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In the language of thermodynamics, living systems are areas in space that increase their internal order by creating disorder in their surroundings. Transport physiology attempts to find out more about the boundaries of this entropy minimum. In concrete terms: how can nutrients be absorbed against large gradients, while metabolic wastes are excreted in concentrated form? Why doesn't Na⁺ leak out of a frog sitting in a pond? A combination of functional studies and molecular biological investigations have identified the mechanisms involved to maintain gradients over epithelia and cell membranes. The lipid bilayer around cells is enriched by cholesterol, while cells of epithelia are interjoined by tight junction proteins. These barriers ensure that transport of almost all substances is regulated by the expression and activity of specific transport proteins.

Channels are transmembrane proteins that form a pore which mediates passive flux of cations, anions, or polar substances such as H₂O or CO₂ down the electrochemical gradient. In certain channels – such as ROMK or ENaC – binding of the substrate (e.g. K⁺ or Na⁺) to aminoacid residues precisely delivers the energy for dehydration, resulting in the high selectivity required to maintain gradients for these ions across cells and epithelia. Other channels are promiscuous, with substrates of anion channels typically including Cl⁻, HCO₃⁻ and acetate. In gating, a charged protein residue intermittantly occludes the mouth of the pore. Membrane voltage or second messengers change gating behaviour, so that transport rates can be rapidly adjusted.

The structure of transporters is very similar, but the binding site is accessible from one side only, preventing free diffusion. Gating mediates a rapid switch between inside- and outside-facing orientations, so that ions and substrates can be selectively bound on one side and released on the other. In primary active transport via the Na⁺/K⁺-ATPase, this conformational shift is energized by phosphorylation, delivering the energy required to build electrochemical gradients for K⁺ and Na⁺. These gradients subsequently energize co- and antiport of other substrates. Finally, Claudins within the tight junction regulate the permeability of the paracellular pathway for absorption or recycling of ions. In epithelial transport, channels, transporters, and paracellular proteins work in concert to mediate the uptake of energy and the efflux of entropy that make life possible.
LRRC8/VRAC channels not only conduct halides, but also organic compounds like taurine (2), cisplatin and other anti-cancer drugs (5), and neurotransmitters (6), with the substrate specificity depending on the subunit composition (5,6). Our structure-function analysis shows that both the second half of the first extracellular loop (4) and the extreme amino-terminus (7) of LRRC8 proteins participate in VRAC’s pore. LRRC8/VRAC channels are not only involved in cell volume regulation, but are, for instance, also involved in cancer drug resistance by a dual mechanism that includes its role in apoptosis and drug uptake (5). VRAC also enhances β-cell glucose sensing and insulin secretion by depolarizing β-cells upon glucose-induced cell swelling (8). VRAC is needed for proper development of male germ cells in a cell-autonomous manner (9). Many other important functions are likely to emerge.

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**Keynote Lecture 04**

**RNA and DNA binding proteins control the composition of calcium channels in nociceptors in normal and neuropathic pain states**

D. Lipscombe1,2, E. J. López Soto1,2
1Brown University, Robert J. & Nancy D. Carney Institute for Brain Science, Providence, US
2Brown University, Department of Neuroscience, Providence, US

