area (VTA) are mainly spared. The mechanisms underlying this age-dependent and region selective loss are unclear. However, activity-related metabolic stress, and dysfunctional Ca²⁺ signaling constitute important factors. DA neurons display an autonomous pacemaker activity which is crucial for dopamine release and movement control. In SN DA neurons, this activity generates oscillatory increases in free cytosolic Ca²⁺ levels which are thought to impart mitochondrial stress and render these neurons more vulnerable to degenerative stressors. This stressful Ca²⁺ driven mode of action distinguishes SN DA from resistant VTA DA neurons. Particularly in distal dendrites of SN dopaminergic neurons, these Ca²⁺ transients are sensitive to inhibitors of L-type voltage-gated Ca²⁺ channels (LTCGs), such as isradipine. Consistent with a role for activity-related Ca²⁺ signals in triggering neuronal demise in PD is epidemiological evidence, correlating use of LTCG blockers with a reduced risk for developing the disease later in life. But a recent phase-III clinical trial with isradipine to investigate its potential for neuroprotection in PD patients was negative, and in PD animal models, there is no agreement regarding the effect of isradipine on PD-related lesion induction.

We combined single cell molecular techniques, brain slice patch-clamp recordings and Ca²⁺ imaging with pharmacological and genetic tools to analyze the role of all brain voltage-gated Ca²⁺ channels (VGCCs) for DA neuronal viability in PD. We identified Cav2.3 R-type Ca²⁺ channels, which have yet to be studied in the context of PD. As a new key regulator of SN DA neuronal viability in vivo, we demonstrated that Cav2.3 KO reduced activity-associated somatic Ca²⁺ transients and Cav2.3 dependent after-hyperpolarizations in SN DA neurons, and afforded their full protection from degeneration in vivo in a neuropathin PD mouse model (chronic MPTP/probenecid). In conclusion, Cav2.3 emerges as a novel target for combating Ca²⁺ dependent neurodegeneration in PD.

**Effect of exposure to low doses of ozone on the expression of IL-17A and its receptor during the process of progressive neurodegeneration in the hippocampus of rats**

D. Rebolledo-Solleiro¹, I. Cruz-Guzmán¹, Solleiro-Villavicencio¹, G. Roldán-Roldán¹

¹Universidad Nacional Autónoma de México, Facultad de Medicina/Departamento de Fisiología, Mexico City, Mexico

In populated cities, air pollution is one of the main risk factors for the incidence of various health problems. Ozone is one of the most important air pollutants due to its abundance and toxicity, therefore, the inhalation of this gas produces a state of oxidative stress, which is considered a critical factor in the development of neurodegenerative diseases. Recently, our work group reported that during the initial stage of exposure to low doses of ozone (7 and 15 days) an acute CNS damage occurs, which is accompanied by a Th17IL-17A systemic response that can be self-regulated. However, if the exposure is prolonged (30 days), the damage caused is irreversible and the inflammatory response, characterized by an increase in the hippocampus of IL-17A is no longer self-limiting. However, we still do not know the cellular source and targets of the secreted IL-17A in the hippocampus of the animals of this experimental model. Thereby, the aim of this study was to evaluate the effect of chronic exposure to low doses of ozone on the expression of IL-17A and its receptor (IL-17RA) in neurons, microglia, astrocytes and T cells in rat hippocampus.

For this purpose, we used 72 Wistar rats, divided into 6 groups (n = 12): control group (without ozone) and groups exposed to ozone (0.25 ppm, 4 h daily) for 7, 15, 30, 60 and 90 days, respectively. Six subjects from each group were processed to quantify IL-17A by ELISA, and the remaining 6 were processed for immunohistochemistry (against IL-17A or IL-17RA and GAP, Iba-1, NeuN or CD3). The data obtained by ELISA test showed a significant increase in the concentrations of IL-17A in the groups of 7, 15, 30 and 60 days of exposure, compared with the control (P <0.05). Furthermore, they indicate that hippocampal neurons are the cells that showed the greatest immunoreactivity against IL-17A and IL-17RA between 7 and 90 days of ozone exposure.

With these data we conclude that exposure to ozone induces an increase in the expression of IL-17A in hippocampus. Also, data suggest an autocrine secretion of this cytokine by hippocampal neurons of rats under a state of oxidative stress induced by chronic exposure to low doses of ozone. This work was supported by DGAPA IN206691 to H.S-V and IN221417 to S.R-A.

**Autoregulation as a potential pathway of miRNA-host-gene interaction linked to neuropathic pain development**

M. Zeiler¹, M. Kress¹, K. K. Kummer

¹Medical University of Innsbruck, Department of Physiology and Medical Physics/Division of Physiology, Innsbruck, Austria

MicroRNAs (miRNAs) are 22-25nt non-coding RNA fragments that are implicated in miRNA regulation via degradation or translational inhibition. Currently, ~1900 miRNA genes have been experimentally found or predicted by potent computational miRNA prediction algorithms. Potential miRNA:mRNA interactions are identified both by experimental validation and computational prediction. Although the relevance of those miRNAs for different pathologies, including neuropathic pain, is recently emerging, the direct contribution of most miRNAs is currently not understood.

**Question**

The aim of the current study was to identify an underlying autoregulatory mechanism of intragenic miRNAs on host-genes and a potential link to neuropathic pain development.

**Methods**

Custom written Python, and R pipelines were applied to classify miRNAs based on the location in the genome and 4 groups (intragenic, antisense, overlapping, extragenic) were defined based on the location of the miRNAs in respect to protein-coding genes. The Diana Tools microCDS algorithm was used to predict possible miRNA::mRNA interactions and an iterative randomized model (RM) was applied to evaluate the native miRNA::mRNA interaction effect. Small and long RNA sequencing signatures of dorsal root ganglia (DRG) in a mouse model of neuropathic pain were generated and correlation/regression analyses of intragenic miRNA::host gene interactions performed.

**Results**

This analysis revealed significant upregulation of specific miRNAs in DRG and a significant downregulation of target mRNAs, which supports the hypothesis of a potential autoregulatory mechanism of intragenic miRNAs.
Computational analysis of miRNAs genomic location revealed that the majority of miRNAs were located within intronic regions of protein coding genes across species. The microCDS prediction algorithm predicted that ~60% of intragenic miRNAs were located within intronic regions of protein coding genes across species. The sensitized C-fibers of the acutely inflamed joint to innocuous (control 127±28 APs/15s, after 3 hours 14±8 APs/15s) and revealed a number of dysregulated intragenic miRNAs as well as host genes. Significant correlations for intragenic miRNA-host gene interactions were identified.

Conclusions: Correlation analyses of DRG signatures enabled us to identify possible co-expression and autoregulation patterns of intragenic miRNAs: host gene interactions. Together, our analysis indicated underlying autoregulatory mechanisms of intragenic miRNAs to their host genes, which was conserved across species. These might be involved in the development of neuropathic pain and therefore give rise to novel treatment strategies.

B 03-2
Electrophysiological impact of Nav1.7 variant R1150W on induced pluripotent stem cell-derived nociceptors
C. Rüsseler1, V. Sahin1, S. Sontag1, P. Hautvast1, R. Goetzke2, W. Wagner2, N. Üçeyler2, M. Zenke3, A. Lampert3
1RWTH Aachen University, Institute of Physiology, Aachen, Germany
2RWTH Aachen University, Institute for Biomedical Engineering, Stem Cell Biology and Cellular Engineering, Aachen, Germany
3University of Würzburg, Department of Neurology, Würzburg, Germany

About 13% of the Caucasian population harbor the Nav1.7 R1150W polymorphism, which may render the carrier more prone to develop chronic pain. The impact of this variant on nociceptor excitability is still unclear and only a fraction of carriers with r.1150W develops severe pain symptoms during their lifetime, such as small fiber neuropathy (SFN). The onset of SFN can be correlated with a reduction of intraepidermal small nerve fibers in subgroups of SFN patients, which seems contrary to the chronic pain syndrome these patients suffer from. The specific genetic background of the affected patients may play a crucial role and needs further investigation.

We reprogrammed the fibroblasts of a SFN patient and a healthy control carrying the Nav.1.7/R1150W polymorphism into induced pluripotent stem (IPS) cells and differentiated them into nociceptors using an optimized protocol. Furthermore, patient-specific and healthy control cells were co-cultured with mature IPS cells and co-cultured nociceptors to investigate the pathophysiologically relevant of the patient-specific genetic background, we performed patch-clamp analysis and multi-electrode recordings to assess the impact of the genetic variant on cellular excitability of the human nociceptors. We investigated the co-culture behavior by immunofluorescence staining for different marker expression.

In our cultures IPS cell-derived Schwann cells and nociceptors showed interaction and aligned in accordance with formation of a remark bundle-like structure. Patch-clamp recordings revealed an enhanced excitability of patient derived IPS cell-derived nociceptors. These neurons display a reduced firing threshold and other features in accordance with enhanced neuronal excitability. In conclusion, IPS cell-derived nociceptors and Schwann-cells can be co-cultured to study the impact of a pain-linked polymorphism on nociceptor excitability. Our data support the link between the R1150W polymorphism and neuron excitability and could be a factor for increased pain symptoms in the investigated patient.

B 03-3
Mechanonociception in C-fibers but not in Aδ-fibers is controlled by the Transient Receptor Potential vanilloid 4 ion channel in the normal and inflamed joint of the rat
F. Richter, G. Segond von Banchet, H. – G. Schäible
University Hospital Jena, Institute of Physiology 1 / Neurophysiology, Jena, Germany

The Transient Receptor Potential vanilloid 4 ion channel (TRPV4) is an important sensor for osmotic and mechanical stimuli in the musculoskeletal system, and it is also involved in processes of nociception. It has not been studied yet whether this receptor also participates in the mechanonociception of the normal or of the acutely inflamed joint.

Healthy adult Wistar rats were anesthetized with sodium thiopentone (100 mg/kg, i.p.). The knee joint was mechanically stimulated by innocuous (20 mmHg) or noxious (40 mmHg) rotations of the lower leg against the fastened femoral bone for 15 s each. Action potentials were recorded from nerve fibers that were classified as C- or as Aδ-fibers by their conduction velocity (<1.4 m/s or <10 m/s, respectively). The TRPV4 antagonist RN-1734 and the TRPV4 agonists RN-1747, 4αPD, and GSK 1016790A were used. A volume of 0.1 ml each was injected into the joint cleft. Acute joint inflammation was induced by injection of kainic acid into the joint cleft and recordings were performed 7 hours after induction of inflammation. In addition, the expression of TRPV4 receptors was studied on cultured dorsal root ganglion (DRG) neurons by immunohistochemistry.

The intratheatrical injection of RN-1734 at 500 µM into the knee joint reduced the responses of C-fibers of the normal joint to noxious mechanical stimulation (control 214±55 APs/15s, after 3 hours 53±30 APs/15 s, respectively) and the responses of the sensitized C-fibers of the acutely inflamed joint to innocuous (control 127±28 APs/15s, after 3 hours 14±8 APs/15s) and noxious (control 215±30 APs/15s, after 3 hours 74±42 APs/15s) mechanical stimulation. In line with this, the local mechanical thresholds in the receptive fields increased. The responses of nociceptor Aδ-fibers were not significantly altered by RN-1734. The lower dose of 20 µM RN-1734 had similar effects on C-fibers as the higher one. The intratheatrical application of the TRPV4 agonist 4αPD, GSK 1016790A, and RN-1747 did not consistently alter the responses of Aδ- and C-fibers to mechanical stimulation of the joint nor did they induce ongoing activity. Staining for TRPV4 confirmed that a proportion of DRG neurons (mainly small- to medium-sized) expressed the TRPV4 receptor.

We conclude that TRPV4 on channels are involved in the responses of C-fibers to noxious mechanical stimulation of the normal joint, and in the enhanced sensitivity of C-fibers to mechanical stimulation during inflammation of the joint.

B 03-4
Metabotropic mechanosensing in a proprioceptor
S. Dahnt1, T. Langenhahn2, R. J. Kittel3
1Leipzig University, Institute of Biology, Department of Animal Physiology, Leipzig, Germany
2Leipzig University, Carl Ludwig Institute for Physiology, Leipzig, Germany
3Leipzig University, Rudolf Schönherr Institute of Biochemistry, Division of General Biochemistry, Leipzig, Germany

Drosophila melanogaster utilizes specialized organs for the perception of mechanical cues which are essential for survival. These so-called chordotonal organs (CHO) consist of multicellular units (scopulae) with each unit containing one bipolar, monociliated neuron. They are required for sensing tactile, proprioceptive and auditory stimuli. One particular CHO is the lateral pentascolopidial chordotonal organ (lch5), which is located laterally in the body wall of each larval hemisegment and consists of five scopula.

Previous studies showed that CIRL, an adhesion-type G protein-coupled receptor (GPCR), shapes the perception of sensory lch5 neurons on the level of the receptor potential. Within the GPCR superfamily (GPCR) a group compromising 32 members in humans with crucial functions in a variety of physiological processes. Latrophils form a well conserved subfamily of GPCRs and CIRL is the sole latrophil homolog in Drosophila. In this study, electrophysiological recordings of lch5 axon bundles were conducted to further elucidate the interaction between CIRL and the receptor potential generating TRP (transient receptor potential) channels.

Furthermore, we are currently establishing a novel approach to discriminate between individual lch5 neurons in order to unravel possible differences in activation modalities. To this end, MARCM (mosaic analysis with a repressible cell marker) combined with optogenetic inhibition will be used to stochastically silence individual neurons and thereby subtracting their contribution to compound recordings in a reversible manner.

B 03-5
Pain and brain network oscillations in freely moving mice
J. Zhang1, S. Ponsel1, Y. Yanovsky1, M. Piil2, J. Brankov1, A. Draguhn1
1Heidelberg University, Institute of Physiology and Pathophysiology, Heidelberg, Germany
2Heidelberg University, Institute of Medical Biometry and Informatics, Heidelberg, Germany

The assessment of pain mainly depends on either communication with the subject or secondary reactions to painful stimuli. Currently there is no objective way to diagnose pain. Changes of brain network oscillations are often used to search for a signature of pain. Pain was induced in mice by injecting capsaicin into one hind paw and saline injection served as control. Local field potential (LFP) activity was recorded in pain-related brain areas, including anterior cingulate cortex (ACC), primary somatosensory cortex, posterior insula, ventral posterolateral thalamic nucleus, parietal cortex, central nucleus of the amygdala and olfactory bulb during capsaicin-induced tonic pain or after saline injection. Power, cross-frequency coupling (CFC) and coherences between brain regions were calculated for multiple frequency bands. Capsaicin-induced allodynia was verified with von Frey hair tests. In the isilateral ACC CFC between low (1-12 Hz) and fast frequencies (80-120 Hz) increased during capsaicin-induced pain compared to post-saline condition. In addition, several interregional cotemporally increased after capsaicin injections mainly in frequency ranges below 30 Hz. By using the elastic net, a variable selection method, and a logistic regression classifier, pain (capsaicin) could be distinguished from non-pain (saline) when parameters of all three analyzing methods: CFC, power and coherence were used. Furthermore, pain classification was still successful with parameters in frequency bands below 30 Hz, which can be obtained in clinical EEG. These findings in mice may help to find a signature of pain in human EEG.
**Update on PGE2 induced sensitization: PI3Kγ and GRK2 as important tools in EP3 evoked self-control of nociception**

A. Bader, S. K. Shulga, C. C. Kienle, A. Eitner, R. Wetzker, H. G. Schild, L. K. Li, X. Su, Y. Kovalchuk, S. Wang, K. Li, O. Garaschuk

1. Jena University Hospital, Institute of Physiology I/Neurophysiology, Jena, Germany
2. Jena University Hospital, Clinic for Anaesthesiology and Intensive Care, Jena, Germany

Prostaglandin E2 (PGE2) is a proinflammatory mediator and known to sensitize primary nociceptive afferents mainly by activation of the G-protein-coupled receptors EP2 and EP4. Sensitized neurons show decreased excitation thresholds and increased responses to noxious stimuli. However, these neurons also express the G-protein-coupled PGE2 receptor subtype EP3 which counteracts nociception during inflammation. Therefore, EP3 receptor activation could limit the sensitizing effect of PGE2 but how the EP3 receptor interacts with EP2 and EP4 dependent signaling networks is not clarified.

We used the whole cell patch clamp method to analyze TTX-resistant sodium currents of primary sensory neurons (DRGs). PGE2 or agonists to EP2 and EP4 receptors, which stimulate cAMP production, induce a significant increase of these currents. This could be inhibited by coapplication of a specific EP3 agonist. Interestingly, using an ELISA-based cAMP measurement, EP3 activation had obviously no effect on intracellular cAMP levels increased by EP2 and EP4 activation. In Western-Blot analyses of DRG protein lysates, no decrease of either EP2 or EP4 dependent activation of PKA and Erk1/2 by concomitant EP3 activation could be observed. A decrease of cAMP was proofed by inhibitors for adenylate cyclase, PKA, or Erk1/2 which blocked EP2 and EP4 dependent sensitization. Thereby an upstream regulatory function of PKA for Erk1/2 activity can be assumed.

Notably, selective EP3 activation lost its ability to inhibit PGE2 dependent sensitization in DRGs of P2X3-/- mice but was maintained in neurons carrying a kinase-dead version of P2X3. P2X3, activated by G-protein coupled receptors, can activate phosphodiesterases in a kinase-independent manner and thereby providing AMP for AMP-activated Kinase (AMPK) activity. Indeed, an inhibitor of AMPK activity could block the EP3-dependent inhibition of EP2-induced sensitization.

While inhibition of EP2 activity through EP3 is lost in P2X3-/- DRGs, its inhibitory capacity on selective EP4-induced sensitization was still intact. EP4 is known to strongly undergo internalization processes and G-protein subunits could also recruit APRK-coupled receptor kinase 2 (GRK2) to enable II. Inhibition of GRK2 diminished EP3 dependent receptor sensitization of EP4- induced sensitization. Our immunocytochemical analysis of EP4 stimulated DRG cells revealed an accelerated internalization of EP4 during concomitant EP3 receptor stimulation.

**Identification of molecular markers for subpopulations of intrinsically photosensitive retinal ganglion cells**

M. Lindner, P. K. Reardon, M. J. Gilhooley, S. Hughes, M. Hankins

University of Oxford, Oxford, UK

Melanopsin (OPN4) expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) represent a third class of ocular photoreceptors. These inner retina photoreceptors characteristically mediate a range of non-image forming responses to light, including circadian photentrainment, regulation of sleep, and the pupillary light response. Recent evidence additionally indicates a role for ipRGCs in image forming vision. ipRGCs are not a homogenous population of cells but rather are comprised by various subgroups with distinct physiological functions. To-date, ipRGC subgroups are defined by morphological criteria, which are not free of ambiguity. This inhibits the rigorous study of individual subgroups in physiological experiments.

To classify individual ipRGC subpopulations and identify specific molecular markers, we performed an in-depth analysis of the 804 ipRGCs (Opn4-positive) cells contained in a published single-cell RNA-sequencing dataset (Rheaume et al, 2018). Using the k-nearest neighbors classification algorithm we were able to identify six genetically distinct clusters of ipRGCs by a data-driven approach. Subsequently, we identified candidate molecular markers that were not only specific for each ipRGCs cluster but showed high specificity across all classes of non-intrinsically photosensitive retinal ganglion cells. To independently validate these biologically obtained markers, we performed immunostaining on retinal cross sections collected from ipRGC reporter mice. Indeed, immunostaining against the identified markers (e.g. Klf4, Atdlc) labeled distinct subpopulations of ipRGCs, which we could now correlate with the available morphological classification. Most strikingly, amongst the genes selectively expressed in individual clusters as identified by our data-driven approach, several were involved in receptor signaling (e.g. Gna14, Gabrg3) indicative for unique signaling pathways and regulatory mechanisms employed in individual IP3G clusters.

Taken together, the identified markers will facilitate the study of the physiology of the distinct ipRGC subpopulations. Moreover, the present dataset of differentially expressed genes may provide a starting point for studying ipRGC subtype specific signaling processes.
Combined, we expect to gain deep knowledge of the role of HIF-2α during physiological and pathological processes in synaptic transmission.

**B 04-2**

**Hypoxia enhances endothelial ICAM-1 expression through upregulation of arginase-II and mitochondrial oxidative stress**

X. Liang, P. Arullampalam, Z. Yang, X. - F. Ming

University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular System, Fribourg, Switzerland

Hypoxia plays a crucial role in the pathogenesis of cardiovascular diseases. Mitochondrial enzyme arginase type II (Arg-II) is reported to lead to endothelial dysfunction and enhance the expression of endothelial inflammatory adhesion molecules such as intercellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1). In this study, we investigate the role of Arg-II in hypoxia-induced endothelial inflammation and the potential underlying mechanisms. Exposure of the human endothelial cells to hypoxia induced a time-dependent increase in Arg-II, HIF1α, HIF2α, and ICAM-1 expression, whereas no change in the expression of ICAM-1 and E-selectin was observed. Similar effects were obtained in cells treated with a hypoxia mimetic Dimethylisosoyglycine (DMOG). Silencing HIF1α, but not HIF2α, reversed hypoxia-induced upregulation of Arg-II. Moreover, silencing Arg-II prevented the ICAM-1 upregulation induced by hypoxia or DMOG. Furthermore, the endothelial cells treated with DMOG enhanced monocyte adhesion, which was inhibited by silencing Arg-II. Lastly, silencing Arg-II prevented hypoxia-induced mitochondrial superoxide production in endothelial cells, and hypoxia-induced ICAM-1 upregulation was reversed by mitochondrial electron transport inhibitor rotenone. These data demonstrate that hypoxia enhances ICAM-1 expression and monocyte-endothelial interaction through HIF-II mediated upregulation of Arg-II expression leading to increased mitochondrial reactive oxygen species production. These effects of hypoxia on endothelial cells may play a key role in cardiovascular diseases. Our results suggest that Arg-II could be a promising therapeutic target to prevent hypoxia-induced vascular damage/dysfunction.

**B 04-3**

**Application and effects of artificial oxygen carriers in pO2-controlled kidney cell culture**

A. Stokvis1, M. Cantore1, J. Fandrey, K. B. Ferenz2

1University Duisburg-Essen, CeNIDE, Duisburg, Germany
2University of Duisburg-Essen, Institute of Physiology, Essen, Germany

Kidneys donated for transplantation that experience shortage of oxygen often suffer from ischemia and reperfusion injury resulting in delayed graft function or even transplant rejection. Hypoxia-Inducible-Factor (HIF) is normally activated to mediate the cellular adaptation ensuring survival under hypoxic conditions. However, during acute ischemia the HIF system may not be mobilized sufficiently before damaging mechanisms are activated. We hypothesize that the application of nanoscaled albumin-derived artificial oxygen carriers (A-AOCs) will improve the performance of the ischemic kidneys by bridging the vulnerable phase of oxygen deficiency.