The majority of multi-exon genes are subject to alternative splicing, whereby each gene has the capacity to generate multiple mRNA and protein isoforms depending on cell state. Cell-specific alternative splicing regulates numerous essential cell functions and aberrant splicing is now implicated in many diseases. The Cacna1b gene that encodes the main functional subunit of voltage-gated CaV2.2 calcium (CaV2.2) channels contains several alternatively spliced exons. Cell-specific factors regulate alternative exon inclusion and the composition of the CaV2.2 channel mRNA pool. The diversity of CaV2.2 channel splice isoforms is especially notable in the nervous system. In sensory neurons, CaV2.2 channels control transmission of noxious stimuli at nociceptor terminals in the dorsal horn of the spinal cord. CaV2.2 channels are major targets of many drugs and neurotransmitters that inhibit channel activity via activation of G-protein coupled receptors, including morphine. Inhibitors of presynaptic nociceptor CaV2.2 channels downregulate nociception. Nociceptor-specific alternative splicing of Cacna1b pre-mRNA generates a family of CaV2.2 channel splice isoforms that have higher surface density and greater sensitivity to inhibition by G-protein coupled receptors, compared to those expressed in other neurons. We have now identified the cell-specific mechanisms that regulate the expression and exon composition of CaV2.2 channel splice isoforms in nociceptors. We show that the coordinated actions of both RNA and DNA binding proteins contribute to exon recognition and inclusion during alternative pre-mRNA splicing to generate a family of CaV2.2 channel splice isoforms with properties that define nociceptor CaV2.2 channel currents. The normal pattern of alternative splicing of Cacna1b pre-mRNA is disrupted in nociceptors following peripheral nerve injury. We describe the molecular changes that result in abnormal alternative splicing of Cacna1b pre-mRNA in nociceptors to impact CaV2.2 channel properties and contribute to neuropathic pain pathology. Funded by NIH grant NS055251.
Optogenetics and chemogenetics have become highly instrumental in neuroscience research as they allow the highly specific activation and inhibition of genetically defined neuron populations with unprecedented spatial and, in case of optogenetics, also temporal resolution. Here, we have used these techniques both in mouse spinal cord slices and in behaving mice to study circuits and mechanisms of itch transmission. In our study, we were primarily interested in the function of a subpopulation of excitatory dorsal horn neurons defined by the expression of gastrin-releasing peptide (GRP) and in the role of GRP as a synaptic signaling molecule. Chemogenetic activation or diphtheria toxin mediated ablation of spinal GRP neurons demonstrated that these neurons are critical elements of a spinal circuit of itch but do not contribute to pain signaling. We then used optogenetic activation of GRP neurons in spinal cord slices to examine details of the synaptic communication between GRP neurons and their postsynaptic target neurons, the GRP receptor (GRPR) expressing neurons. These experiments demonstrated that baseline synaptic coupling between GRP and GRPR neurons is too weak to elicit suprathreshold excitation. Only when GRP neurons were stimulated repetitively to fire bursts of action potentials, GRPR neurons depolarized progressively and became excitable by GRP neurons. In behaving mice, similar optogenetic stimulation paradigms applied to spinal GRP neurons were needed to provoke itch-like behaviors. These results established a spinal gating mechanism for itch that requires sustained repetitive activity of preneuronal GRP neurons and postsynaptic GRP signaling to drive GRPR neuron output.

Examples will be shown for the application of machine-learning in clinical and experimental pain research. Main applications of machine-learning in this field include (i) supervised classification problems such as pain phenotype prediction from complex case data, (ii) unsupervised methods such as pattern detection in complex pain-related data to observe clinically relevant subgroups of patients that require individualized multimodal treatments, and (iii) knowledge discovery and exploration of pain-related data.

References

Acknowledgement
Landesanstalt für Entwicklung wissenschaftlich-ökonomischer Exzellenz (LOEWE), LOEWE-Zentrum für Translationale Medizin und Pharmakologie.
Maximizing host defence while minimizing nephron damage: IL-1 and IL-22 in kidney injury

H.-J. Anders
LMU Munich, Nephrology, Munich, Germany

The resident immune cells in the kidney are mainly mononuclear phagocytes that populate the renal interstitial compartment from early development. While their contribution to renal function during homeostasis remains unknown, they are actively involved in all phases of kidney injury and repair. During the early phase of kidney injury they secrete a plethora of factors that on one end intend to fight the injurious trigger, following a program originating from host defence. In that IL-1 and IL-1-dependent innate immunity is essential and initiates the sequential recruitment of bone marrow-derived immune cells and the crescendo of innate immune inflammation during the early and late injury phase, a process named “reinflammation”. At the same time the resident immune cells secrete interleukins (a misnomer in this context) that maintain epithelial integrity. For example, IL-22 is exclusively secreted by intrarenal immune cells, while IL-22 receptors are mainly expressed by epithelial cells. This lecture will report the contrasting roles of these two paradigmatic interleukins secreted by resident intrarenal immune cells that try to maximize host defence while minimizing collateral damage to the nephrons of the kidney.

Tubular-glomerular crosstalk: a new functional link between tubular lesions and a compromised function of the glomerular filtration barrier

H. Castrop
University of Regensburg, Institute of Physiology, Regensburg, Germany

Albuminuria is a hallmark of kidney disease of various etiologies. Acute lesions of the renal tubular system (acute tubular necrosis) are frequently accompanied by albuminuria, which is considered to be the consequence of a compromised tubular reabsorptive function. Net urinary albumin excretion, however, is determined by glomerular albumin filtration and subsequent tubular reabsorption. Therefore, we hypothesized that in situations of insults to the tubular system, a tubular-glomerular crosstalk may lead to an increased filtration of albumin, and, consequently, may contribute to the development of albuminuria.

To test this hypothesis, we used intravital multiphoton microscopy in rats to determine the glomerular sieving coefficient (GSC) of albumin after ablation of single cells of the proximal tubule in an otherwise healthy kidney. Single tubular cells were ablated by focused laser exposure. The GSC of albumin was determined after i.v. injection of Alexa-546-labeled albumin as the ratio of the fluorescence intensities in Bowman’s space/the glomerular capillary lumen, corrected by the background intensities. The density of the glomerular endothelial glycocalyx was determined after i.v. application of wheat germ agglutinin.