To examine the potentially protective effects of A-AOCs in this context, human kidney cell lines were exposed to different hypoxia-reoxygenation protocols and the accumulation of HIF subunits was measured in protein extracts using Western blotting. This way the cellular reaction on application of either A-AOCs or medium alone, both preoxygenated (20% O₂) were compared. In addition, we established a cellular system in which we measured and controlled the percentage O₂-fraction (fO2). The accumulation, translocation and degradation of the HIF subunits in the nucleus was visualized by means of immunofluorescence. Furthermore, we performed cell viability and cytotoxicity assays (e.g. MTT assay) to exclude toxic effects of the A-AOCs.

To examine the potentially protective effects of A-AOCs in this context, human kidney cell lines were exposed to different hypoxia-reoxygenation protocols and the accumulation of HIF subunits was measured in protein extracts using Western blotting. This way the cellular reaction on application of either A-AOCs or medium alone, both preoxygenated (20% O₂) were compared. In addition, we established a cellular system in which we measured and controlled the percentage O₂-fraction (fO2). The accumulation, translocation and degradation of the HIF subunits in the nucleus was visualized by means of immunofluorescence. Furthermore, we performed cell viability and cytotoxicity assays (e.g. MTT assay) to exclude toxic effects of the A-AOCs.

**B 04-4**

**Regulation of Synaptic Plasticity by the Hypoxia-inducible Factor-2 alpha in Hypoxia**

T. Quinting, J. Fandrey, T. Schreiber

University Duisburg-Essen, Institute of Physiology, Essen, Germany

Sufficient oxygen supply is fundamental for normal brain functions and to avoid hypoxia. In acute hypoxia, neuronal cells adapt in different ways to the decreased oxygen supply for protection of neurons including synaptic signaling decrease or changes in excitation and inhibition of neuronal and glial cells. Key factors of the cellular response to low oxygen are the heterodimeric transcription factors “hypoxia-inducible factors” (HIF-1, HIF-2 and HIF-3). HIFs alter the expression of oxygen-related genes and play an important role during brain development and neural regeneration after hypoxic events as it has been shown for HIF-1. However, the exact role of HIF-2 in the brain remains unknown.

As several target genes of HIF-2 are known to have a significant part at the synaptic terminal, this study will investigate the role of HIF-2a (oxygen-sensitive subunit) in synaptic formation and function during normoxia and hypoxia. By using brain-specific HIF-2a knockout mice we showed that synaptic plasticity is highly oxygen-dependent and Hif-2a KO led to altered expression of genes involved in synaptic transmission.

To analyze synaptogenesis in vivo, immunocytochemistry for synaptic markers in brain slices will be performed for wildtype or knockout mice during brain development. Additionally, differences in mature synapses will be analyzed using electron microscopy. We furthermore develop an in vitro neuros sphere system to study changes in expression of synaptogenic factors using qPCR. To investigate the involvement of HIF-2α in specific cell populations, the formation of synaptic contacts and the expression of synaptic regulating molecules will be studied in hippocampal neuron-astrocyte co-cultures. Two further approaches will explore the role of HIF-2α in regulating synaptic transmission. First, experiments will be carried out on acute tissue slices of wildtype or knockout hippocampi to measure field excitatory postsynaptic potentials and long-term potentiation. Additionally, we will conduct optogenetic studies by using our neuron-astrocyte co-culture. Excitatory neurons will be transfected with specific light-sensitive plasmids and co-transfected with a calcium sensor to measure synaptic transmission by Ca²⁺ increase in postsynaptic cells.
Interestingly, although we had previously seen effects of a single knock-out we observed no differences between WT and double Hif-1α KO mice. Our data suggest that additional knockout of Hif-2α might reverse the disease ameliorating effect of myeloid Hif-1α knockout in experimental colitis. Therefore, HIF-2 seems to have antagonistic functions to HIF-1 in stimulated myeloid cells.

Role of hypoxia on lipid signaling
T. Schönberger, U. Glaser, K. Prost-Fingerle, J. Fandrey
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

Question: The transcription factor hypoxia inducible factor-1 (HIF-1) controls and mediates transcriptional adaption in response to low oxygen conditions in multiple diseases. In tumor development and progression activated HIF-1 modulates angiogenesis, adaption of the tumor metabolism to low nutrient and oxygen levels, genetic instability and metastasis. In addition, HIF activation plays an important role for the function of tumor-associated macrophages in hypoxic tumor regions. Furthermore, sphingolipids are known to be oxygen-independent regulators of HIFs and are important cell mediators in tumor and inflammatory hypoxia. An imbalance of the pro-survival signaling metabolite sphingosine-1-phosphate and the pro-apoptotic ceramide within the sphingolipid metabolism can be found in multiple diseases. We aim to investigate the connection between hypoxia and HIF activation on lipid signaling by studying mouse embryonic fibroblasts (MEFs) derived from mice with altered sphingolipid levels. Beyond that, HIF-1α activation also plays an important role for the function and recruitment of macrophages to injured tissue and is upregulated in tumor associated macrophages, promoting tumor angiogenesis and invasiveness. Therefore, we want to investigate the connection between sphingolipid metabolism and HIF-1α accumulation and expression in bone marrow derived macrophages (BMDMs).

Methods: We are studying MEFs and BMDMs from mice with wild type and altered sphingolipid metabolism regarding hypoxic response and sphingolipid levels under normoxic (N2, 21% O2) and hypoxic conditions (H2O, 1% O2). Therefore, we are using Western Blot, immunocytochemistry staining, RNA analysis and mass spectrometry.

Results: Our recent findings indicate a change in the HIF response mechanisms in case of altered sphingosine-1-phosphate levels in MEFs. Further preliminary results indicate an influence of acid ceramidase levels on HIF1α RNA expression and on specific HIF-1α target genes of BMDMs, when exposed to an inflammatory stimulus.

Conclusion: Taken together we want to unravel the connection between HIF activation and the sphingolipid pathway resulting from our findings in differently altered MEFs and macrophages.

Importance of hypoxia-inducible factors (HIF-1 and HIF-2) for the pathophysiology of inflammatory bowel disease
E. Hammel, J. Fandrey, S. Winning
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

400,000 people in Germany are affected by inflammatory bowel diseases (IBD). The most common IBD types, Crohn's disease and ulcerative colitis, are not curable and their causes are mostly unknown. Inflammation is characterized by a lack of oxygen (hypoxic inflammatory hypoxia). The cellular adaptation to hypoxia is regulated by transcription factors called hypoxia-inducible factors (HIF-1 and HIF-2). Currently, the function of HIF-1 in IBD is much better understood than that of HIF-2. Previous work of our group has shown that loss of MEF11I-1α ameliorates dextran sodium sulfate (DSS)-induced colitis in mice. Based on this work, initially we worked with Hif1α+/+ x Hif2α+/+ mice (WT) and Ly2-Cre/Hif1α+/+ x Hif2α+/+ mice (KO). To induce acute inflammation, mice received 2.5% (w/v) DSS (MP Biomedicals, MW 36-50 kDa) in drinking water for six days while control mice received normal drinking water. The course of IBD was recorded by measuring body weight, stool consistency and occult blood in the feces, which are important for the Disease Activity Index (DAI) used as clinical observation parameter. During DSS-treatment the DAI increased on day 4, indicating colitis progress, while control mice remained unaffected. DSS-treated animals also showed a 1.5 fold higher colon weight to length ratio and a 4-fold higher Histology Score characterizing destroyed tissue. Furthermore, treated animals compared to control mice showed a significantly higher mRNA expression of the proinflammatory cytokine interleukin-10 (IL-10). Interestingly, although we had previously seen effects of a single HIF-1α knock-out we observed no differences between WT and double HIF-1α and HIF-2α KO mice. Our data suggest that additional knock-out of HIF-2α might reverse the disease ameliorating effect of myeloid HIF-1α knockout in experimental colitis. Therefore, HIF-2 seems to have antagonistic functions to HIF-1 in stimulated myeloid cells.

Targeted manipulation of nuclear transport processes inhibits hypoxia signaling pathways and 3D spheroid growth of cancer cells
F. Kosy, M. von Fallois, Y. Landesman, R. Depping
University of Lübeck, Institute of Physiology, Lübeck, Germany

3D spheroid growth of cancer cells
Changes in the O2-fraction (F02), M. von Fallois
University of Lübeck, Institute of Physiology, Lübeck, Germany
2University Medical Center Schleswig-Holstein, Clinic for Radiotherapy, Lübeck, Germany
3Karyopharm Therapeutics, Newton, US

Hypoxia interacts with the expression of Nrf2 in rat lung tissue
E. Hammel, S. Oter1, A. Korkmaz2
1University of Ulm, Institute of General Physiology, Ulm, Germany
2Gülhane Military Medical Academy, Department of Biophysics, Ankara, Turkey
3Gülhane Military Medical Academy, Department of Biophysics, Ankara, Turkey
4University of Health Sciences Medical School, Department of Undersea and Hyperbaric Medicine, Ankara, Turkey
5University of Health Sciences Medical School, Department of Physiology, Ankara, Turkey

Introduction: The interaction of hyperbaric oxygen (HBO) exposures with oxidant/antioxidant pathways is long been of particular interest. The hypoxia-inducible factor-1alpha (HIF-1α) and the nuclear factor erythroid 2-related factor 2 (Nrf2) are two important transcription factors that target proteins playing role in these pathways. In this experimental study, the potential interaction of normobaric and hyperbaric hypoxic exposures with these transcription factors was tested in rat lung tissue.

Methods: A total of 45 male Sprague-Dawley rats were equally divided into 5 groups: one unexposed group for determining control values, two normobaric and two hyperbaric oxygen (%100) exposure groups. Normobaric (NBO) and HBO was administered at two different modalities as 5 and 10 consecutive days, once daily; exposure time was 90 minutes and clinical observation parameter. During HBO-treatment the DAI increased on day 4, indicating colitis progress, while control mice remained unaffected. DSS-treated animals also showed a 1.5 fold higher colon weight to length ratio and a 4-fold higher Histology Score characterizing destroyed tissue. Furthermore, treated animals compared to control mice showed a significantly higher mRNA expression of the proinflammatory cytokine interleukin-10 (IL-10). Interestingly, although we had previously seen effects of a single HIF-1α knock-out we observed no differences between WT and double HIF-1α and HIF-2α KO mice. Our data suggest that additional knock-out of HIF-2α might reverse the disease ameliorating effect of myeloid HIF-1α knockout in experimental colitis. Therefore, HIF-2 seems to have antagonistic functions to HIF-1 in stimulated myeloid cells.
Results: Lung tissue MDA, GPx and HIF-1α resulted at comparable values. SOD tended to increase in both HBO groups but mice were inhaled with Sph-loaded oxygen carriers 30 minutes after infection or left untreated, sacrificed 4 h after infection, lungs were homogenized, lysed and colony-forming units in the lungs were determined. Interestingly, Nrf2 expression was found to be significantly decreased (P = 0.006) in lungs were homogenized, lysed and colony-forming units in the lungs were determined.

Conclusion: The increased expression of the redox-sensitive transcription factor Nrf2 with longer hyperoxic exposures up to 100% in a dose-dependent manner. Most importantly, inhalation of Sph-loaded oxygen carriers reduced bacterial load in mice lung infected with P. aeruginosa. These results demonstrate that Sph-loaded oxygen carriers can be used successfully for treating bacterial pulmonary infections of, for instance, cystic fibrosis mice.

B 04-9

Interactions of continuous and intermittent hyperbaric oxygen treatments with oxidative and inflammatory indices in rat lung tissue

S. Sadi,1 K. Simsek,1 K. Ates,1 M. Özler,1 B. Uysal,2 T. Topala,2 A. Korkmaz2,3 S. Öter4
1University of Ulm, Institute of General Physiology, Ulm, Germany
2Gülhane Military Medical Academy, Department of Physiology, Ankara, Turkey
3Gülhane Military Medical Academy, Department of Biophysics, Ankara, Turkey
4University of Health Sciences Medical School, Department of Undersea and Hyperbaric Medicine, Ankara, Turkey

Introduction: Hyperbaric oxygen (HBO) administrations are known to interact with oxidant/antioxidant and inflammatory pathways of living organisms. To avoid oxygen toxicity, HBO is generally administered intermittently with short air respiration intervals at normoxic every half an hour. In this experimental study, the effect of continuous HBO exposure on rat lung oxidant/antioxidant and inflammatory indices was compared with a wide range of intermittent HBO exposure modalities.

Methods: A total of 56 male Sprague-Dawley rats were equally divided into 7 groups. One group was let unexposed for determining control values. The continuous HBO exposure group was set at 2.5 ATA for 2 hours. All of the 5 other groups received HBO at the same pressure and for a total of 2 hours, but 15 min air breathing breaks were set at different intervals: so, HBO was administered for 60, 40-40-40, 30-30-30, 20-40-40 or 60-40-40 min periods. Two hours after HBO exposure the animals were sacrificed and their lungs were harvested for malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) measurements.

Results: Lung tissue TNF-α and IL-1β levels tended to decrease with HBO exposure but reached only significantly high values in the 60-40-40 and 20-40-40 min groups when compared with controls (P = 0.012 for both, and P = 0.039 and 0.003, respectively). Among HBO exposure groups, the inflammatory cytokine levels resulted at comparable values. MDA levels were mainly found to be decreased in almost all HBO groups with the most pronounced level in the continuous exposure groups (P < 0.05 vs. control); these levels were also found to be significantly lower than those in the 40-40-40 and 20-40-40-30-30 min exposure groups (P = 0.05 and 0.028, respectively). Although not significant (P = 0.153), the continuous HBO group also reflected a slight increase of SOD activity which could explain the fall of MDA values. GPX activities also tended to decrease with HBO exposure at different rates.

Conclusion: With regard to the interactions of HBO exposure with oxidant/antioxidant and inflammatory parameters, intermittent or continuous procedures seem not to be of advantage or disadvantage compared to each other.

B 04-10

Development of sphingosine-coupled oxygen carriers as novel drugs for treating pneumonia

K. A. Becker1, J. Jäger1, J. Fandrey1, E. Gubinis1, K. E. Ferenczi2
1University of Duisburg-Essen, University Hospital Essen, Institute for Molecular Biology, Essen, Germany
2University of Duisburg-Essen, University Hospital Essen, Institute for Medicine, Essen, Germany

Pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa often form biofilms that, together with mucus, dead epithelial cells, and neutrophils with concomitant release of DNA, result in hypoxic or anaerobic areas in the infected lungs. Pathogens embedded in hypoxic biofilms are protected from the immune system and from antibiotics. The bioactive lipid sphingosine (Sph) is able to kill various bacteria in vitro, including bacteria grown in biofilms. We aim to use the dual function of Sph as (i) an oxygen carrier (ii) a pro-apoptotic agent. Oxygen carriers are synthesized and loaded with 10 mM Sph. Particle size was controlled using dynamic light scattering while Sph-loading was checked with a Sph-kinase assay investigating the concentration of free Sph in the Sph-loaded oxygen carriers’ supernatant directly after loading. S. aureus were incubated at 37°C for one hour in (i) buffer alone, (ii) with addition of 10 µM Sph or with different amounts of (iii) Sph-loaded oxygen carriers or (iv) control oxygen carriers without coupled sphingosine. To determine bacterial survival, aliquots were plated on agar plates. Furthermore, cystic fibrosis mice were infected intranasally with 10^9 Sph-aeruginosa 762.

Poster Session B | 173
with A-AOCs stayed at normal pressure. Up to 30 min after surfacing animals were supervised for clinical symptoms. Activity demonstrated by a strong increase in right ventricular systolic pressure (RVSP (WT): 30.0 mmHg vs (beta arr1-/-): 35.0 mmHg, p<0.001) and right heart hypertrophy (Fulton index (WT): 27.5 % vs beta arr1-/-: 32.3 %, p<0.05).

Our results demonstrate that the absence of beta arr1 causes an impaired function of the sGC in PASMC and strongly promotes the development of Hx-induced PH.

B 05-2
The Qq protein inhibitor FR900359 is a strong pulmonary vasorelaxant ex and in vivo
A. Seidinger1, A. Böhringer1, G. König1, E. Koseki2, B. Fleischmann1, D. Wenzel1
1University of Bonn, Institute of Physiology, Bonn, Germany
2University of Bonn, Institute for Pharmaceutical Biology, Bonn, Germany

Gq proteins are key regulators of vascular tone in the lung as various agonists induce pulmonary vasoconstriction via Gq activation in health and disease. Previously, we have identified the depsipeptide FR900359 (FR) as a strong and specific pharmacological pan-Gq inhibitor. Here, the effect of FR on pulmonary arterial tone regulation in mouse ex and in vivo is shown.

To examine the effect of FR on pulmonary vascular tone regulation ex vivo we applied isometric force measurements of murine pulmonary arteries in a wire-myograph, precision cut lung slices and the isolated perfused lung model of mouse. The effect of FR on pulmonary blood pressure in vivo was determined after intrastrate application of FR (2.5 µg per mouse) with subsequent serotonin injection (3 ml, iv) by a 1 F Miller catheter.

Isometric force measurements revealed that a single dose of FR (1 µM) induces a strong vasorelaxation in pulmonary arteries after pre-constriction with several Qq-dependent agonists. While FR was able to fully reverse serotonin pre-constriction (0.5 µM, 79.2 ± 2.5 % relaxation), endothelin (ET, 3 nM) and UA6819 (U, 0.1 µM)-dependent constrictions were only reduced by 71.8 ± 1.2 % and 52.2 ± 3.0 %, respectively. However, a combination of FR (1 µM) and the Rho kinase inhibitor Y-27632 (10 µM) completely relaxed the vessels indicating that ET and U act via Gq and G12/13 proteins. We also compared the vasoalatory effect of FR with that of drugs currently used in human pulmonary hypertension (PH) and found that FR was superior (74 ± 2 % (n=7)) at 10 µM FR to 8.8 ± 2.9 %, n=5 (bosentan), 27.5 ± 3.9 %, n=7 (ipratrop, 34.6 ± 3.5 %, n=6 (sildenafil), all p<0.001 vs FR. Apart from the prominent vasoalatory effect in pulmonary arteries we can also show that FR is a powerful relaxant of smaller intrapulmonary arteries as found in lung slices (93 ± 1.2 %, n=7) and the isolated perfused lung model of mouse. Right ventricular catheter measurements demonstrated that FR also reduces a serotonin induced blood pressure increase by 65 % compared to controls in vivo.

Our data illustrate that the pharmacological Qq inhibitor FR is a strong pulmonary vasorelaxant in mice ex and in vivo. The vasorelaxant effect is superior to that of currently used drugs for PH. Additionally, FR is a powerful tool to distinguish Qq-dependent and Qq-independent signaling pathways, which enables to uncover GPCR coupling.

B 05-3
Pharmacological Gq protein inhibition reduces airway hyperresponsiveness and airway remodeling in chronic asthma
J. M. Dietrich1, 2, A. Simon1, M. Mattey1, G. König1, B. K. Fleischmann1, D. Wenzel1
1University of Bonn, Institute of Physiology, Bonn, Germany
2University of Bonn, Research Training Group 1873, Bonn, Germany

Gp proteins play a major role in the regulation of airway tone and are hence interesting targets for the treatment of lung diseases, e.g. asthma. Asthma is characterized by airway hyperresponsiveness, inflammation and remodeling. The degree of airway remodeling reflects the severity of the disease but there are no specific drugs against remodeling available. Therefore, in the current project we examined the effect of the pharmacological pan-Gq inhibitor FR900359 (FR) on asthma, in particular airway remodeling.

We have assessed the effect of FR in a mouse model of chronic ovalbumin (OVA)-induced asthma. For this, Balb/c mice were sensitized with i.p. injections of 20 µg OVA/2 mg Alum on day 0 and 14 and mice were challenged with 1% OVA three days a week for further 3 weeks. FR (5 µg/d) or vehicle were applied via the intratracheal route on OVA challenge. Airway hyperresponsiveness was determined with a Flexivent system, inflammatory cells were quantified in the bronchoalveolar lavage (BAL) and lungs were processed for histology. To examine in vitro effects of FR, a cell growth assay using human lung fibroblasts (HFL-1) was used. After a starving phase of 48 hours, the effect of FR (1 µM) was tested on basal cell growth and after stimulation with growth factors e.g. TGFβ (5 ng/ml) and thrombin (1 U/ml).

Local pulmonary application of FR reduced airway hyperresponsiveness in OVA mice (5.6±4.9 cmH2O/15 mL n=9 (FR) vs 9.2±5.9 cmH2O/15 mL n=6 (vehicle), p<0.001). There was no effect of FR on inflammation as leukocyte counts and in particular the number of eosinophils in BAL were unchanged. Histological investigation of the lung revealed that FR treatment decreased collagen deposition (3.1±0.2 µm2/µm perimeter basal membrane (pbm), n=10 (FR) vs 3.7±0.2 µm2/µm
pbm (vehicle) in asthmatic lungs and reduced goblet cell hyperplasia (0.05±0.01 PAS+ cells/μm pbm, n=11 (FR) vs 1.0±0.0 (vehicle), p<0.05). FB was able to reduce basal growth of collagen producing HFL1 cells (0.8±0.0 relative cell number, n=4) vs 1.0±0.0 (vehicle), p<0.05) in vitro. This was also found upon induction with growth factors (1.4±0.1 relative cell number (FR) vs 2.3±0.2 (vehicle), n=4, p<0.05). These results demonstrate that the pharmacological Gq inhibitor FR reduces airway hypersensitivity as well as airway remodeling in the OVA-dependent chronic asthma mouse model. Interestingly, there is no effect on lung inflammation.

B 05-4 Genetic ablation of the newly identified androglobin leads to primary ciliary dyskinesia A. Keppler, S. Santambrogio, P. Engelke, K. Steiner, D. Hoogewijs

University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular systems, Fribourg, Fribourg, Switzerland

The author has objected to a publication of the abstract.

B 05-5 TGF-β1 disturbs airway epithelia integrity and decreases claudin 3 in tight junctions via activating SMAD 2/3 proteins.

C. Schillig, B. Loichbaum, M. Frick, P. Dietl, O. Wittkeindt

Ulm University, Institute of General Physiology, Ulm, Germany

Pulmonary epithelia form a barrier that separates the air-filled compartment of the airways from the organism. Tight junction (TJ) proteins determine the epithelial barrier function and limit paracellular transport. Their functional properties depend on their claudin composition and hence TJ's claudin content and density is crucial for lung function and must be tightly controlled. During inflammatory lung diseases, lung epithelia are exposed to multiple inflammatory factors such as Transforming Growth Factor beta 1 (TGF-β1). However, its effect on lung epithelial barrier function is poorly elaborated. Herein we investigated the effects of TGF-β1 on TJ in lung epithelia. Human bronchial epithelial cells (hBEpC) were cultivated under air-liquid conditions (ALI). To elaborate TGF-β1 effects on differentiated epithelia, hBEpC were cultivated under ALI control conditions until d18 and subsequently cultivated with either TGF-β1 or ligands that activate the TGF-β pathway. Epithelial permeability was quantified as the transepithelial electrical resistance (TER) and the apparent permeability coefficient (Papp) for sodium fluorescein and fluorescein labeled dextrans (molecular weight, 4 kDa and 20 kDa). Immunofluorescence (IF) experiments were performed to elaborate cellular protein localization of TJs. Only TGF-β1 but neither Activin A nor bone morphogenetic factor 2 decreases TEER of hBEpC epithelia. The with an IC50 for TGF-β1 was 0.3 ng/ml (IC50 coefficient n=4). TGF-β1 increased Papp of Na+fluorescein, 4 kDa and 20 kDa fluorescein labeled dextrans for Na+ (n=4). Inactivation of TGF-β1 signal transduction by Inhibitor of TGF-β1 kinase activator-like kinase 5 (ALK5) ASB-01 attenuated TGF-β1 effect on TEER. Time course experiments revealed that TEER declines from day 4 onwards after TGF-β1 exposure. IF experiments detected that the drop in TEER was accompanied by nuclear accumulation of phosphorylated SMAD2, reduced protein abundance of claudin 3 (cldn3) at the tight junctions and a nuclear accumulation of cldn3. Our results give evidence that TGF-β1 disturbs airway epithelial integrity. This TGF-β1 mediated process involves activation of the SMAD2/3 pathway, which results in a reduction of cldn3 at apical tight junctions and a concomitant accumulation in the nuclei.

Supported by: Ministry of Science, Research and the Arts of Baden-Württemberg (Az: 32-7533-6-10/15/5) and the DFG Pulmosens GRK 2203 to O. H. Wittkeindt.

B 05-6 Mechanical strain induces intracellular calcium signalling in alveolar epithelial type I cells in a caveolin-1 dependent manner

K. Diem, G. Fois, P. Dietl, M. Frick

Ulm University, Institute of General Physiology, Ulm, Germany

Secretion of pulmonary surfactant from alveolar type II (ATII) epithelial cells is essential to maintain lung function. Stretching of the alveoli during lung inflation is the main trigger for surfactant secretion. Yet, the precise mechanisms how mechanical distortion of the alveolus results in surfactant secretion are still elusive. Within this study we investigated whether alveolar type I (ATI) epithelial cells represent the alveolar mechanosensor. ATI cells cover >90% of the alveolar surface area and paracrine signals from ATI cells can stimulate surfactant secretion from ATII cells. Moreover, only ATI, but not ATII cells express caveolae, small plasma membrane invaginations, that function as membrane buffers and may play a role as mechanosensors. To investigate the role of caveolae for the cellular response of ATI cells to mechanical stress, we generated human caveolae-1 knockout (Cav1 KO) ATI cells using CRISPR/Cas9. Cells were cultured on flexible substrates (PDMS membranes) in mono- or co-culture with primary rat ATI cells and were stretched intermittently with increasing amplitudes to a maximum of 75% longitudinal distortion. ATI cells responded to increasing stretch amplitudes with a rise in intracellular calcium (Ca2+), whereby at ATI cells responded more frequently (27.4 % responders) and at lower thresholds (20 % stretch) to mechanical stress than Cav1 KO cells (12.4 % responders, 40 % stretch). This suggests that caveolae serve as a hub for downstream Ca-signalling in response to mechanical deformation of ATI cells. Subsequent experiments in Ca2+-free bath solution revealed that the rise in Ca2+ entry mechanism, likely via pannexin hemichannels.

Our data support the hypothesis that caveolae within ATI cells are potential mechanosensors. Our findings indicate that caveolae induce downstream Ca2+-signalling in response to mechanical deformation of ATI cells. Furthermore, co-culture with ATI cells increased the response of ATI cells (Ca2+) to mechanical stretch, adding to the insight that communication between ATI and ATII cells facilitates surfactant secretion.

B 05-7 Interleukin-13 increases Lipopolysaccharide sensitivity of human airway epithelia

H. Schmidt, C. Schillig, O. H. Wittkeindt, P. Dietl

Ulm University, Institute of General Physiology, Ulm, Germany

The airway epithelium forms a barrier that protects the organism against airborne pathogens. It is covered by an apical liquid layer, the airway surface liquid (ASL). ASL volume homeostasis is crucial for normal lung function and requires transepithelial ion and water transport. Chronic inflammation in lung diseases as Asthma, COPD or Cystic Fibrosis has been shown to damage the airway epithelium and compromise its protective function against pathogens. Infectious exacerbations are a frequent and potential lethal complication in these diseases. The mechanisms that account for increased susceptibility to infections are not completely understood. In this study, we investigated the effect of chronic exposure to the T2 cytokine Interleukin-13 (IL-13) on pathogen mediated effects on fluid transport across airway epithelia. Primary human bronchial epithelial cells were cultivated as air-liquid interface and investigated for transcription levels of pathways involved in infection recognition. Chronic exposure to IL-13 resulted in significant up-regulation of TLR-like receptors, TLR4 expression. Inhibition of the JAK/STAT 6 pathway signifying abolished TLR4-regulation. RT-PCR screening for genes related to transepithelial water and ion transport revealed downregulation of the alpha, beta and gamma ENaC subunits, up-regulation of CFTR as well as reduced Aquaporin (AQP) 3 and 5 expression levels. Exposure to the TLR4 ligand LPS only slightly reduced beta ENaC subunit as well as AQP3 and 5 expression and slightly increased CFTR expression. Inhibition of the newly identified androglobin leads to primary ciliary dyskinesia

B 05-8 A novel pulmonary fibroblast model to study the pathophysiology of idiopathic pulmonary fibrosis

J. K. Nemeth, P. Dietl, M. Frick

Ulm University, Institute of General Physiology, Ulm, Germany

Idiopathic pulmonary fibrosis (IPF) is a fatal disease of the lower respiratory tract characterized by aberrant fibroblast activation and progressive fibrotic remodelling of the lungs. Chronic or repetitive injury of the distal lung leads to activation and proliferation of "quiescent" fibroblasts and their differentiation into myofibroblasts. This is accompanied by excessive extraacellular matrix (ECM) formation in the lung interstitium, resulting in tissue scarring and loss of lung function, ultimately leading to respiratory failure. However, the exact pathological mechanisms of IPF remain unknown. To a large extent, this results from the lack of suitable models to mimic the onset and progression of IPF. It is our aim to establish a representative in vitro model for investigating pathophysiological mechanisms of IPF. To this end, we generated a novel pulmonary fibroblast cell line (10-4A) and characterized its suitability for fibrosis studies.
Fibroblast-to-myofibroblast transformation is dependent on biochemical cues (e.g. TGF-β1), mechanical properties of the cell substrate and ECM components. Therefore we investigated the influence of varying substrate stiffness (9 kPa to 2 GPa), TGF-β1 (5 ng/ml) and various ECM components to induce a myofibroblast phenotype in 10-4A cells. We analyzed the expression of specific markers for myofibroblasts (COL1A1, ACTA2), lipofibroblasts (PLIN2) as well as a general mesenchymal cell marker (VIM) on the transcript (qRT-PCR) and protein (immunocytochemistry, western blot) level over a 2 weeks time-course. We compared the results to the response of primary isolated fibroblasts. Our results indicate that 10-4A cells show a response comparable to primary fibroblasts and are a suitable model for studying fibrotic changes underlying the pathophysiology of IFP. In addition, 10-4A cells allow for selective genetic editing to specifically address signaling pathways in IFP development or induced reporter gene expression for high throughput screens.

P2X4 is activated in an autocrine fashion upon exocytosis by ATP stored in lamellar bodies G. Fötz1, V. Winkelmann1, L. Bareis1, L. Staudenmaier1, E. Hecht1, C. Ziller1, K. Ehinger1, J. Schymeinski1, C. Kranz1, M. Frick1 1University of Ulm, Institute of General Physiology, Ulm, Germany 2University of Ulm, Institute of Analytical and Bioanalytical Chemistry, Ulm, Germany 3Boehringer Ingelheim Pharma GmbH & Co. KG, Immunology and Respiratory Research, Biberach an der Riß, Germany

Vesicular P2X receptors are expressed on the limiting membrane of lamellar bodies (LBs), large secretory lysosomes storing lung surfactant in Alveolar type II pneumocytes. Upon exocytosis ATP dependent activation of P2X receptors results in a local, fusion-activated cation entry (FACE) facilitating fusion pore dilation, surfactant secretion and fluid resorption from alveoli. Despite ATP importance in the alveoli, and hence lung function, its origins in the alveoli are still elusive. Within this study we demonstrate that ATP is present in LBs themselves at a concentration of ~1.9 mM. P2X4 receptors are expressed on intraluminal alveolar epithelial cells. However, the rise in intravascular pH upon opening of the fusion pore results in receptor activation by vesicular ATP. We also provide evidence that ATP is stored in LBs by transport via vesicular nucleotide transporter (VNTU). In summary, our data demonstrate that agonist (ATP) and receptor (P2X4) are located in the same intracellular compartment (LB), protected from premature degradation (ATP) and activation (P2X4) but ideally suited to result in most efficient receptor activation to induce FACE upon LB exocytosis.

Establishing a Co-Culture model of pulmonary fibrosis J. K. Nemeth, A. Schundner, P. Dietl, M. Frick 1University, Institute of General Physiology, Ulm, Germany

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease with poor prognosis and survival. Chronic organ injury disturbs normal wound healing in the distal lung and results in organ fibrosis and ultimately lung failure. Under healthy conditions, alveolar type II (ATII) cells are able to repair damage of the alveolar epithelial barrier, whereas chronic insults lead to progressive fibrosis. Alveolar type II cells, disturbed epithelial/mesenchymal homeostasis, proliferation and differentiation of lung fibroblasts into myofibroblasts and excessive extracellular matrix (ECM) deposition. Molecular and cellular mechanisms and initial triggers of disturbed epithelial/mesenchymal interactions are only sparsely understood. Amongst these are the well-examined activation of TGF-β and/or changes in the biophysical properties of the ECM. To investigate the pathophysiology of fibrosis with high resolution we established an in vitro model resembling epithelial/mesenchymal interactions. Co-cultures of primary rat lung fibroblasts and ATII-cells grown on opposing sites of a 0.4 μm Transwell filter were maintained at air-liquid conditions. Preliminary data confirm that our model is able to recapitulate the effects of TGF-β1 and/or changes in the ECM stiffness (5 kPa vs 2-4 GPa) on fibroblast activation and differentiation. The model also reveals temporal and functional differences of the effects of TGF-β1 and changes in the ECM on myofibroblast differentiation. Direct co-culture of ATII cells and fibroblasts also reveals an increased proliferation rate but no differentiation of mesenchymal cells, indicating an opportunity to evaluate early stage epithelial/mesenchymal communication after injury. The next step will be to further delineate the interplay between ECM, epithelial cells and fibroblasts, in particular to differentiate the effects on/from epithelial cells or fibroblasts, respectively. Additionally, we will use a newly developed lung on a chip system (Alveolix AG, Bern, Switzerland) mimicking breathing motion and providing an in vivo like environment. Our model will not only be helpful to obtain a better understanding of pathological processes in disease but also provide a useful screening/research tool for drug development.

Secretion of pulmonary surfactant from alveolar type II cells depends on dynamin 2 E. Wirsching1, M. Poppi1, T. Feidler1, N. Hobit1, P. Dietl1, M. Frick1 1Ulm University, Institute of General Physiology, Ulm, Germany

Secretion of pulmonary surfactant from alveolar type II cells is essential for normal lung function. Surfactant, a mixture of proteins and phospholipids, is stored in alveolar type II (ATII) cells and released into the alveolar lumen by exocytosis. Regulated secretion via exocytosis entails a sequence of highly regulated steps leading to fusion of secretory vesicles with the plasma membrane (PM), opening of a fusion pore and finally content release. Within this study, we found that LB exocytosis depends on dynamin 2 (Dnm2), a large GTPase best known for its prominent role in endocytosis, but not exocytosis. To delineate the function of Dnm2 for LB exocytosis, we infected primary ATII cells with adenoviruses expressing either wt or mutant Dnm2. Mutants targeted the different functional domains of Dnm2, including Dnm2-K44A, Dnm2-T61D, Dnm2-T65A and Dnm2-T141A (point mutations in the GTPase domain). Dnm2-K535A (point mutations in the plekstrin homology domain), Dnm2-K685A, Dnm2-R724A (point mutations in the GTPase activating domain) and Dnm2-DPRD (removed prolin rich domain). We used high-resolution life cell imaging to identify the impact of wt and mutant Dnm2 on LB exocytosis. Specifically we analyzed differences in the kinetics of LB fusion with the plasma membrane following stimulation with 100 µM ATP and 300 nM PMA, as well as kinetics of fusion pore opening. Our results demonstrate that LB exocytosis depends on the expression of a functional GTPase domain of Dnm2.

Cystic fibrosis transmembrane conductance regulator (CFTR) loss as a new mechanism of edema formation and therapeutic target in lung infection L. Erfling1, L. Zou1,2, B. Gutbier1, B. Schneider1, K. Reppe1, J. Lienau2, A. Hocke2, W. Liedtke2, M. Witzenrath2, W. M. Kuebler1 1Charité - Universitaetsmedizin Berlin, Institute of Physiology, Berlin, Germany 2Charité - Universitaetsmedizin Berlin, Institute of Infectious Diseases and Pulmonary Medicine, Berlin, Germany 3German Heart Center Berlin, Berlin, Germany 4Duke University, Department of Medicine, Neurology, and Neurobiology, Durham, US

Rationale: Infection as in sepsis or pneumonia is the most common cause of the acute respiratory distress syndrome (ARDS), a potentially fatal lung disease characterized by hyperinflammation and loss of endothelial barrier function. Infectious and inflammatory stimuli can cause rapid downregulation of the Cl channel cystic fibrosis transmembrane conductance regulator (CFTR) from the cell surface, and inhibition of CFTR was found to increase lung endothelial permeability in vitro. We hypothesized that loss of CFTR may present an important pathomechanism in endothelial barrier failure such as in pneumonia-induced ARDS, and aimed to elucidate the molecular signaling mechanisms underlying this effect.

Methods and Results: CFTR expression is downregulated in human and murine lung tissue following Streptococcus pneumoniae infection. Weight gain measurements and real time fluorescence imaging in isolated perfused lungs revealed that CFTR inhibition increases endothelial permeability in parallel with intracellular Ca2+ concentration ([Ca2+]i). Inhibition of the Cl sensitive with no lysine kinase 1 (WNK1) by tyrphostin or WNK463 replicated the effect of CFTR inhibition on endothelial permeability, while WNK1 activation by temozolamide blocked the effects of CFTR inhibition. In line with endothelial leak, inhibition of CFTR also increased endothelial Ca2+ ([Ca2+]i) transients and permeability in response to inhibition of either CFTR or WNK1 were equally prevented by inhibition of transient receptor potential vanilloid 4 (TRPV4), an endothelial Ca2+ channel negatively regulated by WNK1. Consistently, mice deficient in TRPV4 (Trpv4-) developed less lung edema and protein leak as compared to their wild type controls following infection with S. pneumoniae.

Conclusion: Lung infection with e.g. S. pneumoniae causes rapid loss of CFTR that promotes lung edema formation via intracellular Ca2+ accumulation, inhibition of Cl-sensitive WNK1 and subsequent disinhibition of TRPV4, ultimately resulting in edothelial Ca2+ influx and vascular barrier failure.
Regulation of TALK-2 Two-pore domain potassium channels by intracellular pH, membrane lipids and pharmacological compounds

E. Riel, B. Jürs, J. Langer, M. Schewe, T. Baukrowitz
Christian-Albrechts University of Kiel, Institute of Physiology, Kiel, Germany

Two-pore domain potassium (K₂P) channels play an important role in cellular electrical excitability and are regulated by divers physiological stimuli including voltage, temperature, lipids, protons and certain permeant ions (e.g. Rb⁺ and Cs⁺), as well as pharmacological compounds.

The TALK-2 K₂P channel expressed in the brain, endocrine pancreas and heart has been shown to be activated by extracellular alkalinisation and nitric oxide. Even though, they represent a potential pharmacological target for the treatment of severe diseases such as atrial and ventricular arrhythmias their gating mechanisms are widely unexplored.

Here, we present novel pharmacological activators like 2-APB, Ordofine, DCP1B and three other Negative Charged Activators (NCAs). Furthermore, we show that TALK-2 channels are not only regulated by extracellular pH, but also by intracellular pH alkalinisation. To explore the impact of physiological stimuli on the channel, we studied their regulation by membrane lipids such as LC-CoA and PIPs. In contrast to other K₂P channels (e.g. TREK-1) that were activated by both LC-CoA and PIPs, we observed that the TALK-2 K₂P channel was activated robustly by LC-CoA, but showed only a slight activation by PIPs.

In the observations described above, we discovered another interesting difference of the TALK-2 channel compared to the remaining members of this family. In K₂P channels all stimuli are thought to finally converge at the selective filter representing the principal gate in K₂P channels. The accessibility of the channel pore can be probed by testing for the state dependence of inhibition by pore blockers e.g. quaternary ammonium (QA) ions or the modification of cysteine in the pore using MTSET-reactants. Unlike all other K₂P channels, we report here that the non-activated TALK-2 channels are insensitive to inhibition by the large QA ion Tetraethylammonium (TMA⁺). However, TPA sensitivity increased when the channel was activated by 2-APB but not when activated by the permeant ion Rb⁺. Suitable observations were made testing for TPA-TEA modulation of an introduced pore cystein. However, the inhibition by smaller QA ions was similar with and without PKC activating towards a structural constriction at the pore entrance of the channel.

These findings identify a unique structural gating behaviour within the K₂P channel family, raising interesting questions towards further gating mechanisms and structural diversity in K₂P channels.

ATP-dependent potassium channels modulate synaptic as well as intrinsic properties of GABAergic interneurons in the hippocampal area CA1

M. - E. Burkart, J. Ellers, K. Lippmann
Leipzig University, Carl-Ludwig Institute for Physiology, Faculty of Medicine, Leipzig, Germany

ATP-dependent potassium channels (K₂P channels) act as an essential mediator between cellular metabolism and electrical activity by coupling K⁺ membrane conductance to intracellular ATP levels. In pancreatic β-cells rising levels of intracellular ATP lead to closure of K₂P channels, membrane depolarisation and to exocytosis of insulin-filled vesicles. Gain-of-function mutations of KCa2.2 channels cause impaired release of insulin and, in severe cases, cognitive dysfunction and epilepsy, i.e. DEND syndrome (Delay and Epilepsy with Neonatal Diabetes). While the development of diabetes in this syndrome is well understood, the pathophysiology of the neurological symptoms remains to be elucidated. We have shown that epilepsy can be caused by a dysfunction of hippocampal GABAergic interneurons resulting in over-excitation of pyramidal cells. Particularly parvalbumin-expressing (PV⁺) fast-spiking interneurons play a pivotal role in epileptogenesis and cognitive comorbidities. Since predominantly interneurons express KCa2.2 channels we hypothesise that these channels are key players not only for the diabetes but also for the neurological symptoms of the DEND syndrome.

To determine the influence of K₂P channels on GABAergic inhibition we performed patch-clamp recordings of either pyramidal cells or PV⁺ interneurons in acute hippocampal slices of mice. We modulated the channel activity by bath-applying either the KCa2.2 channel opener diazoxide (300 µM) to mimic the gain-of-function mutations underlying DEND syndrome or the K⁺ channel blocker tetraethylammonium (500 µM) to test its therapeutic potential.

Measurements of miniature inhibitory post-synaptic currents (mIPSCs) in pyramidal cells reveal that activation of KCa2.2 channels by diazoxide diminishes spontaneous release of GABAergic vesicles (Fig. 1 A-C). However, closing the channels with tetraethylammonium reversed this effect. In contrast, tetraethylammonium itself only slightly increases mIPSCs release, indicating that the channels are mainly closed under resting conditions (Fig. 1 D). When recording from PV⁺ interneurons, we found that diazoxide decreases the membrane resistance and hyperpolarizes the membrane towards the K⁺ equilibrium potential. Thus, our data provide clear evidence that KCa2.2 channels modulate synaptic as well as intrinsic properties of GABAergic cells in the hippocampus.

Proteasomal degradation of KCa2.2 channels is involved in emergence of acute epileptiform activity.

S. Müller, V. Sudmann, X. Guli, T. Kirschstein, R. Köhling
Rostock University Medical Center, Oscar Langendorff Institute of Physiology, Rostock, Germany

Voltage-independent, Ca²⁺-activated K⁺ channels (KCa2.2) are powerful regulators of cellular excitability by generating an afterhyperpolarizing potential (AHP) following prolonged excitation. Superfusion hippocampal brain slice preparations with the GABA A receptor blocker gabazine (GZ) induces epileptiform activity actively. In this epilepsy model, the AHP has previously been shown to be significantly decreased. Here, we asked the question whether KCa2.2 protein degradation occurs in this model, and which pathways are involved. To test this, we applied either GZ alone or GZ together with inhibitors of proteasomal and lysosomal protein degradation pathways, Z-Leu-Leu-Leu-CHO (MG132) and chloroquine (CQ), respectively. Using western blot analysis, we showed a significant decrease of total KCa2.2 protein content in GZ-treated slices which could be rescued by co-incubation management with MG132 and CQ. In HEK293 cells transfected with a green fluorescent protein-tagged KCa2.2 construct, we demonstrated that proteasomal rather than lysosomal degradation was involved in KCa2.2 reduction. To test for functional significance, we recorded epileptiform afterdischarges at hippocampal Schaffer collateral-CAL synapses and confirmed that the GZ-induced increase was significantly attenuated by both MG132 and CQ with MG132 being significantly more effective than CQ. Epileptiform afterdischarges were nearly completely prevented by co-application of protein degradation inhibitors. Furthermore, epileptiform afterdischarges could be rescued by using the KCa2.2 blocker UCL 1654, demonstrating involvement of KCa2.2. We conclude that KCa2.2 degradation by proteasomal rather than lysosomal pathways plays a major role in the generation of epileptiform afterdischarges.
cells. Activation of the TALK1 channels was measured by the patch-clamp technique in whole-cell mode. In order to test the influence of different pH values on the activation of the TALK1 or the TALK1 mutated channels during recordings we exchanged the extracellular solution in the range from pH 5 to pH 11. Our results demonstrate that the wild type TALK1 channel senses the changes in the extracellular pH, exhibiting strongly high-pH activated outwardly rectifying currents. In the R233V mutated channel, the pH dependency was greatly diminished. Even more interestingly, the R233E mutant shows reverse pH dependent activation with strong acidic activated outwardly rectifying currents. Mutating the neighboring alanine did not influence the transduction despite the wild type channel. We propose that the sensing of extracellular pH changes is accomplished by the positively charged arginine 233, located near the selective pore. The positively charged lysine 84 does not seem to be involved in the pH sensing. Since TALK1 channels have a substantial expression in the beta cells of pancreatic islets, we will next test the hypothesis that the TALK1 channel is involved in the fine tuning of beta cell excitability as a resting or a background channel.

**Biophysical characterisation and interplay of voltage-gated calcium currents and A-type potassium currents in substance nigra dopaminergic neurons**

**A. Gaifullina**, **S. Rinné**, **H. Köpke**, **J. Leonardt**, **M. Bedoya**, **M. Schewer**, **A. K. Kiper**, **T. Baulac**, **N. W. Gonzalez**, **B. L. de Groot**, **N. Decher**

1Max-Planck-Institute for Biophysical Chemistry, Biomolecular Dynamics Group, Göttingen, Germany
2University of Taka, Center for Bioinformatics and Molecular Simulations, Taka, Chile
3Christian-Albrechts-Universität Kiel, Institute for Physiology, Kiel, Germany

Our results demonstrate that the wild type TALK1 channel senses the changes in the extracellular pH, exhibiting strongly high-pH activated outwardly rectifying currents. In the R233V mutated channel, the pH dependency was greatly diminished. Even more interestingly, the R233E mutant shows reverse pH dependent activation with strong acidic activated outwardly rectifying currents. Mutating the neighboring alanine did not influence the transduction despite the wild type channel. We propose that the sensing of extracellular pH changes is accomplished by the positively charged arginine 233, located near the selective pore. The positively charged lysine 84 does not seem to be involved in the pH sensing. Since TALK1 channels have a substantial expression in the beta cells of pancreatic islets, we will next test the hypothesis that the TALK1 channel is involved in the fine tuning of beta cell excitability as a resting or a background channel.
amplitude and decreased extent of inactivation of voltage-gated K⁺ currents, suggesting that ARTD10 regulated Kv1.1 also in hippocampal neurons. Nevertheless, OUL35 increased excitability of hippocampal neurons. The present work demonstrates that Kv1.1 is indirectly regulated by ARTD10 through PKC-dependent de-phosphorylation. Our work identifies a completely new regulator of Kv1.1 that may have an important role in the regulation of neuronal excitability.

**B 06-9**

**Influence of Ba²⁺ on currents through hKv1.3_V388C mutant channels**

M. L. Haag, S. Grissmer

Universität Ulm, AG Grissmer / Angewandte Physiologie, Ulm, Germany

The exchange of the amino acid valine with cysteine at position 388 (Shaker position 438) in Kv1.3 channels was reported to (1) result in channels with two different ion conducting pathways: First, the central K⁺-selective o-pore sensitive to block by peptide toxins and second, a o-pore pathway that is characterized by an inward current at potentials where the central o-pore would normally be closed (more negative than -100 mV). For a more detailed characterization of the o-pore we used the whole-cell recording mode of the patch clamp technique (2) to test the influence of different externally applied Ba²⁺-concentrations on mammalian cells transfected with cDNA encoding hKv1.3_V388C mutant channels. Therefore, the normal bath solution (in mM: 160 NaCl; 4.5 KCl; 2 CaCl₂; 1 MgCl₂; 10 HEPES; pH 7.4) contained specific concentrations of BaCl₂ ranging from 0.1 to 10 mM, while the pipette solution contained mainly KF (in mM: 145 KF, 1 CaCl₂, 2 MgCl₂, 10 HEPES; pH 7.2). Starting from a holding potential of -120 mV the cells were first depolarized to 40 mV for 100 ms and then hyperpolarized in steps of ten from -100 to -200 mV for 500 ms. This protocol yielded inward tail currents carried by Na⁺ that were largest at -200 mV. Extracellularly applied Ba²⁺ reduced the tail current amplitudes in a concentration-dependent manner, i.e. amplitudes at -200 mV were reduced by 10 mM BaCl₂ to 26% of control values, whereas 1 mM BaCl₂ reduced the tail current amplitudes at -200 mV to 44% of control values. In addition to the concentration-dependency of the Ba²⁺-block we observed a slight voltage-dependent unblock of Ba²⁺ at more hyperpolarized potentials. For example, in the presence of 10 mM Ba²⁺, the tail current amplitudes at -160 mV were 16% of control values compared to 26% at -200 mV. From these results we conclude that Ba²⁺ might enter the o-pore at hyperpolarized potentials to block current and at stronger hyperpolarizations might overcome the block and might penetrate through the o-pore.

(1) Prütting S et al. 2011. A novel current pathway parallel to the central pore in a mutant voltage-gated potassium channel. J Biol Chem. 286(22):20031–20042.

(2) Haag M. L. Haag, S. Grissmer. 2006-9

B 06-10

**Pharmacological characterization of the o-pore in tetrameric hKv1.3_V388C mutant channels**

A. S. Schmaizl, S. Grissmer

University of Ulm, Department of Applied Physiology, Ulm, Germany

The replacement of the amino acid valine with the smaller cysteine at position 388 (Shaker position 438) in the mutant voltage-gated K⁺ channel hKv1.3_V388C opened a new pathway (o-pore) behind the central o-pore. At potentials more negative than -100 mV, where the central o-pore would normally be closed, a large inward current through the o-pore appeared being carried by different cations like Na⁺, Li⁺, Ca²⁺, and NH₄⁺ (1) whereas the current through the o-pore showed faster inactivation. For the pharmacological characterization of the o-pore we performed measurements with the patch-clamp technique in the whole cell recording mode using external bath solutions containing mainly Na⁺ (160 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES). For each experiment drugs were added to the external bath solution and the internal bath solution contained mainly KF (145 mM KF, 2 mM MgCl₂, 10 mM EGTA and 10 mM HEPES). To simultaneously measure current through both o- and o-pore and determine the blocking effect we used depolarizing pulses from the holding potential of -120 mV to +40 mV for 100 ms to show currents through the o-pore, followed by hyperpolarizing pulses to -180 mV to show currents through the o-pore-blocking peptide toxins acting at the external vestibule of the channel such as CTK, KTX and AγTX blocked the o-pore with five times lower affinity than hKv1.3_Wt channels and showed no blocking effect on the o-pore of the mutant channel. Channel blockers acting from the intracellular side like the channel blocker PAP-1 blocked currents through both the o- and the o-pore with high affinity similar to wildtype (K₅ = 2 nM). The phenylalkylamine blocker verapamil and its derivative norverapamil acting also from the intracellular side of the channel reduced currents through both pores with the same affinity and similar to wildtype. Methoxyverapamil and aminoverapamil could also reduce both currents, however with somewhat different affinities for the o-pore compared to current through the o-pore indicating a possibility to distinguish the location of the o-pore- and o-pore-exit into the internal vestibule.
The melastatin-like Transient-Receptor-Potential-7 protein regulates Cyclooxygenase-2 expression in human myeloid leukemia cells

S. Hampel, W. Nadolni, K. Hötting, M. Fratelli, S. Belabbanov, I. Bockhoff, R. Greil, T. Gudermann, S. Zierler

Ludwig-Maximilians Universität München, Walter Shrub Institute of Pharmacology and Toxicology, München, Germany
Paracelsus Medical University Salzburg, Department of Medicine III, Austria

We recently acknowledged the ubiquitously expressed melastatin-like transient-receptor-potential-7 protein (TRPM7) as modulator of immune system homeostasis. TRPM7 combines a cation channel, conducting calcium, magnesium and zinc, with a serinethreonine kinase, phosphorylating i.e. annexin A1, myosin II and Smad2. TRPM7 has been implicated in several human diseases, such as stroke, cardiac diseases and altered immune responses and it also has been associated with the growth of many malignancies. As TRPM7 is essential for leukocyte proliferation, development and differentiation, it is a likely candidate to regulate proliferation and survival of leukemia cells. Many types of hematopoietic malignancies such as chronic leukemia, including chronic myeloid leukemia (CML) are highly expressed cyclooxygenase-2 (COX-2), a key modulator of inflammation. Expression of COX-2 enhances survival and proliferation of malignant cells, while negatively influencing anti-tumor immunity. Therefore, COX-2 selective inhibitors have promising therapeutic potential in patients suffering from CML. Here, we show that targeting TRPM7 in human myeloid leukemia cells results in reduced constitutive and inducible COX-2 gene expression and activity. Using a human TRPM7-deficient leukemia cell line (HAP1), we were able to link this defect to impaired NFAT, NFκB, SMAD, Akt and mTOR signaling cascades. Genetic inactivation of the TRPM7 kinase by introducing a point mutation at the active site of the kinase (K164R) in HAP1 cells revealed that the reduction in Akt phosphorylation is kinase-dependent and that TRPM7 kinase activity is a key regulator of COX-2 gene expression. Using pharmacological blockade of TRPM7 in primary human myeloid leukemia (CML) cells, we were able to confirm its promoting effect on COX-2 gene expression. Pharmacologic inhibition of TRPM7 led to reduced constitutive COX-2 expression in human CML cells. Also LPS-induced COX-2 expression was reduced in CML cells upon TRPM7 blockade. For both inducible and constitutive COX-2 expression the phosphorylation of Akt was essential and significantly diminished following TRPM7 inhibition. Our results identify TRPM7 as novel regulator of COX-2 expression and may pave the way for new therapies against chronic leukemia.

Interactions between opposite subunits stabilize autoinhibition in HCN2 channels

M. Kondapuram, S. Yüksel, C. Sattler, B. Freig, H. Gohlke, R. Schmader, K. Benndorf, J. Kusch

Universität zu Köln, Institut für Physiologie II, Jena, Germany
Heinrich-Heine Universität, Institut für Pharmazeutische und Medizinische Chemie, Düsseldorf, Germany

HCN ion channels are gated by hyperpolarizing voltages and cyclic-nucleotide (CN) binding to intracellular CN binding domains (CNBDs). The four CNBDs are connected to the gate-forming transmembrane S6-helices via so-called C-linkers (CLs). The empty tetrameric CL-CNBD region exerts an autoinhibiting effect on the channel gate which is relieved upon CN binding. Based on the recent HCN1 channel structure (Lee and MacKinnon, Cef, 2017) and on MD simulations, we hypothesized that a highly conserved lysine residue (K464 in mHCN2) at the loop linking the A'-helix and B'-helix of the CL enables functionally relevant interactions with the opposite subunit. As potential interacting partners for K464 we identified M155 in the second a-helix of the HCN domain and E247 in the loop between the S2 and S3 transmembrane segments. Electrophysiological characterization of myotonic dystrophy type 1 (M D1) mutant channel was carried out in inside-out macropatches excised from Xenopus laevis oocytes. Steady-state and non-steady-state parameters were analyzed in the absence and in the presence of [cAMP]o. Together with distance distributions obtained by MD simulations the data suggested that in HCN2 channels not binding cAMP, K464 interacts with both the backbone carbonyl of M155 and the side chain of E247, thereby stabilizing autoinhibition. For K464E where this stabilization is missing, confocal patch-clamp fluorometry showed that the binding affinity for non-activated channels is similarly high as for activated channels, which is basically different to WT channels where voltage-induced activation enhances the binding affinity for cAMP. This suggests that in K464E the CL-CNBD adopts already the conformation of the WT channel activated by cAMP. In summary, the data show that in HCN channels opposite subunits functionally interact to stabilize autoinhibition. Breaking these interactions either by cAMP-binding or mutagenesis allows the CL-CNBD to adopt a conformation in which autoinhibition is relieved and channel activation is promoted.

Uncoiling the cyclic-nucleotide-binding domain from the channel gate in HCN2 channels

S. Yüksel, M. Kondapuram, T. Schwabe, T. Zimmer, M. Lell, A. Schweinitz, M. Bonus, H. Gohlke, R. Schmader, J. Kusch, K. Benndorf

University Hospital Jena, Institute of Physiology II, Jena, Germany
Heinrich Heine University Düsseldorf, Institute for Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany

HCN pacemaker channels are dually gated by hyperpolarizing voltage and cyclic-nucleotide (CN) binding to intracellular cyclic nucleotide-binding domains (CNBDs). Each CNBD is connected to the gate, a right-handed S6-helix bundle, via a C-linker (CL). Movement of the voltage sensor causes a leftward unwinding of the helix bundle to open the pore. Functional and structural data suggest that this unwinding is supported by cAMP binding, promoting a leftward rotation of the CL domain located beneath the S6 gate. In the absence of cAMP this CL disk autoinhibits channel opening. To study the autoinhibition and its cAMP-induced release in more detail, we constructed HCN2 channels carrying an additional amino acid sequence (AAAS) between S6 and the first helix (A') of the CL to uncouple pore and CNBD-CL portion. The AAAS consisted of one, two, three, four, or five glycines following S441. 441-1G, 441-2G, 441-3G, 441-4G, 441-5G. Recordings were performed in inside-out macropatches from Xenopus laevis expressing the channels. The results of patch-clamp and confocal patch-clamp fluorometry experiments showed that: (1) The S6 helix bundle is a functional gate. (2) Already a single additional glycine destroyed the cAMP-induced enhanced opening of the gate opening. Our current results also showed that there is no cAMP-effect on gate opening for the longest AAAS (441-5G). (3) Autoinhibition was reduced in proportion to the number of inserted glycines, and was completely abolished with four glycines (441-4G). (4) Voltage-dependent activation in 441-4G did not increase the binding affinity for a fluorescent cAMP derivative, as typical for wild-type channels (Kusch et al., Neuron, 2010). Together, these data led us to conclude that the interactions which inhibit the leftward rotation of the CL-CNBD portion are partially intact with the constructs 441-4G, 441-2G and 441-3G but completely damaged in the construct 441-4G. Mechanically, these data suggest that autoinhibition is controlled in regions remote from the S6 helix bundle whereas the cAMP-induced release of autoinhibition requires a tight mechanical coupling between the S6 helix bundle and the A'-helix of the CL.

Reference:
Kusch, J. Bissp, C. Thon, S. Schulz, E. Nache, V. Zimmer, T. Schwade, F. Benndorf, K. (2015) Interdependence of receptor Activation and Ligand Binding in HCN2 Pacemaker Channel, Neuron, 67, 75-85.

Interactions between opposite subunits stabilize autoinhibition in HCN2 channels

M. Kondapuram, S. Yüksel, C. Sattler, B. Freig, H. Gohlke, R. Schmader, K. Benndorf, J. Kusch

Universität zu Köln, Institut für Physiologie II, Jena, Germany
Heinrich-Heine- Universität, Institut für Pharmazeutische und Medizinische Chemie, Düsseldorf, Germany

HCN ion channels are gated by hyperpolarizing voltages and cyclic-nucleotide (CN) binding to intracellular CN binding domains (CNBDs). The four CNBDs are connected to the gate-forming transmembrane S6-helices via so-called C-linkers (CLs). The empty tetrameric CL-CNBD region exerts an autoinhibiting effect on the channel gate which is relieved upon CN binding. Based on the recent HCN1 channel structure (Lee and MacKinnon, Cef, 2017) and on MD simulations, we hypothesized that a highly conserved lysine residue (K464 in mHCN2) at the loop linking the A'-helix and B'-helix of the CL enables functionally relevant interactions with the opposite subunit. As potential interacting partners for K464 we identified M155 in the second a-helix of the HCN domain and E247 in the loop between the S2 and S3 transmembrane segments. Electrophysiological characterization of myotonic dystrophy type 1 (MD1) mutant channel was carried out in inside-out macropatches excised from Xenopus laevis oocytes. Steady-state and non-steady-state parameters were analyzed in the absence and in the presence of [cAMP]o. Together with distance distributions obtained by MD simulations the data suggested that in HCN2 channels not binding cAMP, K464 interacts with both the backbone carbonyl of M155 and the side chain of E247, thereby stabilizing autoinhibition. For K464E where this stabilization is missing, confocal patch-clamp fluorometry showed that the binding affinity for non-activated channels is similarly high as for activated channels, which is basically different to WT channels where voltage-induced activation enhances the binding affinity for cAMP. This suggests that in K464E the CL-CNBD adopts already the conformation of the WT channel activated by cAMP. In summary, the data show that in HCN channels opposite subunits functionally interact to stabilize autoinhibition. Breaking these interactions either by cAMP-binding or mutagenesis allows the CL-CNBD to adopt a conformation in which autoinhibition is relieved and channel activation is promoted.
Conclusion: Our data suggest that CNP modulates HCN2 pacemaker channels. We propose that these findings might be relevant to understand the electrical activity in oligodendrocytes and demyelinating processes.

B 07-6
Role of Inositol Hexakiphosphate Kinases in Mammalian Cellular Phosphate Sensing
B. Haykir1, A. Saliardi2, N. Hernando1, C. A. Wagner1
1University of Zurich, Physiology Institute, Zurich, Switzerland
2University College London, MRC Laboratory for Molecular Cell Biology, London, UK

Phosphate (Pi) is essential for life and human Pi homeostasis is tightly regulated by intestine, kidney, bone and parathyroid glands via Na+-dependent Pi cotransporters and/or hormones including parathyroid hormone, fibroblast growth factor 23 and calcitriol. Although there is a huge amount of research on Pi regulation, how Pi is sensed by mammalian cells is still unknown, since most of the Pi-sensing genes identified in yeasts and bacteria are not conserved in mammals. However, inositol polyphosphates and their kinases, which respond to changes in ambient Pi in yeast by regulating the amount of Pi transporters, are found in eukaryotic genome including human. In yeast, a single kinase (Sac1) is responsible to convert inositol hexakiphosphate (IP6) to inositol pyrophosphate 7 (IP7), whereas, in mammals, there are three kinases (IP6k1, IP6k2 and IP6k3).

The aim of this project was to investigate the role of IP6ks in cellular Pi-sensing in mammals. For that, we have studied the effect of dietary/ambient Pi on the renal mRNA expression of IP6ks in vivo (mice) and in an in vitro model of renal proximal epithelia (Opossum kidney (OK) cells). Additionally, we investigated the effect of IP6ks inhibition on the well-known adaptation of OK cells to ambient Pi.

Kidneys and OK cells express preferentially IP6k1 and IP6k2 mRNAs, with undetectable levels of IP6k3, respectively. The mRNA expression of IP6k2 was lower in kidneys of mice fed acutely (12 hr) with a low Pi diet as compared with mice fed high Pi. Similarly, IP6k2 expression was lower in OK cells acutely exposed (4 hr) to low ambient Pi than in cultures incubated with high Pi. Upon chronic administration (5 days for mice and 24 hr for cells), this regulation disappeared in both models. Dietary/ambient Pi either provided acutely or chronically did not alter the expression of the two other kinases neither in vivo nor in vitro. Using a radioactive uptake assay, we found that an inhibitor of the kinases, N6-[4-nitrophenyl]-N2-[3-(triﬂuoromethyl)phenyl]ethyl]-7-H-purine-2,6-diamine (TNP), decreased the basal Pi-uptake in OK cells in a dose-dependent manner. TNP prevented the acute adaptation to low Pi, but had no effect on the long-term adaptation. Together, these results suggest that IP6ks, particularly IP6k2, may be involved in sensing acute reduction of ambient Pi.

B 07-7
Cell surface abundance of CaV1.2 is regulated by CaVβ ‐ depending endocytic turnover
R. Conrad, D. Kortzak1, P. Hidalgo1,2
1Forschungszentrum Jülich, Institute of Complex Systems (IC54), Jülich, Germany
2Heinrich-Heine-Universität Düsseldorf, Cellular Biophysics, Düsseldorf, Germany

L-type voltage-activated calcium channels (VACC) are major contributors to the entry of calcium into excitable cells that in turn, triggers a variety of cellular functions including cardiac contractility. Their cell surface channel abundance has to be tightly regulated in order to coordinate the variety of calcium signals. The balance between anterograde and post-endocytic trafficking determines VACC's cell surface expression, however, the mechanisms underlying their intracellular transport are poorly understood. Canonically, CaV1.2 channel consists of a large CaVα1.2 pore-forming subunit and two accessory subunits the β- and αd-subunit. CaVβ is mandatory for targeting the channel complex to the plasma membrane.

We here studied the post-endocytic trafficking of CaV1.2 and the role of CaVβ in HL-1 cardiomyocytes using fluorescence spinning disk confocal microscopy to visualize extracellularly labeled channels and evaluate their endocytic turnover. We found that channels newly inserted into the plasma membrane become internalized with an average time constant of 7.5 min and recycle via Rab11a endocytic recycling compartment. Disruption of the actin cytoskeleton, but not of microtubules, reroutes channels from recycling towards lysosomal degradation. Impairment of CaVβ-dissociation from CaVα1.2 by generating a CaVα1.2-CaVβ-complex covalently linked to CaVβ decreases the internalization rate, and thus, increases the stability of the channel at the plasma membrane.

Our findings establish a central role for post-endocytic sorting in determining the CaV1.2 cell surface abundance via a Rab11a/adin-mediated recycling itinerary depending on the dynamic interaction of CaVβ with CaV1.2. This novel mechanism for the homeostatic regulation of voltage-dependent calcium influx allows rapid, precise and energy-saving adjustment of calcium influx and thus of heart’s contraction.

B 07-8
Purinergic signalling affects the activity of the bile acid-sensitive ion channel
S. Wiegreffe, S. Gründer, D. Wiemuth
RWTH Aachen University, Institute of Physiology, Aachen, Germany

Background: The bile acid-sensitive ion channel (BASIC) is a cation channel belonging to the DEG/ENaC family of ion channels. It is expressed in brain, intestinal tract and liver where it is mainly found in cholangiocytes, the epithelial cells of bile ducts. Despite the identification of various electrophysiological features, e.g. its activation by bile acids, its physiological function in the organism remains unknown.

Methods: To identify further electrophysiological characteristics of BASIC in epithelial processes and to establish a functional cell model, we generated normal rat cholangiocytes (NRC) in Ussing chambers and measured transepithelial currents in response to various substances including bile acids. Furthermore, we generated and included an NRC-Hep cell line in our experiments to elucidate the functional role of BASIC.

Results: The apical application of different bile acids induced reversible, transepithelial Na+-currents. Furthermore, we generated and included an NRC-Hep cell line in our experiments to elucidate the functional role of BASIC. This purinergic reaction and the bile acid induced currents, which were potentiated by previous application of ATP and partly inhibited by amiloride, an inhibitor of DEG/ENaC channels. In addition, we observed a stronger reaction to ATP in BASIC-expressing cells. These data support a functional role of BASIC in pH regulation of bile and bicarbonate-umbrella formation for protection of cholangiocytes in case of increased bilary flow by interaction with other transport systems.

Conclusion: Our data suggest a connection between P2X4-mediated signalling pathways and the function of BASIC in epithelia and further support a cell model, where BASIC functions in pH regulation of bile and bicarbonate-umbrella formation.

B 07-9
Interaction of the sphingosine-1-phosphate pathway with purinergic receptors
D. Zahiri, M. Klapa, M. Markwardt
M. University Halle, JB Institute for Physiology, Halle, Germany

Cells release ATP in many ways. One of them is via the cell volume-sensing anion channel VRAC induced by activation of voltage-sensitive calcium (S1P) receptors. We have previously reported about this S1P-induced ATP release measured by voltage clamp and luciferase assay. Here, we investigated whether the S1P-induced ATP release can affect cell functions like cell migration by activating purinergic P2X or P2Y receptors. The microglia cell line BV-2 has been used to conduct the experiments. In order to assess the effects of a S1P-induced ATP release we used scratch assays (also wound healing assay). S1P, like ATP and ADP, stimulates cell migration into the scratch area. The inhibition of S1P receptors and of the downstream G-proteins reduced the cell migration. Antagonists of VRAC, which lead to reduced ATP release, were also able to diminish the cell migration. Furthermore, direct inhibition of ATP-gated P2X4 or P2X7 receptors or ADP-stimulated P2Y12 receptors blocked the stimulating effects of S1P on cell migration. We conclude that there is an interaction between S1P receptors and purinergic receptors mediated by a S1P-induced ATP release via VRAC and that the amount of released ATP is capable to stimulate cell migration of BV-2 microglia cells via activation of P2X4, P2X7 and P2Y12 receptors.

B 08 | Novel Techniques and Molecular Analysis of Channels

B 08-1
Effect of ProToxin-II on Sodium ion channel dimers
A. Kalia, A. Lampert
RWTH Aachen University, Department of Physiology, Aachen, Germany

Voltage-gated sodium (Nav) channels initiate action potentials in excitable cells and play an important role in the detection and transmission of sensory information to the CNS and perception of pain. Genetic studies have identified loss-of-function and gain-of-function mutations in Nav1.7 that result in congenital insensitivity to pain with chronic pain syndromes, respectively. This clinical evidence provided the basis for Nav1.7 as a target for developing novel pain therapeutics. Peptide toxins that target voltage sensor domains (VSDs) have been used to probe the complex gating properties of Nav channels. ProToxin-II (ProTox-II) is a 30-residue disulfide-rich peptide isolated from the Thunexipilla pruniaria that
has unusually high affinity and selectivity toward the human Nav1.7 channel. ProTx-II acts by inhibiting Nav channels through the reduction of peak current and the induction of a depolarizing shift in the voltage-dependence of activation.

It was reported that sodium channel α-subunits not only assemble as dimers but that this physical interaction also results in coassembly of voltage-gated sodium channels by concatemer formation. As a first approach, we generated concatemers of rNav1.7 ion channels with strongly altered gating properties and will apply it in an asymmetric manner to channels with strongly altered gating properties and to various Na\(^+\) channels, measuring in the whole-cell patch clamp mode with standard current-clamp techniques. As a result, we found that ProTx-II inhibits Nav1.7 and reduces peak current with high potency. Difopein treatment significantly reduced the effect of ProTx-II on Nav1.7 channel expression. This study further highlights the role of dimORIZATION in modulating the sodium channel gating and shows that sodium channel pharmacology is affected by their dimerization.

B 08-2

Studying coassembly of voltage-gated sodium channels by concatemer formation

F. Stumpff, Freie Universität Berlin, Institute of Veterinary Physiology, Department of Veterinary Medicine, Berlin, Germany

Voltage-gated Na\(^+\) (Nav) channels are responsible for initiation and propagation of electrical signals in excitable cells, and even minor alterations of their functional properties give rise to a broad array of diseases affecting muscle as well as the peripheral and central nervous systems. Recent findings [1] have challenged the traditional assumption that Nav-channels are operating as large monomeric pore-forming protein α subunits, just modulated by auxiliary β subunits. Apparently, Nav channels can form homo- and hetero-dimers with some functional cross-talk between them. This has far-reaching consequences for Nav function as such and also for the interpretation of Nav-mediated human diseases. Using rat skeletal muscle Nav-channel (Nav1.1) as a model system, we find that Nav1.1 channels co-assemble with other channel types, like αβ1.5 and TASK-1. This approach might yield innovative anti-arrhythmic compounds having a more promising risk–benefit ratio than currently available drugs in clinic.

B 08-3

A “receptophore model” for local anesthetics binding site in cardiac ion channels

W. González, Center for Bioinformatics, Simulations and Modeling (CBSM), Tacla, Chile

An “receptophore model” for local anesthetics binding site in cardiac ion channels has unusually high affinity and selectivity toward the human Nav1.7 channel. ProTx-II acts by inhibiting Nav channels through the reduction of peak current and the induction of a depolarizing shift in the voltage-dependence of activation.

It was reported that sodium channel α-subunits not only assemble as dimers but that this physical interaction also results in coassembly of voltage-gated sodium channels by concatemer formation. As a first approach, we generated concatemers of rNav1.7 ion channels with strongly altered gating properties and will apply it in an asymmetric manner to channels with strongly altered gating properties and to various Na\(^+\) channels, measuring in the whole-cell patch clamp mode with standard current-clamp techniques. As a result, we found that ProTx-II inhibits Nav1.7 and reduces peak current with high potency. Difopein treatment significantly reduced the effect of ProTx-II on Nav1.7 channel expression. This study further highlights the role of dimORIZATION in modulating the sodium channel gating and shows that sodium channel pharmacology is affected by their dimerization.

B 08-4

TRPV3 mutation Gly573Ser and its wild type are functionally expressed in the membrane of HEK-293 cells

H. Lieber, F. Lieber, G. Spender, F. Stumpff

Freie Universität Berlin, Institute of Veterinary Physiology, Department of Veterinary Medicine, Berlin, Germany

In Omslet syndrome of humans, a gain of function mutation of the TRPV3 channel leads to severe hyperkeratosis of the skin. Literature suggests that the excess influx of calcium via this mutated protein causes apoptosis [2]. In contrast, it has recently been shown that the mutation prevents trafficking of the channel protein to the membrane, with cell death apparently caused by an alteration on the lysosomal level [3]. We investigated these contradictory hypotheses. HEK-293 cells were either transiently transfected with a pPRBS2-AcGFP1 vector with vector construct including a strep-tagged wild type TRPV3 (WT) sequence or its Gly573Ser mutant. Successful expression was confirmed by immunodetection (n = 8) of the strep tag and with a commercially available antibody (n = 7) in western blots for WT. The functionality of the WT was investigated via whole cell patch clamp experiments. We could show significant effects using the three TRPV3 agonists menthol (p = 0.002), thymol (p < 0.001), and 2-APB (p < 0.001) in overexpressing WT cells but not in controls. To localize the channel, immunohistochemical stainings of WT, Gly573Ser, and controls were performed. In contrast to controls, cells over-expressing WT showed distinct staining of the cell membrane, in conjunction with a cysteine downstream expression of GFP. Individual Gly573Ser mutant cells also showed clear staining of the channel membrane with the TRPV3 antibody. However, no mutant cells expressed GFP, suggesting that cell death had taken place before a successful transfection and expression of GFP could occur. For this reason, patch clamp experiments were not possible in the mutant. To quantify cell death rate, trypan blue staining was used as a marker for cell viability. Cells expressing the mutant TRPV3 were shown to have a significantly higher death rate than either control HEK-293 cells or those expressing the WT. When the TRP antagonist ruthenium red was added to the medium, a significant (p < 0.001) increase in the cell count of expressed mutant was observed versus that in WT-only controls.

In conclusion, our experiments support the thesis of a functional expression of both TRPV3 and its mutant Gly573Ser in the cell membrane of overexpressing HEK-293 cells.

Funding: German Science Foundation (DFG), Sonnenfeld Stiftung

B. Nitsu et al., J. Physiology. 2014;592(2):295-304.
Z. Lin et al., Am. J. Hum. Genet. 2012;90(3):558-64.
M. Yadav et al., Channels. 2017;11(3):196-208.

B 08-5

Melastatin-like Transient Receptor Potential 7 Influences T Cell Activation through Modulation of Stored-Operative Calcium Entry

K. Hoeting 1, W. Stuber 2, A. Dietrich 1, T. Gudermann 1, A. Splietko 2, S. Zierler 3

1LMU München, Walther-Brendel-Zentrum für Experimentelle Medizin, Planegg-Martinsried, Germany
2Universität Marburg, School of Medicine, Marburg, Germany
3Philipps-University of Marburg, Institute for Cell Biology, Marburg, Germany

The melastatin-like transient receptor-potential-7 protein (TRPM7) is a ubiquitously expressed cation channel, conducting Ca\(^{2+}\) and Mg\(^{2+}\) and Zn\(^{2+}\). The protein contains a cysteine-α-kinase of which a few in vitro substrates have been identified, including annexin A1, myosin II and, as recently discovered, SMAD2. TRPM7 has been implicated in cellular and systemic Mg\(^{2+}\) homeostasis. In lymphocytes it has been linked to store-operated calcium entry (SOCE), the primary mechanism of Ca\(^{2+}\)-entry into the cell. However, the mechanism by which TRPM7 induces SOCE remains unclear. To understand the role of the dual-function protein in Ca\(^{2+}\) signaling of lymphocytes, we are utilizing a TRPM7 kinase-deficient mouse model (Trpm7\(^{-/-}\)), the human Jurkat T cell line, as well as primary human CD4\(^{+}\) lymphocyte subsets and stimulate them with antibodies against CD3 and CD28. Using ratimonic Ca\(^{2+}\) imaging with cell membrane permeable Fura-2, Jurkat T cells were able to show that SOCE induced by TRPM7 is inhibited in the mutant. Although we could not detect differences in T cell receptor (TCR)-activated calcium signaling, proliferation of Trpm7\(^{-/-}\) T cells was normal compared to controls. As a translational approach, we used human Jurkat T cells and pharmacologically inhibited TRPM7 activity, utilizing the small molecules NS593 and waixicenic A. While basal colloidal Ca\(^{2+}\) concentration in Jurkat T cells is relatively low, Ca\(^{2+}\) entry following TCR activation was significantly reduced. Similar effects could be shown in distinct subsets of primary human CD4\(^{+}\) T cells: Ca\(^{2+}\) entry was impaired in pro- as well as in anti-inflammatory T cells. In addition to reduced Ca\(^{2+}\)-influx, characteristic oscillations of cystolic Ca\(^{2+}\) concentrations were significantly reduced in primary T cells treated with NS593. However, in all T cell subtypes used, Ca\(^{2+}\) release from the endoplasmatic reticulum (ER) was not significantly

Poster Session B | 191

Poster Session B | 192
changed, indicating that indeed SOCE is affected by TRPM7 inhibition. We are further examining the impact of TRPM7 inhibition on Ca²⁺ dependent NFAT translocation, resulting gene transcription, and proliferation. These insights will help to evaluate whether TRPM7 is an important immunological modulator and thus a potential drug target for the treatment of inflammatory diseases.

B 08-6

Birds can shake too: wet chicken shakes depend on the cold and menthol receptor T. Selescu†, R. A. Bivoreanu†, A. Malinac‡, E. T. Wei‡, A. Babes§
†University of Bucharest, Faculty of Biology, Department of Anatomy, Animal Physiology and Biophysics, Bucharest, Romania
‡University of California, Berkeley, School of Public Health, Berkeley, US
§Jena University Hospital, Friedrich Schiller University Jena, Biomolecular Photonics Group, Jena, Germany

An important behavior for thermoregulation in warm blooded animals consists in powerful shaking, efficient in evicting water from wet fur or feathers. While in mammals this behavior is well characterized as “wet dog shakes” (WDS), in birds it received much less attention. The Transient Receptor Potential Melastatin type 8 (TRPM8) ion channel is the main molecular transducer of low temperatures in mammalian skin. From the multitude of TRPM8 agonists, only cold is known to trigger WDS in rodents. As avian TRPM8 is cAMP-insensitive, it was not possible to test the role of TRPM8 in shaking or feather ruffling in birds. The novel TRPM8 agonist 1-disopropylphosphorylethylene (Cryoa-1, C1) made this possible. We compared the natural shaking of water-sprayed chickens with the effect of C1 administration. Human, rat and chicken TRPM8 were expressed in HEK293 cells. Primary cultures of sensory neurons were obtained from dorsal root ganglia (DRG) of Wistar rats and Plymouth Rock chickens. Calcium imaging was performed with Calcium Green-1 AM and whole-cell membrane currents were recorded. For in vivo experiments C1 was dissolved in polyethylene glycol and C1 in Ringer’s solution. Animal behavior was recorded up to 30 minutes after compound injection. All three TRPM8 orthologs tested were robustly activated by C1 (10 μM) and these calcium transients were abolished by the TRPM8 antagonist AMB (1 μM). C1 activated inwardly-rectifying currents with a reversal potential close to 0 mV. In both rat and chicken, AMB blocked C1, but not the TRPM8 agonist WS-12 (5 μM). In rats, intraperitoneal injections of C1 (33 mg/kg) evoked WDS and jumping/escape behavior with a shorter onset time compared to cAMP (1 mg/kg).

In chickens we first evaluated the natural shaking behavior evoked by 10 water sprays (~ 0.9 ml each) on the neck and rostral back. This produced 1-5 powerful body shakes within 5 minutes. Subcutaneous axillary injections of C1 (33 mg/kg) in chickens produced up to 4 powerful shakes and feather ruffling in the first 2 minutes, followed by a few jumping/escape attempts succeeded by long-lasting freezing behavior (up to 30 minutes). The similarity between the shaking behaviors recorded in rats and chickens exposed to the novel TRPM8 agonist C1 could support the hypothesis of a common origin of these behaviors in a common ancestor and the hypothesis of a shared evolutionary origin of hairs and feathers.

B 08-7

Mapping the binding site of the opioid neuropeptide Big Dynorphin on ASIC1a
L. Leisle†, M. A. Margreiter†, G. Rossetti‡, S. Grünner†
†RMTH, Physiology, Aachen, Germany
‡RMTH, Jülich Supercomputing Centre, Jülich, Germany

In a number of neurological diseases acid-sensing ion channel 1a (ASIC1a) activity promotes neurotoxicity during prolonged acidosis. The opioid neuropeptide Big Dynorphin shows an overlapping expression pattern with ASIC1a in the nervous system and has also been implicated in neuronal damage. Recent work demonstrated that Big Dynorphin shifts ASIC1a steady-state desensitization to more acid pH values, thus enhancing ASIC1a activity and acidosis-induced neuronal death. To better understand the molecular interaction of ASIC1a and Big Dynorphin, we set out to identify the binding site of the ligand on the receptor. Employing the chemical toolbox of genetic code expansion, we incorporated 4-Azido-L-Phenylalanine at more than 50 positions on the surface of ASIC1a in live cells. Upon UV irradiation, this non-canonical amino acid creates covalent bonds with molecules in close vicinity (specifically ≤3 Å – a distance characteristic for specific interactions). Our site-directed screen confirmed published indications that Big Dynorphin and Phosphatidyl in 1 binding sites on ASIC1a partially overlap. Thus, this basic neuropeptide binds to the acidic pocket of ASIC1a. We identified 17 positions around the acidic pocket as spatial constraints for the receptor-ligand interaction. Currently, we are performing in silico docking of the peptide onto the receptor to complete a detailed conformational model for the ASIC1a-Big Dynorphin complex. Our results will help to target the ASIC1a-Big Dynorphin interaction as a potential treatment in different neurological diseases, in particular in ischemic stroke.

B 08-8

Studying ligand binding and gating of rod CNG channels by FRET
K. Groeneveld, T. Gensch, C. Fahlke
§Jena University Hospital, Friedrich Schiller University Jena, Institute of Physiology II, Jena, Germany
*Jena University Hospital, Friedrich Schiller University Jena, Biomolecular Photonics Group, Jena, Germany

Cyclic nucleotide-gated channels (CNG) constitute the last step in the signal transduction cascade of photoreceptors and olfactory sensory neurons, translating to sensory stimuli into electrical signals. Ligand binding to CNG channels promotes an allosteric conformational change that leads to pore opening. A detailed knowledge of the ligand binding and activation mechanism of these channels is essential for understanding basic cellular functions and pathological processes. Herein we propose a novel approach to measure binding and gating of individual subunits by combining the patch-clamp technique with Förster resonance energy transfer measurements. To this aim we characterized a fluorescently-labelled cGMP (P1-cGMP), used as FRET acceptor, and eGFP-labelled rod CNG channels, as FRET donor. CNG channels were expressed in Xenopus oocytes and their function was tested using inside-out patches. When the labelled ligand binds to the channel’s binding site, energy from the donor fluorophore (eGFP) is transferred to the acceptor fluorophore (P1-cGMP). FRET efficiency was determined by measuring the decrease of donor fluorescence, allowing in this way the quantification of ligand binding. The efficiency of P1-cGMP to activate CNGA1-eGFP channels was close to that of the physiological ligand cGMP. The potency of P1-cGMP to activate the CNGA1-eGFP channels was by a factor of ~10 higher than that of cGMP. The eGFP-tag, which was inserted into the C-terminus close to the CNG-binding domain had only a minor influence on the channel’s function.

Poster Session B | 193
B 08-10
Elucidating Zinc Binding to the Voltage-Gated Proton Channel hHV1 Using Computer Simulations
C. Jardin, B. Musset
Klinikum Nürnberg Medical School, Institute for Physiology, Nuremberg, Germany

H1 voltage-gated proton channels are proton-specific ion channels with unique properties. For example, they are massively voltage-dependent and strongly inhibited by Zn2+. Our previous work demonstrated that one Zn2+ can stably be accommodated in the binding site of a single hH1 dimer. The consecutive experimental measurements were evaluated in agreement with this hypothesis. We tested this hypothesis and investigated the determinants of Zn2+ binding at the molecular level using computational approaches: molecular modeling, molecular docking, and multiple molecular dynamics simulations. Our results support the hypothesis enunciated above: The modeling and docking simulations show that the hH1 channels can form a dimer that present an appropriate interface for two Zn2+ binding sites, each involving a pair of equivalent histidine residues from each monomer. The molecular dynamics simulations reveal that two Zn2+ can stably be accommodated in the proposed binding sites. The zinc ions are coordinated by the histidine and additional acidic residues. Comparison with another possible dimer conformation and with the monomeric form of the channel also reveals why the dimer conformation hypothesized above is more able to coordinate zinc ions.

B 08-11
Activation and deactivation kinetics of class C GPCRs studied with confocal patch-clamp fluorometry
T. Kukal1, R. Schmauder1, T. Schwabe2, U. Zabel2, M. Lohse2, K. Benndorf1
1Friedrich Schiller University Jena, Institute of Physiology 2, Jena, Germany
2University of Würzburg, Institute of Pharmacology and Toxology, Würzburg, Germany

G-protein-coupled receptors (GPCR) constitute the largest and pharmacologically most important family of cell surface receptors. For the light receptor, rhodopsin, activation kinetics have been determined with a resolution of about 1 ms [1]. For other GPCRs, time constants in the range of 30-80 ms were reported [2]. Herein we studied the kinetics of activation and deactivation of mGluR1.Conformational changes between and within receptor subunits were followed by FRET. To study kinetics between two subunits, CFP and YFP were inserted into the i2-loop of each monomer[2], the “quality control system” of GABAR. We used control subunit composition of mGluR1 dimers. Kinetics within one subunit were studied by placing CFP in the C-terminus and YFP in the i2-loop. We measured the kinetics of mGluR1 sensors in isolated outside-out membrane patches from Xenopus laevis oocytes. Glutamate was applied by using a piezo device which generated a solution exchange within ~300 μs. This approach allowed us to study both activation and deactivation kinetics. The signal change was recorded by a confocal microscope. Our data show a fast intermolecular activation kinetics of ~1 ms which is by one order of magnitude faster than data published previously[2, 3]. These kinetics are not limited by the instrument’s response. Notably, for concentrations above 1 mM the activation speed was independent of the concentration, suggesting that the observed kinetics reflect in fact inter-subunit rearrangements and not ligand binding. Deactivation proceeded with a time constant of 41 ms. The activation kinetics within a receptor subunit were around 20 times slower. The observed deactivation kinetics were determined to be in the time range of tens of ms for the inter-subunit sensor, and hundreds of ms for the intra-subunit sensor; suggesting that activation and deactivation follow different pathways. The fast kinetics requires two functional binding sites. This was concluded from a 6-7 times slower activation of the inter-subunit sensor when disabling one of the two binding sites with the YADA mutation[6].

1 Hofmann et al. Trends in Biochemical Sciences 34: 540-552 (2009)
2 Hlavackova et al. Science Signaling 5 (237): ra99 (2012)
3 Marcogli et al. PNAS 11388-11393 (2009)
4 DeFlee B. et al. Nature 535, 183-186, doi: 10.1038/nature18324 (2016)
5 Stüts, D.P. et al. Nature 535, 448-452, doi: 10.1038/nature18360 (2016)
6 Kniazef et al. Nat Struct Mol Biol 11, 706-713, doi:10.1038/nsmb794 (2004)
neurons. Molecular insights into cell-specific neuronal defense mechanisms might serve as novel neuroprotective therapeutic strategies.

B 09 | Epithelial Barrier and Oxygenation

B 09-1 Heterologous expression of claudin-5 in Xenopus laevis oocytes
N. Brunner, S. Amasheh
Freie Universität, Institute of Veterinary Physiology, Berlin, Germany

Outline: The tight junction protein claudin-5 features barrier-tightening properties, and represents a major component of the blood brain barrier (1). We used the established heterologous expression system of Xenopus oocytes (2) to investigate the contribution of claudin-5 to the contact area of clustered oocytes and to evaluate the isolated effect of claudin-5 to cell-cell-interaction.

Methods: Oocytes were harvested from 4 adult female African claw frogs via surgical laparotomy and injected with 1 ng mRNA encoding for human claudin-5, or Phnase-free water as controls, respectively. After 3 days, oocytes were devitellogened and clustered in pairs of claudin-5-expressing and control oocytes as follows: ctrl-ctrl (n=53), claudin-ctrl (n=20), and ctrl-claudin (n=34) respectively. Width of contact was measured after 1h, 24h and 48h after clustering via bright field microscopy and the area of contact was calculated by using the circle equation (A = π × (measurement/2)²). Additionally, membrane protein fraction from injected oocytes were obtained and used for Western blot analysis, and oocytes were prepared for immunohistochemical staining.

Results: After injection of mRNA, Western blots of the membrane fraction revealed claudin-5 specific signals, whereas the water injected controls were negative. In accordance with these results, immunohistochemical staining revealed specific signals for claudin-5 in the plasma membrane. The contact area showed a time-dependent increase over time in all tested combinations. Statistical testing revealed no significant differences between claudin-5-claudin-5, claudin-ctrl, and ctrl-ctrl (Mann-Whitney-U, p > 0.05).

Conclusion: In our study, for the first time, we were able to expand the heterologous Xenopus laevis oocyte expression system towards the analysis of tight junction proteins to claudin-5. The injection of claudin-5 mRNA resulted in the integration of the protein into the plasma membrane, but the contact area did not change in contrast to oocytes coexpressing claudin-1, -2, and -3, as reported recently (2).

References:
1. Amasheh S, Schmidt T, Mahn M, Florian P, Markertz J, Tavallali S, Gitter AH, Schulze JD, Fromm M (2005). Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells. Cell Tissue Res. 321: 89-96.
2. Vitzthum C, Stein N, Brunner N, Knittel R, Faller-Becker P, Amasheh S (2019). Xenopus oocytes as a heterologous expression system for analysis of tight junction proteins. Faseb J. 33: 5312-5319.

B 09-2 Claudin 1 expression is induced by GATA3 in basal cells of human airway epithelium
R. Lochbaum, C. Schilpp1, P. Braubach1, M. Frick2, P. Dietl1, O. H. Wittekindt1
1Ulm University, Institute of General Physiology, Ulm, Germany
2Medizinische Hochschule Hannover, Institut für Pathologie, Hannover, Germany

Claudins are pivotal for epithelial barrier function and essential to establish an air-liquid-interface (ALI) in pulmonary epithelia. Epithelial barrier function is disturbed especially in inflammatory lung diseases and hence its repair is highly desired. The transcription factor GATA3 is involved in several repair processes including epithelial cell differentiation and is related to nuclear factor-kappaB (NF-κB) signaling. However, the role of GATA3 on claudin regulation in airway epithelia is unknown. Effects of ALI and liquid-liquid-interface (LLI) on TJ properties were investigated in NCI-H441 cells and in primary human Tracheal Epithelial Cells (TECs). Transepithelial electrical resistance (TEER) was measured to determine the paracellular permeability. Semi-quantitative RT-PCR, western blot- and immunofluorescence experiments were performed to identify differentially regulated TJ proteins and changes in GATA3 expression. Luciferase reporter gene assays were used to verify activation of Claudin 1 (Claudin1) by GATA3. Overexpression and silencing of GATA3 were performed to further investigate its impact on controlling Cldn1 expression. Human lung slices were analyzed for Cldn1 and GATA3 expression using HRP/DAB-immunohistochemistry.

LLI cultivation of NCI-H441 epithelia reduced TEER by approximately 40% and was in line with reduced Cldn1 expression (versus ALI). GATA3 became upregulated in ALI versus LLI cultivated epithelia. Overexpression of GATA3 increased Cldn1 expression in LLI, while silencing of GATA3 expression reduced Cldn1 in ALI-cultivated NCI-H441 epithelia. Immunochemistry revealed Cldn1 localization at TJ and along the lateral membrane of HTEpCs. GATA3 is localized both in differentiated and basal cells, with higher amount in the latter ones. Basal cells of HTEpCs had an increased Cldn1 promoter activity in luciferase assay experiments when GATA3 was overexpressed. NF-κB inhibitor IMD-0354 reduced Cldn1 promoter activity. Cldn1 localization in human lung slices agreed with Cldn1 localization in HTEpE cells. Evidently GATA3 enhances Cldn1 expression in human airway epithelium and in basal cells. GATA3 may link NF-κB signaling to epithelial barrier function and appears to be important in epithelial repair of the airways during inflammatory lung diseases. Supported by: Ministry of Science, Research and the Arts of Baden-Württemberg (Az: 32-7533-6-1015/5) / DFG, Pulmosens GRS2203 to O. H. Wittekindt.

B 09-3 Elevated apical surface liquid volume disturbs paracellular permeability in NCI-H441 epithelia
N. Schlüter, R. Lochbaum, C. Schilpp, P. Dietl, O. H. Wittekindt
Ulm University, Institute of General Physiology, Ulm, Germany

The pulmonary epithelium forms the surface of the airways and alveoli. It separates the air-filled compartment of the lung from the interstitium and establishes the organo-tropic-air interface (ALI) of the lung, which is essential for appropriate lung function. Chronic respiratory diseases disturb the ALI and result in elevated surface liquid volumes. Compensation of the increased apical volume requires ATP-dependent water resorption, which is believed to induce metabolic distress. In many epithelia metabolic distress activates AMP activated protein kinases (AMPK) that induce functional changes of tight junctions (TJ) and epithelial permeability. Herein we focus on the effect of elevated apical surface liquid volumes on AMPK expression and TJ permeability of pulmonary epithelia. NCI-H441 cells were used as a well-characterized model of the pulmonary epithelium. Cells were cultivated on transwell filters at submerged conditions for 4 days. Afterwards, cells were kept at ALI conditions for 5 additional days to achieve epithelial differentiation. Liquid-liquid interface (LLI) conditions were established by adding a defined volume of medium onto the apical surface. Cells were investigated on day 2 of LLI. Control conditions remained at ALI conditions. Epithelial permeability was quantified by measuring transepithelial electrical resistance (TEER) as well as the apparent permeability coefficient for 4Ka dextran. Expression level of claudins and AMPK were measured by semi-quantitative RT-PCR.

Compared to ALI cultivated cells, LLI reduces TEER by approximately 45% and increases the apparent permeability coefficient. This indicates that LLI increases paracellular permeability. Paracellular permeability is limited by claudin composition of TJs. Hence we investigated changes in claudin expression levels of LLI versus ALI cultivated epithelia. We observed an overall increase in claudin expression in cells exposed to LLI. We further detected elevated expression of AMPK, metabolism in LLI subjected cells.

Our results show that LLI increases paracellular permeability in NCI-H441 epithelia caused by disturbance of TJ function. We assume that claudin upregulation occurs as a compensatory mechanism. Further experiments will reveal the role of AMPK in claudin modulation in LLI exposed epithelia. Supported by: Ministry of Science, Research and the Arts of Baden-Württemberg (Az: 32-7533-6-1015/5) / DFG, Pulmosens GRS2203 to O. H. Wittekindt.

B 09-4 Innate Immune Response Elicitation in an in-vitro model of Acute Lung Injury reveals a Cusp Catastrophe
M. Fauler, E. Wirsching, M. Frick
Ulm University, Institute of General Physiology, Ulm, Germany

Acute Respiratory Distress Syndrome (ARDS) or Acute Lung Injury is a frequent cause of respiratory failure in critically ill patients. It occurs in approximately 10% of patients in intensive care units with various underlying diseases. Despite intense basic and clinical research, mortality remains high. It is caused either by direct alveolar epithelial and endothelial damage or indirectly via an exuberant activation of the innate immune response, e.g. in the course of a systemic inflammatory response syndrome, leading to a breakdown of the alveolar air-blood-barrier and formation of an intra-alveolar exudative oedema. Although many of the cellular and humoral factors involved in the activation and control of the immune system in ARDS have been identified, how these are functionally integrated is not well understood.

Therefore, we have developed an in-vitro model of the air-blood barrier that enables a detailed investigation of systemic dynamics aspects during the immune activation process at high time and spatial resolution. We use isolated alveolar epithelial cells and alveolar macrophages from rat lungs cultivated on porous cell culture inserts to high transepithelial electrical resistances. An innate immune response is elicited by Lipo polysaccharide (LPS) in the presence of response-modulating cytokines, enzyme inhibitors or signalling molecule receptor blockers. Breakdown of the epithelial barrier integrity is monitored by impedancediagnostic spectroscopy at narrow time steps and immunofluorescence imaging.
The LPS concentration response is not continuous but reveals a threshold phenomenon. Based on enzyme inhibition and specific receptor blocking data, we propose a core mechanism of innate immune response elicitation focusing on a bi-lan network motif of mutual inhibition and positive auto-feedback for the stabilisation of either an inactivated or activated system state. The role of the purinergic system and arachidonic acid derived lipid mediators is elucidated within this pathophysiological concept. A diffusion-reaction model is utilized to identify the activation threshold from experimental data and its modulation by cytokines.

This approach reveals a cusp catastrophe as the functional backbone of immune response activation. Relevance and implications of this type of bi-stability in system behaviour for the development of new pharmacological treatment strategies are discussed.

B 09-5

The hypoxia-inducible factor 2 regulates brain remodeling after ischemic injury

T. Lee, J. Fandrey, T. Schreiber
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

Ischemic hypoxia results from insufficient blood flow and causes ATP depletion and rapid cell death in consequence of lacking adequate amounts of oxygen and nutrients. Contrary to previous assumption, the brain is capable of modest regeneration. Moreover, it was shown that hypoxia and hypoxia-inducible factor (HIF) are key factors in neural regeneration. Especially HIF-2α is distributed tissue specific and expressed in the developing brain. It modulates gene activity in response to low oxygen and protects neural progenitor cells and neural differentiation processes. But in general, the role of HIF–α during neural development is poorly understood.

To investigate the impact of HIF on neural regeneration, we established a murine neurensphere culture for wildtype (WT) and HIF-2α-knockout (KO). With this 3D model, we want to unravel the signaling pathways of HIF-2 under hypoxia and especially its function in basic processes of brain development like neural progenitor cell proliferation, migration, differentiation and apoptosis. This is fundamental to understand and clarify the role of HIF-2 in brain regeneration after ischemia such as an ischemic stroke. With focus on the signaling pathway of HIF, we challenged proliferating and differentiating neurenspheres with up to 4 h oxygen/glucose-deprivation (OGD: 0.2 % O2; glucose free medium) to simulate an ischemic stroke in vitro. Afterwards, we analyzed the migration capability of the cells. Before OGD, WT cells had significant better abilities to migrate than the KO. After OGD, neurenspheres migrated significantly less in general, but not different considering the genotypes. Additionally, mRNA analyses showed a strong effect on the expression of genes involved in neurogenesis. Here, genes like Ngr1, which has a protective function against astroglisis, NeuroD1, which is important for neuron differentiation, or Gnr1, which is critical for neuronal connectivity and survival, significantly differ between WT and KO cells after challenging the spheres with OGD.

These data suggest a restricted capability to regenerate from ischemia without HIF-2α and decode its role in (re)modeling the CNS.

B 09-6

Characterization of sFLT-1 in human retinal microvascular endothelial cells under hypoxic and normoxic conditions

Characterization of sFLT-1 in human retinal microvascular endothelial cells under hypoxic and normoxic conditions

J. Ernesti, R. Deppezing, M. Ranjar1
1Department of Ophthalmology, University Hospital Schleswig-Holstein, Lübeck, Germany

Pathogenic ocular angiogenesis, the increased formation of aberrant blood vessels in vascular beds in the eye is the result of severe retina-associated disorders like proliferative diabetic retinopathy (PDR) or wet age-related macular degeneration (wet AMD) [1]. Although this eye diseases are common causes for loss of sight in Europe and North America the molecular details of neovascularization in the posterior eye segment is not fully understood [1,2]. In the last couple of years treatments based on anti-vascular-endothelial-growth-factor (VEGF) immunotherapies were established to slow down the chronic disease process by inhibiting the proangiogenic influences of VEGF-A on endothelial cells (EC) [3]. VEGF-A immunotherapies are based on the fact that both PDR and wet AMD are associated with hypoxia as a stimuliatory factor for choroidal and retinal neovascularization (CNV, RNV) [2,3]. The lack of oxygen in affected cells triggers the transcriptional upregulation of several genes including proangiogenic growth factors like VEGF-A [4, 5]. As a result, aberrant vessel sprouting on pre-existing blood vessels damages the surrounding tissue [1]. One of the proteins which regulate VEGF-A induced vessel sprouting is a soluble isomer of Fms-Related Tyrosine Kinase 1 (sFlt-1), a high-affinity tyrosine kinase receptor for VEGF-A [6]. Release of sFlt-1 followed by binding of VEGF A in spatial proximity to the sprouting vessels leads to localized VEGF-A gradient around the leading tip cell and therefore supports vessel sprouting [7]. Our work focused on the quantitative characterization of sFlt-1 in human retinal microvascular endothelial cells (HRMEC) under normoxic and hypoxic conditions with or without VEGF A. By using tube-formation assays (TFA) as a two-dimensional model for the initial steps of angiogenesis in cell culture, we show that hypoxia decreases the total tube length of HMVEC, while the proangiogenic influence of VEGF-A on tubulogenesis is weak. The expansion of this experiments by combining TFA with ELISA and Immunocytochemistry (ICC) allows us to compare secretion, intracellular availability and storage in the hepatine sulfate proteoglycan-containing matrix of sFlt-1 between the above-mentioned conditions.

B 09-7

Mitochondrial complex IV mutation increases ROS production and reduces lifespan in aged mice

G. Reichart1, J. Mayer1, C. Zehm1, T. Tokai1, F. Lange1, S. Baltuch1, S. Ibrahim3, R. Köhling1
1Research Institute of Physiological Medicine Center, Oscar Langendorff Institute of Physiology, Rostock, Germany
2Rostock University Medical Center, Institute of Medical Biochemistry and Molecular Biology, Rostock, Germany
3Lübeck University Medical Center, Department of Dermatology, Lübeck, Germany

Question: Large-scale miRNA defects are widely known to have a negative impact on lifespan and tissue integrity. The presently study asks the question whether also relatively isolated and specific point mutations in the mitochondrial genome can have observable effects on lifespan and tissue function. Here we examined the effect of single nucleotide polymorphism (SNP) in the complex IV gene.

Methods: We characterized the canine complex IV mouse strain C57BLXj-mtNOD carrying an electron transport complex IV mutation that leads to an altered cytochrome c oxidase subunit III. Using MitoSOX Red fluorescence we measured brain superoxide levels in different age groups. Additionally we analyzed mitochondrial function by gene expression analysis and mitochondrial dynamic network formation. Finally we investigated the impact of the complex IV mutation on learning and memory as well as lifespan.

Results: 24-month-old mutant mice showed elevated mitochondrial superoxide production and a reduced gene expression of superoxide dismutase 2. Together with the decreased expression of the fusion-relevant gene Fis1, these data confirmed that the agemy mtNOD mouse had a mitochondrial dysfunctional phenotype. On the functional level, we found a markedly poor physical constitution to perform the Morris water maze task at the age of 24 months. Moreover, the median lifespan of mtNOD mice was significantly shorter than of control animals.

Conclusion: Our findings demonstrate that a single nucleotide polymorphism in complex IV leads to mitochondrial dysfunction that translates into survival.

B 09-8

Chemogenetic control of acute H2O2 production in cells with D-amino acid oxidase (DAAO)

N. Mueller, K. Noack, P. F. Malacarne, F. Rezende, K. Schröder, R. P. Brandes
Goethe-University Frankfurt, Vascular Research Centre, Frankfurt, Germany

Background: Hydrogen peroxide (H2O2) has important redox signaling functions in the vascular system in response to growth factors, cytokines and calcium signals. These conclusions are often based on in vitro studies where exogenous H2O2 is added in supra physiological concentrations to cells or its production is induced by chemical compounds with uncharacterized effects. In order to define the endogenous H2O2 production on cell function, we here tested a chemogenetic way of controlled intracellular production.

Methods: To induce acute intracellular production of H2O2 in HEK cells (human embryonic kidney cells) and HUVEC (human umbilical vein endothelial cells) D-amino acid-oxidase (DAAO) was overexpressed by a lentiviral system. This enzyme converts D-amino acids into inimino acids and produces H2O2 as a byproduct. Subsequently, H2O2 production was increased by providing D-Ala-Lys-D-Ala (D-ALAa) Treatment with D-ALAa but not L-ALAa (1-100 μM) led to increased intracellular production of H2O2 as measured by chemiluminescence with LuminoHR and Ampex redb. PE-Glylated catase (250μM) reduced this signal by approx. 50% whereas the DAAO inhibitor 4h-ido (3,2-[4-hydroxy-5- carboxylic acid (1μM-L) completely blocked H2O2 production. DAAO-derived H2O2 (10 μM), D-ALAa, 10μM) had intracellular signaling function as it increased oxidation of peroxidase-3 and phosphorylation of p38 MAP kinase.

Conclusion: The DAAO system is a valuable tool to study dynamic changes in physiological redox signaling.

B 09-9

The role of hypoxia-inducible-factors on the microbial composition of mice with analysis of functional consequences

F. Wichmann, J. Fandrey, S. Winning
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

Colorectal cancer (CRC) is one of the most common types of cancer worldwide, with nearly two million new cases in 2018. A considerable fraction of cases are deemed to be associated with changes in the composition of intestinal microbiota (IM). Additionally, a connection between poor prognosis in colorectal cancer treatment and overexpression of hypoxia-inducible factor (HIF) could be established. Though manipulation of HIF via floatal transplantation has already shown to be
effective in ameliorating chronic intestinal inflammation, the understanding of interplay between microbiota and gut epithelium and its effect on tumorigenesis remain on an unsatisfactory level. To examine the effect of IM on intestinal epithelium with special regard to the influence on HIF, we developed a co-culture model of the rather protective species Lactobacillus acidophilus or the potentially harmful Enteroarcoccus faecalis and colonic organoids derived from wildtype (WT) and Factor-Inhibiting-HIF-knockout (KO) mice. Used mice were also split into two groups, one of which received servings of acyclovir (ACV) and dextran sodium sulphate (DSS) to induce colitis in addition. With this setup, we want to untangle the influence of IM on CRC and the immune signaling pathways under tumor hypoxia and expression of HIF. This is essential to understand the contribution of microbiota to pathogenesis of cancer tissue and to discover future treatment options for CRC.

We want to expose described co-cultures to anoxia (0.1% O2) or hypoxia (3% O2) for up to 8 hours to simulate tumor hypoxia and consequent cellular conditions in vitro. After exposure, we want to analyze protein and mRNA levels of HIF and targeted genes or interacting proteins of HIF in the organoid culture. Due to recent studies on protective functions of bacterial species in intestinal diseases, we expect these studies to reveal a deeper insight into the interplay of HIF and IM in development and progress of CRC.

B 09-10
Hematopoietic Hypoxia-inducible factor 2α deficiency ameliorates pathological retinal neovascularization via modulation of endothelial cell apoptosis.

A. Klotzsche - ven Aemili^{1}, I. Korovina^{2}, A. Neuworth^{2}, B. Sprott^{2}, B. Wieloch^{2}, T. Chavakis^{2}, A. Drussena^{1}

1TU-Dresden, Institute of Physiology, Dresden, Germany
2TU-Dresden, Institute for Clinical Chemistry and Laboratory Medicine, Dresden, Germany

A hallmark of proliferative retinopathies, such as retinopathy of prematurity (ROP), is a pathological neovascularization orchestrated by hypoxia and the resulting hypoxia-inducible factor (HIF)-dependent response. We addressed the question whether pathological retinal neovascularization in the murine model of ROP, the oxygen-induced retinopathy (OIR) model, is regulated by Hif2α in hematopoietic cells. Briefly, in the OIR-Model seven-days old mice were exposed to 75% O2 for 5 days, and then returned to normoxic conditions. Eyes were collected at postnatal day 17 and neovascularization was quantified by counting of epiretinal nuclei in PAS stained whole mounts. In order to study hematopoietic-specific deletion of Hif2α, Var-cre (Stadtfeld et al., 2005) mice were crossed with Hif2αf/f (Guerard et al., 2007) mice. Uterinates from Var-Cre-Hif2αf/f and hematopoietic Hif2αf/f mice were used in the experiments. We found that a hematopoietic-specific deficiency of Hif2α ameliorated the pathological neovascularization in the OIR-model, which was associated with enhanced endothelial cell apoptosis. Enhanced apoptosis was concluded from increased cleaved caspase-3 staining in the pathological endothelial cells in retinal whole mounts. Further in-vivo experiments will perhaps blaze a trail for miR663 as a novel treatment for anemia seen in chronic inflammatory diseases. This study is supported by The Scientific and Technological Research Council Of Turkey (TUBITAK, no: 216S729), from the Eskişehir Osmangazi University Scientific Research Projects Committee (no: 2017-1635), Turkey and from Erwin-Risch Stiftung, Germany.

B 09-11
Pharmacological prolyl-4-hydroxy domain enzyme inhibition increases a stem cell-like population in the kidney.

A. Jatto, K. Brechtle, A. Zieseniss, D. M. Katschinski

University Medicine Göttingen, Institute for Cardiovascular Physiology, Göttingen, Germany

Inhibition of the prolyl-4-hydroxy domain (PHD) enzymes is thought to induce tissue protective effects for example in case of ischemia. Cells with a pro- or cell stem-like behavior have been shown to take part in tissue regenerative effects in multiple disease models. We analyzed, if the PHD inhibitor roxadustat (rox) affects the stem cell like antigen-1 (Sca1) positive side population in various tissues. C57Bl/6J male mice were injected with rox for 7 or 14 days, a third group was injected 7 days and kept 7 days without the inhibitor (7 on/off). Various organs and tissues (liver, kidney, atrium, left and right ventricle, M. biceps femoris, peripheral blood and bone marrow) were harvested. Sca1+ cells were subsequently quantified by FACS sorting. Among all tissues analyzed, the kidney and bone marrow showed the highest total count of Sca1+ cells, whereas in liver and blood samples the total count of these cells was significantly lower. In rox-treated mice a significant increase in the total number of Sca1+ cells by 10% was found after injection of the compound for 7 days compared to control. A further increase in number after 14 days of injections could not be detected. Upon exposure and treatment a significant difference comparing controls to rox-treated Sca1+ cells was found. Animals treated with rox showed an increase in erythropoietin (Epo) RNA expression in the kidney as well as in serum Epo levels. Total RNA expression profiles of the Sca1+ cells showed markers for mesenchymal stem cells (Pax9, CD20, CD105, CD44, CD90 and CD146) to be found. Chondrogenesis, osteogenesis and adipogenesis could successfully be induced in the Sca1+ population (BMDMs) with conditioned medium from Sca1+ cells treated with or without rox led to a more anti-inflammatory phenotype.

In conclusion, the application of rox increases a Sca1+ population that holds the potential to support function and healing as well as hematopoiesis in case of kidney insufficiency. This study is supported by R. Ozyurt, A. Karadag, N. Erkasap1

1Eskişehir Osmangazi University, Pathology Department, Eskişehir, Turkey
2University of Lübeck, Institute of Physiology, Lübeck, Germany

Question: In chronic inflammatory diseases, pro-inflammatory cytokines such as TNF-α are present in high amounts in the circulation and are associated with anemia in most cases. Experimental studies have shown that TNF-α inhibits the synthesis of Erythropoietin (Epo), the main stimulant of hematopoiesis. The underlying mechanisms still remain elusive. Our aim was to figure out which micro-RNAs are involved in the Epo repression by TNF-α.

Methods: First we determined the optimal dose of TNF-α in HepG2 cells that has no cytotoxic effect by MTT assay and that inhibits Epo synthesis by qRT-PCR and ELISA. Then we performed the micro-RNA array study with TNF-α (20 ng/ml) treated cells and a control group. The array data results were confirmed by qRT-PCR. We compared miR663 group with the miR663 mimics (30 pmol) for 24 hrs, other groups only treated with transfection reagent, followed by treatment of TNF-α and miR663 groups with TNF-α for 24 hrs, control group incubated with normal medium. We analyzed Epo mRNA level by qRT-PCR. Next, we co-cultured HepG2 with UT-7 cells with TNF-α dependent HIF663 mimics prevents the Epo-repression by TNF-α more UT-7 cells would survive. Percentage of apoptotic UT-7 cells were determined by TUNEL assays. Statistical analyses were performed by one-way Anova and Tukey test for post-hoc. P-values smaller 0.05 were considered as statistical significant.

Results: According to our array study, TNF-α significantly decreases miR663 expression. After transfection of miR663 mimics into HepG2 cells, TNF-alpha was unable to decrease Epo mRNA amounts. Furthermore, miR663 mimics transfection in HepG2 cells resulted in a lower apoptosis rate of Epo dependent UT-7 cells in co-culture experiments.

Conclusions: Our study showed that miR663 is involved in Epo mRNA production and that is able to prevent or reverse the inhibitory effect of TNF-α. In our co-culture study, we showed that TNF-α inhibition over Epo synthesis in HepG2 cells raised apoptosis of Epo dependent UT-7 cells and transfecting HepG2 cells with miR663 mimics decreases apoptosis of UT-7 cells. Further in-vivo experiments will perhaps blaze a trail for miR663 as a novel treatment for anemia seen in chronic inflammatory diseases.

This study is supported by The Scientific and Technological Research Council Of Turkey (TUBITAK, no: 216S792), from the Eskişehir Osmangazi University Scientific Research Projects Committee (no: 2017-1635), Turkey and from Erwin-Risch Stiftung, Germany.

B 09-13
Protein kinase B regulates mitochondrial bioenergetics in isolated heart mitochondria.

T. C. Steemer, A. Heinen, E. Lachmann, S. Gödecke, A. Gödecke

Heinrich-Heine Universität, Institut für Herz- und Kreislaufphysiologie, Düsseldorf, Germany

Introduction: Protein Kinase B (AKT) is a key regulator of multiple cellular processes including cardiac glucose uptake and metabolism. The influence of AKT on mitochondrial bioenergetics is only fragmentarily understood. A potential isofrom-specific effect was proposed as the loss of the AKT1 isoform causes a reduction of mitochondrial complex V activity in ruptured mitochondrial membranes (Yang et al, 2013). However, it is unknown whether 1) AKT regulates oxidative phosphorylation in intact mitochondria, and 2) this potential regulation is isofrom-specific.

Methods: Experiments were performed using male mice with inducible, cardiomyocyte-restricted knock out of either AKT1 (ICM-AKT1) or AKT2 (ICM-AKT2), or wild type animals (WT). The knock out was induced by daily 4-hydroxytamoxifen injections for five consecutive days, and cardiac mitochondria were isolated by differential centrifugation 15 min after in vivo insulin (3 U/kg, i.p.; +/– insulin) treatment. ADP stimulated state 3, resting state (4), and uncoupled respiration (OCR/CO2) were measured using complex I substrate pyruvate/glutamate/malate. In addition, respiratory control ratios (RCR) were calculated as state 3/state 4.

Poster Session B | 201

Poster Session B | 202
B 10-1 Differential adaptation of endothelia to acute and chronic changes in pressure

J. Feis
University of Witten/Herdecke, Institute of Physiology, Pathophysiology & Toxicology, Witten, Germany

Blood pressure is the key parameter used to determine cardiovascular health. While chronic pressure elevations are associated with endothelial dysfunction and end organ damage, transient short-term pressure increases may even be linked to beneficial effects. Despite the considerable variations of blood pressure in health and disease, little is known about the molecular and cellular effects of haemodynamic stress on endothelial cells. Especially short-term pressure variations and their impact on endothelial function are poorly investigated.

A combination of quantitative fluorescence microscopy, atomic force microscopy and molecular perturbations was used to characterize the specific response of endothelial cells to acute (1h) or chronic (24h) pressure application (100mmHg).

Endothelial cells react in a two-phase-response to increased haemodynamic stress. While chronic pressure elevations lead to endothelial cortical stiffening caused by apical F-actin formation, short-term pressure exposure stiffer endothelia via an increase in myosin contractility. Pressure-dependent myosin activation could be prevented by application of blockers for mechanosensitive ion channels (GmATP-4, amiloride). Additional knock-down studies suggest the involvement of specific endothelial cell marker genes.

The described two-phase pressure response may participate in the differential effects of transient and chronic changes in blood pressure.

B 10-2 Bayesian parameter estimation and model selection for impedance spectroscopy data of endothelial monolayers

F. Zimmermann, F. V. Härtel, A. Das, T. Noll, P. Dieterich
TU Dresden, Medizinische Fakultät Carl Gustav Carus, Institut für Physiologie, Dresden, Germany

Question: Endothelial barrier function is frequently studied in vitro measuring the complex electrical resistance of endothelial monolayers cultured on a permeable filter. The transendothelial resistance (TER) as typical parameter of interest, however, can only be determined indirectly based on a mathematical model. Models usually comprise a sequence of a resistance in parallel with a capacity (RC-circuit), each for the cell layer (including the TER), one resistance (R) for the medium and a constant phase element (CPE) for the electrodes. The validity of such a model and its alternatives have been discussed, but a technique for quantitative comparison is still missing. Thus, we aim to develop a method that allows to select the best mathematical model for observed experimental data and to perform a valid estimation of parameters and their uncertainties.

Method & Results: We established an algorithm based on Bayesian data analysis. It allows the inclusion of experimental uncertainties and prior knowledge of the model parameters as well as the calculation of model probabilities. Phase and absolute values of impedance data (3 Hz-100 kHz) were acquired over time by a commercial device (cellZscope®, nanoAnalytics, Münster). Uncertainties of the data were estimated based on stationary measurements. The algorithm was applied to measurements of pure medium, of medium and raw filter, and of human endothelial monolayers (HUVEC) grown on a filter and stimulated with different agents. Bayesian model evidences showed that medium and filter are best described by three RC-circuits in series, which is in contrast to usually applied models. The cell monolayer requires another RC-circuit and modifies the filter properties. Thus, parameters of filter or medium from measurements without cells cannot be used as direct references for those with cells. Parameter estimation of the TER was robust with small uncertainties and a concentration-dependent decrease was observed in response to thrombin (0.1-2 nM). In addition, the model comparison indicated that even high concentrations of thrombin did not fully disrupt the endothelial barrier.

Conclusion: A novel algorithm has been implemented, which allows a valid parameter estimation and the selection of models with different complexity under various experimental conditions to characterize the endothelial barrier function. This algorithm can be extended to more complex setups like co-cultures of different cell types.

B 10-3 A Meta-Analysis Of Vitamin D-Dependent Transcriptomes Suggests Cell Type-Independent Gene Regulation By A Conserved 3D Chromatin Structure

T. E. Warren, S. Seuter, R. P. Brandes
1Goethe-University Frankfurt, Institute for Cardiovascular Physiologie, Frankfurt am Main, Germany
2CPI, Cardio-Pulmonary Institute, Frankfurt am Main, Germany

Question: Is the regulation of gene expression by 1,25-dihydroxyvitamin D via changes in 3D chromatin structure conserved across cell types?

Background: 1,25-dihydroxyvitamin D (1,25D)–mediates most of its signalling through the vitamin D receptor (VDR). VDR binds to vitamin D response element (VDRRE) in the promoter region of many genes resulting in a context-dependent gene induction or inhibition. 1,25D changes the transcription of monocytes, in some cases acting on sets of genes organized into topologically associating domains (TADs). 1,25D also modulates the transcription of other cell types, including airway smooth muscle cells, fibroblasts and bronchial epithelial cells. To elucidate conserved vitamin-D regulated genes across these cell types, a meta-analysis of RNA-sequencing data from seven separate studies was performed.

Methods: A meta-analysis of 1,25D-regulated transcriptomes was carried out using Salmon and DESeq2 to reanalyze raw sequencing data via a common pipeline. Gene clustering analysis, gene ontology analysis, motif enrichment analysis, and network construction based on a distance probability matrix was then performed on a conserved gene set which was differentially regulated across multiple datasets.

Results: Clustering analysis of differentially regulated genes present in at least three datasets revealed that genes regulated by 1,25D are located in groups of close proximity on a chromosomal scale. Gene ontology enrichment analysis suggests a conserved role for 1,25D in regulation of the extracellular matrix formation across cell types. Motif enrichment analysis, rather than an expected enrichment of the vitamin responsive element (VDRRE), showed a specific motif enrichment.

Conclusion: The present data suggest that, throughout many cell-types, 1,25D alters the expression of genes organized into TADs. VDR binding activates numerous signaling networks, so the VDR binding signature is lost in the noise of a general transcription alteration response.

B 10-4 Sulfhydration of β3 integrin controls its cell surface expression and mechanotransduction in endothelial cells

S. I. Bibli, J. Hu, V. Rambimboavony, B. Fishtaller, M. S. Leisegang, A. Weiger, R. Papapetropoulos, R. P. Brandes, F. Sigala, I. Wittig, I. Fleming
1Goethe University, Institute for Vascular Signalling, Centre for Molecular Medicine, Frankfurt am Main, Germany
2Goethe University, Institute for Cardiovascular Physiology, Frankfurt am Main, Germany
3Biomedical Research Foundation of the Academy of Athens, Clinical, Experimental Surgery and Translational Research Center, Athens, Greece
4National and Kapodistrian University of Athens Medical School, First Propedeutic Department of Surgery, Vascular Surgery Division, Athens, Greece
5Goethe University, Functional Proteomics, SFB 815 Core Unit, Frankfurt am Main, Germany

Background/aim: The expression of the cystathionine g lyase (CSE) in the endothelium is regulated by blood flow. CSE generates polyamines which sulfhydrylate endothelial proteins to preserve endothelial function and inhibit atherosclerosis. The aim of the present study was to investigate the consequences of the CSE on the endothelial cell sulfhydrolome.

Results: In human and murine endothelial cells in situ CSE mRNA and protein levels were lower in areas of laminar versus disturbed flow. Mapping the sulfhydrolome of cultured human endothelial cells exposed to laminar flow versus static conditions or flow plus a polysulfide donor, more than 300 sulfhydrolated proteins were identified. A significant number of peptides were enriched, with 133 integrin the most highly sulfhydrolated target of the group. These effects were also evident in the cultured
and native murine endothelial cells from wild-type versus or CSE endothelial specific inducible knock out mice. The lack of -coupled receptors. These findings indicate a potentially mediated signaling by intracellular cannabinoid receptors. RNA sequencing data revealed a massive upregulation of the nuclear receptor family NR4A in response to AEA. This receptor family is particularly known for anti-inflammatory signaling by inhibition of NF-κB regulated genes. Knockdown of NR4A by siRNA blocked the anti-inflammatory effect of AEA whereas specific NR4A agonists (CsnB, C-DIM12) mimic the anti-inflammatory actions of AEA. In line with this, microscale thermophoresis demonstrated a direct binding of AEA to the NR4A receptors. Furthermore, reporter gene assays in HEK293 carrying the receptor binding motifs, showed that AEA promotes NR4A recruitment to the DNA. This recruitment reduced luciferase expression, an effect enhanced by NR4A overexpression and decreased by AEA knockdown. Thus, AEA may activate NR4A receptors by direct binding and then promotes interaction with nuclear gene repression complexes. Indeed, AEA increased suppressive epigenetic marks like H3K4me1 and reduced H3K27 acetylation at promoters of inflammatory genes as determined by chromatin-immunoprecipitations. Consequent siRNA screening and protein complementation assays identified the nuclear receptor complex NCoR1 as the AEA-dependent NR4A interacting protein.

Conclusion: CSE-derived H2S contributes to vascular homeostasis by supplying and proteins involved in endothelial cell mechanosensing and mechanotransduction. Pharmacological intervention to enhance circulating H2S was able to alter the sensitivity of the endothelium to respond to flow in both murine and human arteries.

**B 10-5**

**Vasorin suppresses osteo-/chondrogenic transdifferentiation of vascular smooth muscle cells via inhibition of TGFβ1 signaling**

T. T. Luong1, M. Estepa1, B. Boehmke1, B. Plesiek1, F. Lang2, K. - U. Eckardt1, I. Alesutan4

1Charite – Universitätsmedizin Berlin, Department of Internal Medicine and Cardiology, Berlin, Germany
2Eberhard-Karls-Universität Tübingen, Department of Physiology I, Tübingen, Germany
3Charite – Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, Berlin, Germany
4Johannes Kepler University, Linz, Institute for Physiology and Pathophysiology, Linz, Austria

**Question:** Vasorin is a transmembrane glycoprotein with high expression levels in vascular smooth muscle cells (VSMCs) that binds directly transforming growth factor (TGFβ) to suppress TGFβ1-dependent signaling. TGFβ1 contributes to the development of medial vascular calcification during hyperphosphatemia, an active process mediated by osteo-/chondrogenic transdifferentiation of VSMCs. Therefore, the present study investigated the effects of vasorin on osteo-/chondrogenic transdifferentiation and calcification of VSMCs.

**Methods:** Experiments were performed in vitro in primary human aortic smooth muscle cells (HAsMSCs) treated with recombinant human TGFβ1 or β-glycerophosphate without or with additional treatment with recombinant human vasorin. Results: Treatment of HAsMSCs with vasorin (VASN) mRNA expression was downregulated following exposure to TGFβ1. Additional treatment with exogenous vasorin suppressed TGFβ1-dependent signaling in HAsMSCs as shown by blunted SM202 phosphorylation and mRNA expression of the downstream targets: TGFβ type 1 receptor (ALK5), plasminogen activator inhibitor (PAI-1) and transcription factor SOX9. In addition, the TGFβ1-induced increase of the osteogenic transcription factor CBF1 mRNA and protein expression as well as of the osteogenic enzyme tissue-specific alkaline phosphatase (ALP) mRNA expression and activity and thus, of osteo-/chondrogenic transdifferentiation of HAsMSCs, were all inhibited by addition of vasorin to the cell culture medium. Furthermore, phosphate treatment suppressed VASN mRNA expression in HAsMSCs. Addition of vasorin did not modify phosphate-induced TGFβ1 mRNA expression, but blunt the phosphate-induced TGFβ1-dependent signaling, osteo-/chondrogenic transdifferentiation as well as calcification of HAsMSCs.

**Conclusions:** Vasorin expression is suppressed by TGFβ1 or phosphate in VSMCs. Vasorin supplementation inhibits TGFβ1-dependent osteo/chondrogenic transdifferentiation and calcification of VSMCs. Thus, vasorin may represent a potential therapeutical target to reduce the progression of vascular calcification during hyperphosphatemic conditions such as chronic kidney disease.

**B 10-6**

**Activation of NR4A receptors: An unexpected anti-inflammatory function of the endocannabinoid Anandamide**

B. Pflüger-Müller1, M. S. Leisegang, R. P. Brandes

Goethe Universität Frankfurt, Kardiologische Physiologie, Frankfurt am Main, Germany

Objective – Endocannabinoids are an important class of lipid mediators whose levels are altered in a variety of different disease states. One of the best-characterized endocannabinoids, which mediates inflammation but the effect in the cardiovascular system is controversal. We therefore set out to identify the impact of AEA signaling in vascular smooth muscle cells. Results – Aortic smooth muscle cells were pretreated with AEA and subsequently stimulated with pro-inflammatory stimuli to test the anti-inflammatory properties of this endocannabinoid. Incubation of murine (H40mSCM) and human aortic smooth muscle cells (HAoSMC) with AEA decreased a subset of inflammation-induced genes on mRNA and protein level.

Interestingly, these effects were specific for AEA and not mediated by peroxisome proliferator-activated receptors (PPARs) or the classical Gα1 coupled receptors. These findings indicate a potentially mediating signaling by intracellular cannabinoid receptors. RNA sequencing data revealed a massive upregulation of the nuclear receptor family NR4A in response to AEA. This receptor is particularly known for anti-inflammatory signaling by inhibition of NF-κB regulated genes. Knockdown of NR4A by siRNA blocked the anti-inflammatory effect of AEA whereas specific NR4A agonists (CsnB, C-DIM12) mimic the anti-inflammatory actions of AEA. In line with this, microscale thermophoresis demonstrated a direct binding of AEA to the NR4A receptors. Furthermore, reporter gene assays in HEK293 carrying the receptor binding motifs, showed that AEA promotes NR4A recruitment to the DNA. This recruitment reduced luciferase expression, an effect enhanced by NR4A overexpression and decreased by AEA knockdown. Thus, AEA may activate NR4A receptors by direct binding and then promotes interaction with nuclear gene repression complexes. Indeed, AEA increased suppressive epigenetic marks like H3K4me1 and reduced H3K27 acetylation at promoters of inflammatory genes as determined by chromatin-immunoprecipitations. Consequent siRNA screening and protein complementation assays identified the nuclear receptor complex NCoR1 as the AEA-dependent NR4A interacting protein.

Conclusion: Activation of NR4A receptors: An unexpected anti-inflammatory function of the endocannabinoid Anandamide

**B 10-7**

**Control of endothelial nitric oxide synthase expression by transcription factors of the Stat family**

C. Rumig1, R. Kerber, M. Hecker

Institute of Physiology and Pathophysiology, Department of Cardiovascular Physiology, Heidelberg, Germany

Background: Endothelial nitric oxide synthase (NOS3) is the major source of the vasodilator and anti-inflammatory nitric oxide (NO) in the vascular system. Its expression is maintained primarily by unidirectional fluid shear stress (FSS) to which solely endothelial cells (EC) are exposed. A single nucleotide polymorphism (T-786C SNP) within the promoter of the human NOS3 gene limits its responsiveness to FSS or anti-inflammatory stimuli. Homozygosity for the -786C variant proved to be an independent and strong predictor for both coronary heart disease and rheumatoid arthritis. Here we have analyzed (epi-)genetic control mechanisms of gene expression through chromatin remodeling in the context of genotype-dependent, temporally different interaction of members of the Stat family of transcription factors with the promoter of the NOS3 gene in human EC.

Methods: After treatment with different drug oligodeoxynucleotides (ODN) mimicking potential Stat binding sites in the promoter of the human NOS3 gene, CC or TT-genotype human EC were exposed to unidirectional FSS (30 dyn/cm²) using a plate and cone viscometer. NOS3 expression was analyzed by quantitative RT-PCR and Western blot.

Results: Position -786 in the promoter of the human NOS3 gene is a hotspot for histone marks, namely H3K4me1 and H3K27ac. Histone deacetylase inhibitors as well as methyltransferase inhibitors attenuated FSS-dependent NOS3 expression in TT-genotype EC but disributed it in CC-genotype EC. Using bioinformatic analysis, we identified different STAT binding sites in the promoter of the human NOS3 gene around position -786. A decay ODN mimicking a positive STAT binding motif at position -850 to -542, i.e. less than 50 nucleotides upstream of the SNP, effectively inhibited FSS-mediated up-regulation of NOS3 gene expression in TT-genotype EC.

Conclusion: Temporal differences in chromatin remodeling in the region of the T-786C SNP may be linked to differential accessibility of the promoter of the NOS3 gene for STAT proteins in human EC, and thus may be responsible for the delay in NOS3 gene expression in human EC hence individuals homozygous for the -786C variant of the T-786C SNP.

**B 10-8**

**Bicarbonate-activated soluble adenyl cyclase (ADCY10) controls cell cycle via phosphatase 2A in human umbilical vein endothelial cells**

W. Waranus1, M. L. Moskopp1, A. Das1, Y. Lidlov2, F. V. Härtel, T. Noll

1Medizinische Fakultät Carl Gustav Carus der Technischen Universität Dresden, TU Dresden, Institut für Physiologie, Dresden, Germany
2Universitätsmedizin Berlin, Center of Cardiovascular Research, Berlin, Germany

Introduction: ADCY10 is ubiquitously expressed in cytoplasm and distinct organelles including cell nucleus. In contrast to its membrane-associated isoforms, which are stimulated by G-protein-coupled receptors, ADCY10 is activated by bicarbonate (HCO3-), which is released in the endoplasmic reticulum during the FSC. Hence, the underlying mechanism is still unclear. Aim: The role of ADCY10 in cell cycle control and cell proliferation is studied in endothelial cells from human umbilical veins (HUVEC).

Methods & Results: Cultured HUVEC were synchronized by serum withdrawal for 18h. Afterwards the cells were exposed to 1µM HCO3- (pH 7.4-8.0; 24 mEq HCO3- / L; Pco2 40 mmHg, pH 7.4; 0.3 mmHg, pH 7.4) for 72 h. H2 inhibition of ADCY10 by 10 µM KI77, a specific pharmacological inhibitor of ADCY10 (optimum concentration tested in pilot experiments), completely ablated cell

**Poster Session B | 205**
proliferation. In addition, ambient Pco2 or presence of KH7 delayed cell cycle progression and arrested the cells in G0 phase (FACS analysis using double staining of Hoechst 33342 (DNA-dye) and Pyronin Y (RNA-dye)). HCO3- increased the cellular cdk1 content (Western blot), a trigger for G0-to-mitotic phase transition. Whereas, inhibition of ADCY10 (ambient Pco2 or 10μM KH7) reduced the cellular cdk1 content, indicating that ADCY10 is involved in the G0-to-mitosis transition. During mitosis, phosphatase 2A (PP2A) was found to be co-localised with ADCY10 in the nucleus (immunofluorescence). Inhibition of ADCY10 decreased PP2A activity, reduced cdk1 expression and caused cells to accumulate in G0 phase. A similar result was observed when cells were exposed to 5 mM okadaic acid, a specific inhibitor for PP2A. Further ADCY10 targets were investigated, showing that the PKA antagonist (0.1 μM H89) but not the Epac antagonist (0.1 μM 8-p-sulph-2’O-Me) unblocked the KH7-induced cell arrest in G0 phase. This indicates that only PKA is a downstream target of ADCY10-mediated cell cycle transition from G0 phase to mitosis.

Conclusions: HCO3--activated ADCY10 induces mitotic cell proliferation by promoting cell cycle progression from interphase to mitotic phase in human endothelial cells via a PKA/PP2A pathway.

B 10-9
Nox1 – more than a subunit of Nox1?
T. Schader1,2, C. Reschke1, J. Graumann1, K. Schröder1,2
1Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Germany
2Max Planck Institute for Heart and Lung Research, Kerckhoff Institute, Bad Nauheim, Germany
3DZHK, Deutsches Zentrum für Herz-Kreislauß-Forschung, Partner Site Rhine-Main, Germany
4CPI, Cardio-Pulmonary Institute, Frankfurt, Germany

Nox1 is an essential subunit of the active NADPH oxidase Nox1 complex, which enables superoxide anion formation in vivo. In endothelial cells Nox1-mediated ROS formation maintains the activity of the Notch signaling pathway. Accordingly, knockout of Nox1 forces the formation of tip cells and thereby enhances angiogenesis in mice. Recently we found that knockout of Nox1 results in an increased proliferation of colon epithelial cells. That effect potentially is not directly related to a reduced ROS formation as in Nox1 knockout mice no such effect has been described. Interestingly, Nox1 overexpression exceeds the expression of the other subunits of the Nox1 complex. Therefore, we hypothesize that Nox1, via its two SH3 domains, not only binds to the Nox1 complex but may interact with other proteins as well. We observed that Erbin (ErbB2-interacting protein) is a close interaction partner of Nox1 and Nox1. For this reason, we investigated the interaction between Erbin and Nox1 using a yeast two-hybrid assay. Erbin binds and regulates the activity of Nox1. Inhibition of Nox1 decreases ROS production and results in a decrease in cell proliferation and cell migration. This indicates that Erbin is a newly identified interaction partner of Nox1. Binding of Nox1 to Erbin prevents phosphorylation of Erbin, which potentially increases the efficiency of the protein to bind unphosphorylated Erbin2, which prevents the activity of e.g. ErbB-receptor. Consequently, EGF-induced Erk and AKT activation is reduced upon Nox1 overexpression. This may explain the positive effect of Nox1 knockdown on proliferation.

B 10-10
Modulation of endothelial chromatin remodelling complexes by long non-coding RNAs
L. A. De1,2, K. Pål1,2, I. Wittig2, J. Heidler2, S. Günther2, M. Looso2, S. M. Leisegang2,3, R. P. Brandes4,5
1Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Main, Germany
2Max Planck Institute for Heart and Lung Research, ECPSP Biospectromatics and Sequencing Facility, Bad Nauheim, Germany
3German Center of Cardiovascular Research (DZHK), Frankfurt am Main, Germany
4CPI, Cardio-Pulmonary Institute, Frankfurt, Main, Germany

Background: Long non-coding RNAs modulate chromatin remodelling complexes and thereby gene expression. The mechanisms underlying the remodulation of these complexes to gene-specific promoters are largely unknown. It was previously shown that the insRNAs Xist, HOTAIR and Kcnq10Itf are required for the Foxc2 promoter of the Polycomb Repressive Complex 2 (PRC2). We previously identified the insRNA MANTIS as a crucial component of the endothelial SWI/SNF complex. MANTIS stabilised the interaction between the core ATPase BRG1 and BRG1-associated factor 155 (BAF155) and thereby maintained its targeting to angiogenesis-associated genes and vascular function. On this basis we hypothesise that a specific network of insRNAs modulates chromatin remodelling through a site- and cell-specific as well as context-dependent recruitment of BRG1.

Methods and Results: In human umbilical vein endothelial cells (HUVEC), siRNA against BRG1 decreased angiogenic function as documented by attenuated sprout length in the endothelial outgrowth assay. Additional protein subunits of endothelial SWI/SNF were identified by mass spectrometry and one of them, Double PHD Fingers 2 (DPFP), was also found to uphold endothelial function. RNA-IP interaction partners of different chromatin remodelling complexes such as EZH2, BRG1, BRM, SMARCA5 and BAF170. This technique recovered (among others) the insRNAs EPHA1-A51, CACNA1G-A51, MALAT1 and NEAT1, which have been implicated in endothelial function.

Importantly, there was a higher degree of insRNA profile overlap between the SWI/SNF complex members BAF170, BRM and BRG1 than with the PRC2 member, EZH2. Subsequent screens with a customised siRNA library revealed that approximately 30% of the studied interacting insRNAs alter endothelial functions like proliferation and migration.

Conclusions: HUVEC contain a significant number of functionality important insRNAs which interact with chromatin remodelling complexes. ChIPseq will reveal whether this interaction results in altered targeting of the complexes.
with heart hypertrophy (epidermal growth factor receptor (EGFR) knockout (KO); chronic angiotensin II infusion) but without significant signs of heart failure. RNA-Seq and gene enrichment analyses identified differentially regulated ion channels 1, 2.

Goethe University, IVS, Frankfurt, Germany

A potential role of dysregulated miR-221/222 levels in cardiac electrical remodeling. To investigate if this regulation has an impact on the physiology of cardiomyocytes, we analyzed Ca²⁺-homeostasis in HL-1 cells by ratiometric fluorescence microscopy. Cell depolarization and subsequent LTCC activation by K⁺ (25 mM) led to a constantly increasing intracellular Ca²⁺ concentration up to a plateau level. In miR-transfected cells the time needed to reach plateau Ca²⁺ levels was prolonged and the overall Ca²⁺ increase (area under the curve) was reduced. These results imply that downregulating LTCC may have an effect on depolarization kinetics in cardiomyocytes.

In conclusion, increased expression of miR-221/222 influences Ca²⁺-homeostasis at least in part by downregulation of LTCCs. MiR-221/222 may therefore contribute to disturbed cardiac excitation generation and propagation.

11-4

Insulin-like growth factor 1 and insulin have different effects on macrophage polarization influencing cardiac regeneration after myocardial infarction

L. Pogge von Strandmann, P. Panjwani, A. Spychala, R. Niederlof, A. Gödecke

Heinrich-Heine-Universität Düsseldorf, Institut für Herz- und Kreislaufphysiologie, Düsseldorf, Germany

Objective – The long non-coding RNA (lncRNA) MANTIS is known to impact on endothelial angiogenic function by recruiting the chromatin-remodelling protein BRG1 to angiogenesis-related genes. Profiling with short RNA-sequencing and Cap analysis of gene expression (CAGE) of FANTOM5 indicated that MANTIS is even higher expressed in both cell types. In vivo treatment of patient in remission of a leukemia with the histone deacetylase inhibitor Panobinostat in the course of a clinical study, reduced MANTIS expression. In contrast, MANTIS expression was increased in CD14 monocytes from patients recovering from various severe operations as well as from subarachnoidal hemorrhage. This might suggest that MANTIS is induced in inflammation. In order to test this, human PBMC-derived macrophages were pre-treated with inflammatory cytokines (e.g. TNFα, lipopolysaccharide).

Bone marrow derived cells were isolated from mice, cultivated with macrophage colony-stimulating factor (mCSF) (10 ng/ml) to enrich macrophages (MΦ), which were then polarized to M1 (LPS/IFNγ) or M2 (IL4/IL13) MΦ, or treated with IGF-1 (1.7 nM) or different concentrations of insulin (1.7 nM – 17 nM) at day 7. qPCR, western blot and flow cytometry were performed to analyze the expression of macrophage polarization markers.

Results Western blot analysis confirmed that M0 MΦ express insulin and IGF-1 receptors. Cells treated with IGF-1 showed upregulation of the M2-markers mannose receptor (CD206), arginase and resistin-like α in qPCR compared to untreated cells. In line with these results, western blot analysis showed elevated phosphoStat1 levels and flow cytometry upregulation of CD206 after IGF-1 treatment. These markers were all induced in cells treated with the M2-polarizers IL4 and IL13. In contrast, the LPS and IFNγ treated cells expressed markers for the M1-phenotype such as TNFα, iNOS and IL12 in qPCR, phosphoStat1 in western blot and CD38 in flow cytometry. None of these markers were upregulated in IGF-1 treated MΦ.

In contrast to IGF-1, insulin treatment did not induce any of the mentioned markers similar to untreated M0 MΦ.

Conclusion While IGF-1 is driving macrophage polarization towards an M2-like phenotype, insulin fails to promote a similar polarization. In conclusion, IGF-1 and insulin exert substantial differences in modulation of macrophage function despite the high level of similarity in intracellular signal transduction.

References:
1. Leisegang et al. (2017). Long Noncoding RNA MANTIS Facilitates Endothelial Angiogenic Function. Circulation 136, 65-79.

11-5

miR-221/222 in cardiac electrical remodeling

M. Knypri, S. Binas, U. Klöckner, S. Rabe, S. Mildenberger, M. Gekle, B. Schreier, C. Grossmann

Martin Luther University of Halle-Wittenberg, Julius Bernstein Institute of Physiology, Halle (Saale), Germany

Cardiac remodeling involves structural and electrical alterations that can lead to fatal conditions like heart failure or sudden cardiac arrest. The underlying mechanisms are not fully understood but there are indications that changes in the expression of microRNAs may play a role. Previously, we could show that miR-221/222 expression is increased in two mouse models with heart hypertrophy (epidermal growth factor receptor (EGFR) knockout (KO); chronic angiotensin II infusion) but without significant signs of heart failure. RNA-Seq and gene enrichment analyses identified differentially regulated ion channels associated to the T-tubule cluster in EGFR KO mice, which were also predicted targets of miR-221/222. This implicates a possible role of dysregulated miR-221/222 levels in cardiac electrical remodeling.

Among those genes were three subunits of the L-type Ca²⁺ channel (LTCC: Cacna1c, Cacnb2, Cacna2d1). To analyze if miR-221/222 target those subunits, dual luciferase 3'-UTR reporter assays were performed. MiR-221 mimics reduced luciferase activity of Cacna1c 3'-UTR, while miR-222 reduced luciferase activity of Cacnb2 and Cacna2d1 3'-UTR. To analyze the effect of miR-221/222 on L-type Ca²⁺ current (I_{Ca,L}) density by whole cell patch clamp recording, transfection with mimics for both miRs decreased I_{Ca,L} density in HL-1 cells significantly while the voltage-dependence of activation was not altered.

To investigate if this regulation has an impact on the physiology of cardiomyocytes, we analyzed Ca²⁺-homeostasis in HL-1 cells by ratiometric fluorescence microscopy. Cell depolarization and subsequent LTCC activation by K⁺ (25 mM) led to a constantly increasing intracellular Ca²⁺ concentration up to a plateau level. In miR-transfected cells the time needed to reach plateau Ca²⁺ levels was prolonged and the overall Ca²⁺ increase (area under the curve) was reduced. These results imply that downregulating LTCC may have an effect on depolarization kinetics in cardiomyocytes.

In conclusion, increased expression of miR-221/222 influences Ca²⁺-homeostasis at least in part by downregulation of LTCCs. MiR-221/222 may therefore contribute to disturbed cardiac excitation generation and propagation.

11-6

MANTIS is a functionally important IncRNA in human monocytic cells

C. Ratiu, R. P. Brandes

Goethe University, Institute for Cardiovascular Physiology, Frankfurt am Main, Germany

Goethe University Hospital, Department of Neurosurgery, Frankfurt am Main, Germany

Max-Planck-Institute for Heart and Lung Research, Department IV Lung Development and Remodeling, Bad Nauheim, Germany

Goethe University Hospital, Department of Hematology/Oncology, Frankfurt am Main, Germany

Goethe University, Institute for Biochemistry I, Frankfurt am Main, Germany

DZHK, German Center for Cardiovascular Research, Partner Site RheinMain Frankfurt, Germany

CPI, Cardio-Pulmonary Institute, Frankfurt am Main, Germany

Objective – The long non-coding RNA (lncRNA) MANTIS is known to impact on endothelial angiogenic function by recruiting the chromatin-remodelling protein BRG1 to angiogenesis-related genes. Profiling with short RNA-sequencing and Cap analysis of gene expression (CAGE) of FANTOM5 indicated that MANTIS is even higher expressed in both cell types. In vivo treatment of patient in remission of a leukemia with the histone deacetylase inhibitor Panobinostat in the course of a clinical study, reduced MANTIS expression. In contrast, MANTIS expression was increased in CD14 monocytes from patients recovering from various severe operations as well as from subarachnoidal hemorrhage.

Bone marrow derived cells were isolated from mice, cultivated with macrophage colony-stimulating factor (mCSF) (10 ng/ml) to enrich macrophages (MΦ), which were then polarized to M1 (LPS/IFNγ) or M2 (IL4/IL13) MΦ, or treated with IGF-1 (1.7 nM) or different concentrations of insulin (1.7 nM – 17 nM) at day 7. qPCR, western blot and flow cytometry were performed to analyze the expression of macrophage polarization markers.

Results Western blot analysis confirmed that M0 MΦ express insulin and IGF-1 receptors. Cells treated with IGF-1 showed upregulation of the M2-markers mannose receptor (CD206), arginase and resistin-like α in qPCR compared to untreated cells. In line with these results, western blot analysis showed elevated phosphoStat1 levels and flow cytometry upregulation of CD206 after IGF-1 treatment. These markers were all induced in cells treated with the M2-polarizers IL4 and IL13. In contrast, the LPS and IFNγ treated cells expressed markers for the M1-phenotype such as TNFα, iNOS and IL12 in qPCR, phosphoStat1 in western blot and CD38 in flow cytometry. None of these markers were upregulated in IGF-1 treated MΦ.

In contrast to IGF-1, insulin treatment did not induce any of the mentioned markers similar to untreated M0 MΦ.

Conclusion While IGF-1 is driving macrophage polarization towards an M2-like phenotype, insulin fails to promote a similar polarization. In conclusion, IGF-1 and insulin exert substantial differences in modulation of macrophage function despite the high level of similarity in intracellular signal transduction.

References:
79.
Rhein, a novel Histone Deacetylase (HDAC) inhibitor with antifibrotic potency

B 11-7

1Rhein, a novel Histone Deacetylase (HDAC) inhibitor with antifibrotic potency
11-7
1B 11-9
1, 2, 4
1, M. Schulz
2C. Carlberg
3R. P. Brandes
4D. Herzfeld de Wiza
5Seuter
61, 2, 3
71, 2
81, 2
91, 2
101, 2
111, 2
121
13Goethe University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt, Germany
142Heinrich-Heine University, Institute of Clinical Biochemistry and Pathobiochemistry, Düsseldorf, Germany
152Heinrich-Heine University, Medical Faculty, Institute for Clinical Biochemistry and Pathobiochemistry, Düsseldorf, Germany
164Heinrich-Heine University, Medical Faculty, Department of Cardiovascular Physiology, Düsseldorf, Germany

Background: CTCF (CCCTC-binding factor) is a transcription factor that plays a major role in chromatin organization by forming chromatin loops. The DNA looping can increase transcription by bringing enhancers and promoters into close proximity. Fibrosis manifests progressively leading to cardiac stiffness and left ventricle dysfunction. Persistence of this pathologic state dramatically affects the survival after cardiovascular events. Although fibrosis depicts a reparative nuclear vitamin D receptor (VDR) is dependent on TADs and that vice versa, activation of the vitamin D receptor induces urgent interest. The rhubarb anthraquinone Rhein, a drug already established as treatment for osteoarthritis, has been shown to effect the fibroblast-to-myofibroblast transition sensitive TADs have been identified and were further segregated into different functional classes by pathway analysis and using CRISPR/Cas9 gene editing to knock out CTCF in THP-1 cells with different guide RNAs we generated lines with a 87-95% reduction of CTCF protein expression. The cells did not show any significant signs of reduced viability or other modulatory shift of secretory products in association to differential regulation of profibrotic pathways. Rhein administration mitigated this hypoxia-mediated modulatory shift. Further, the influence of Rhein on the alteration of the transcriptome linked to the cardiac fibroblast phenotype was associated to changes in the secretory profile. In a holistic approach, the combination of secretomic and transcriptomic pathway analyses, robustly identified TGFβ1, p53 and p21 as upstream regulators. Functionally, Rhein increased p53 and p21 associated to a prolongation of the G2M cell cycle phase and in line with decreased proliferation. Furthermore, Rhein was shown to reduce cellular sensitivity to exogenous TGFβ1 and to intracellularly target TADs and SMADs. Rhein affected transcription of TNFα, IL-1β, IL-6 and VEGF. Contradictorily, Rhein administration led to increased nuclear availability of PML and Smyd1, which are known to be involved in various cellular processes including inflammatory pathways. Interaction of Smyd1 and PML was confirmed by coimmunoprecipitation experiments. Furthermore, we show that Smyd1 is involved in the SUMOylation of Smyd1, a posttranslational modification, which targets Smyd1 to proteasomal degradation. In addition, inhibition of Smyd1 leads to decreased and overexpression of Smyd1 to increased expression of PML and assembly of PML-NBs. Overexpression of an HMT-deficient Smyd1 mutant showed nearly no effect indicating an involvement of H3K4 methyltransferase activity of Smyd1 on the regulation of PML expression. Both PML and Smyd1 expression were increased by stimulation of endothelial cells with pro-inflammatory cytokines (e.g. TNFα, INFγ), where PML expression was lower by simultaneous inhibition of Smyd1 expression. Conclusion: Our data suggest an interplay between Smyd1 and PML in ECs by which Smyd1 positively influences PML expression. An increase in PML availability may in turn lead to an enhanced degradation of Smyd1 itself. By regulating the expression and assembly of PML and Smyd1 the influence of Smyd1 on endothelial cell behavior should be studied in more depth in the future.

B 11-8

Smyd1, a histone methyltransferase, interacts with PML nuclear bodies in endothelial cells

S. Becker, K. G. Steinemann, A. Zakrzewicz, J. Berkholz
Charité, Institute of Physiology, Berlin, Germany

Background: Several cardiovascular diseases have been linked to Smyd1 overexpression and dysfunction. Recently, the expression of Smyd1, an epigenetic modulator known to exhibit histone methyltransferase activity and an established recruiter of histone deacetylases (HDACs), has been identified in vascular ECs, but only little is known about its function in vascular cell biology so far. We hypothesize that Smyd1 extensively influences endothelial cell phenotype by interaction with PML.

Methods and Results: By immunocytochemistry and immunohistochemistry analyses of cultured vascular endothelial cells and arteries isolated from human hearts, we demonstrate a co-localization of Smyd1 and promyelocytic leukemia nuclear bodies (PML-NBs). PML-NBs are dynamic protein aggregates in the nucleus, which can consist of over 100 components and are known to be involved in various cellular processes including inflammatory pathways. Interaction of Smyd1 and PML was confirmed by co-immunoprecipitation experiments. Furthermore, we show that Smyd1 is involved in the SUMOylation of Smyd1, a posttranslational modification, which targets Smyd1 to proteasomal degradation. In addition, inhibition of Smyd1 leads to decreased and overexpression of Smyd1 to increased expression of PML and assembly of PML-NBs. Overexpression of an HMT-deficient Smyd1 mutant showed nearly no effect indicating an involvement of H3K4 methyltransferase activity of Smyd1 on the regulation of PML expression. Both PML and Smyd1 expression were increased by stimulation of endothelial cells with pro-inflammatory cytokines (e.g. TNFα, INFγ), whereas PML expression was lower by simultaneous inhibition of Smyd1 expression.

Conclusion: Our data suggest an interplay between Smyd1 and PML in ECs by which Smyd1 positively influences PML expression. An increase in PML availability may in turn lead to an enhanced degradation of Smyd1 itself. By regulating the expression and assembly of PML and Smyd1 the influence of Smyd1 on endothelial cell behavior should be studied in more depth in the future.

B 11-9

Vitamin D modulates the three-dimensional chromatin structure

S. Seuter, T. M. Warwick1, M. Schulz, A. Neme, C. Carlborg, R. P. Brandes
1Goethe University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt, Germany

2University of Eastern Finland, School of Medicine, Institute of Biomedicine, Kuopio, Finland

Background: CTCF (CCCTC-binding factor) is a transcription factor that plays a major role in chromatin organization by forming chromatin loops. The DNA looping can increase transcription by bringing enhancers and promoters into close proximity, but it can also insulate different functional topologically associating domains (TADs) from each other. Often, CTCF and cohesin act in concert to elicit this function. We hypothesize that regulation of gene expression, as exemplified by the nuclear vitamin D receptor (VDR) is dependent on TADs and that vice versa, activation of the vitamin D receptor induces TAD reorganization. Results: 1,25-dihydroxyvitamin D3(1,25(OH)2) significantly altered the binding of CTCF to approximately 2,100 genomic regions located throughout the genome. Using CTCF ChIP-seq and combined analysis using the ENCODE project data set, we show that CTCF interacts with enhancers, but not with TADs, while the vitamin D receptor interacts with TADs, but not with enhancers. Conclusions: Our data suggest that vitamin D modulates the three-dimensional chromatin architecture, which may be a mechanism to fine-tune the expression of VDR target genes.

B 11-10

The role of the base excision DNA repair enzyme 8-oxo-7,8-dihydroguanine glycosylase (OGG1) in combination with Mut-T-Homologue1 (MTH1) inhibition

J. Vogel, U. Brockmeier, H. Riffkin, P. Kranz, M. Pompe, J. Baumann, K. Goepelt, M. Baumann, E. Metzen
University of Düsseldorf, Institute of Physiology, Essen, Germany

Reactive oxygen species (ROS) oxidize the nucleotide guanine, thus generating 8-oxo-7,8-dihydroguanine (8-oxoG) which causes potentially harmful and carcinogenic G:C to T:A transversion mutations. The base excision repair (BER) enzyme 8-oxoG-DNA glycosylase (OGG1) can remove 8-oxoG preferentially opposite cytosine to initiate BER. Recently, the base excision repair enzyme OGG1-knockdown potentiated apoptosis induction in the unirradiated and TH588 treated, thus MTH1-inhibited cells. Furthermore, treatment with the Mut-T-Homologue1 (MTH1) inhibitor TH588 was reported to induce apoptosis by accumulation of 8-oxoG-GTP in the nucleotide pool. Therefore, we hypothesized that inhibiting OGG1 should also cause DNA damage by 8-oxoG-accumulation. To prove this, we generated a lentiviral OGG1-knockdown in the colorectal cell line HCT116. However, activation of apoptosis in OGG1-depleted cells was not detectable, although the OGG1-knockdown potentiated apoptosis induction in the unirradiated and TH588 treated, thus MTH1-inhibited cells. Additionally, the OGG1-knockdown decreased viability in MITT assays and reduced proliferation in long-term survival assays after TH588 treatment. Stress providing factors like exposure to hypoxia, irradiation and tert-butylhydroperoxide were equally harmful to OGG1 deficient cells and control cells. To boost the effect of 8-oxoG-accumulation in the cells, a double-knockdown of the enzymes OGG1 and MTH1 was generated in HCT116 cells, but the effect could not be enforced. To verify the effect of the double-knockdown, a lentiviral double-knockdown was also generated in SW480 cells, another colorectal adenocarcinoma cell line. Interestingly, in these double knockdown cells we observed higher levels of apoptosis after irradiation and treatment with TH588. Furthermore, the cells proliferated less, and cell survival was reduced in colony formation assays. We conclude that a single knockdown of OGG1 does not have a detectable effect on the cells. Knocking down OGG1 in combination with TH588 potentially is an opportunity to combat tumor cells which are resistant to conventional treatments. Additionally, simultaneous inhibition of MTH1 and OGG1 in SW480 cells has an obvious effect with respect to apoptosis induction and inhibition of cell proliferation. Therefore, we conclude, that inhibition of these enzymes has cell type specific effects and could be a treatment option for a subset of cancer cell lines.
CRISPR-Cas mediated visualization of long non-coding RNAs in human endothelial cells

S. Seredinski1,2,3, M. S. Leisegang1,2,3, R. P. Brandes1,2,3

1Goethe-University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt, Germany
2DZHK Partnersite Rhine-Main, German Center of Cardiovascular Research (DZHK), Frankfurt, Germany
3Goethe-University Frankfurt, Cardio-Pulmonary Institute (CPI), Frankfurt, Germany

Background: The CRISPR-Cas system is a constantly expanding toolbox for editing the genomic landscape of cells. So far, its applications are mainly focused on DNA using CRISPR-Cas9. Targeting of RNA was mostly neglected although RNAs play crucial roles in every cellular process. CRISPR-Cas13a from Leptotrichia wadei (LwaCas13a) exclusively targets and cleaves RNA with the help of a matching guideRNA. Mutation of arginine residues R474 and R1046 to alanine lead to the development of catalytically inactive Cas13a, namely LwadeadCas13a (dCas13a). A fluorescent variant (dCas13a-msfGFP) was obtained by fusion to mstfGFP.

Results: In a first step, the transfection efficiency of the system was optimized for HEK293 cells and human umbilical vein endothelial cells. As documented by fluorescence assisted cell sorting (FACS) transfection efficiency above 24 and 35 percent could eventually be reached using Lipofectamine 3000 for HUVECs and Lipofectamine 2000 for HEK293 cells respectively. Visualization of dCas13-GFP was carried out with a Zeiss LSM800 Laser Scanning Microscope. Different RNAs were studied. Acta1-mRNA (beta-actin) was observed mainly in the cytosol, which is expected for an mRNA. Visualization of the lncRNAs LISPR1 resulted in a signal in the nucleus as well as the cytoplasm. Differential RT-qPCR experiments of the nuclear and cytosolic compartment confirmed this finding. In contrast, the IncRNAs NEAT1 and HIF1α-AS1 were detected in nuclear speckles. For NEAT1, this is in line with the concept that the RNA localizes to nuclear para-species.

Conclusion: CRISPR-Cas13 is a new and powerful tool to target RNA. It will help to elucidate regulatory mechanisms of gene expression by RNA.