The GSC of albumin in the healthy rat kidney was low, averaging 0.008±0.002. Within 20 min after laser ablation of a few proximal tubular cells, the albumin GSC of the affected nephron increased to 0.007±0.006 (p<0.01 vs. baseline; n=25), whereas the albumin GSC of neighboring nephrons remained unchanged. The albumin GSC returned to baseline within 4–6 days, as determined by serial intravital imaging. This normalization of the GSC was accompanied by a functional regeneration of the affected tubule. In parallel with the albumin leakiness of the glomeruli of the affected nephrons, the density of the glomerular endothelial glycocalyx declined, averaging 18±3±5 µM in controls and 14±4±5 µM after tubular lesions (p<0.0001; n=215 regions of interest).

In summary, using intravital multiphoton microscopy, we found a transient increase in the albumin GSC in response to acute insults to the proximal tubule, which may be functionally relevant for the regeneration of the affected tubule. Our data further suggest a crucial role of the endothelial glycocalyx for the glomerular filtration barrier and provide first evidence for a functional modulation of the glomerular filtration barrier by the tubular system.

Immune cell mediated kidney and transplant fibrosis

S. Buchterer, S. Balam, K. Renner, M. Mack
University Hospital Regensburg, Regensburg, Germany

Renal fibrosis is characterized by the accumulation of extracellular matrix around the tubules and a hallmark of chronic renal failure independent of the underlying disease. While various cell types including hematopoietic cells can express collagen, it is unclear to what extent these cells contribute to deposition of collagen and how fibrosis affects renal function. Using cell type specific collagen-I knock-out mice, we show that hematopoietic cells produce 40-50% of the collagen-I in the kidney. Functionally, collagen deposition is essential for renal survival following unilateral ureteral obstruction, while it is deleterious in the chronic model of adriamycin-induced nephropathy.

In addition to directly producing collagen, immune cells are also important inducers/mediators of profibrotic pathways. In recent years it has been shown that T cell-derived IL-3 is a major (indirect) inducer of fibrosis in several animal models including lupus nephritis, myocarditis and transplant rejection. In the later model we have found that the profibrotic cascade induced by IL-3 involves basophils as well as IL-4 and IL-6.

The talk will give an overview about novel concepts and potentially novel therapeutic targets in fibrosis development.

S 02-05
Dickkopf 3 and chronic renal disease

H.-J. Gröne
Philliاعتماد Marburg, Institute of Pharmacology, Marburg, Germany

Tubular epithelial atrophy and interstitial fibrosis are the hallmark of chronic kidney disease (CKD). The Wnt signaling pathways have a bimodal function contributing context dependent to atrophy and fibrosis but also to regenerative processes in acute tubular injury. The Dickkopf (Dkk) family is part of the Wnt pathways. DKK1 has been reported to antagonize the canonical Wnt pathway by binding to the LDL receptor-related protein 6 (LRP6). Recombinant DKK1 inhibited the inflammatory and fibrotic sequelae of ureteral obstruction. In extensive animal and human studies DKK3 has now been shown to be an agonist for canonical Wnt signaling in CKD, it fosters chronic fibrosing inflammation of the renal tubulointerstitial compartment. Genetic- and antibody-mediated inhibition of DKK3 led to a pronounced improvement of tubular differentiation and a reduction in fibrosis. In controlled prospective studies the secreted glycoprotein Dkk3 could be shown to be a reliable non-invasive urinary marker for the progression of CKD of different etiologies in man.

Symposium 03: Single Cells: Sequencing to Function

S 03-01
Mapping timescales of cancer therapy resistance in single cells

S. Shaffer
University of Pennsylvania, Department of Pathology and Laboratory Medicine, Department of Bioengineering, Philadelphia, US

Even within a clonal cancer, individual cells have different responses to drugs; while some cells may be sensitive to a therapy and die, other cells from the same tumor can be resistant and continue to grow. Methods such as single-cell RNA-sequencing provide a snapshot into the underlying molecular variability responsible for different single-cell phenotypes. However, the missing dimension from such end-point based methods is time. Single-cell RNA-seq provides just one point in time of gene expression, but does not capture the dynamics of how gene expression states change with time. In this talk, I will present two approaches that capture the timescales of gene expression states that are associated with therapy resistance in melanoma cells. The first approach, dubbed MemorySeq, leverages the experimental framework of Luria and Delbrück’s fluctuation test and combines it with modern high-throughput RNA-seq. The second approach combines a high-throughput library of transcribed barcodes and single-cell RNA-seq to reveal variation in gene expression within lineages of cells and across lineages. Together, we are using these complementary approaches to capture how heterogeneity in gene expression evolves over time, and how different gene expression states map to the phenotype of drug resistance.

S 03-02
Genome editing in cell based assays

R. Morocka, S. Reuter
Universitätsklinik Jen, Jena, Germany

Reporter gene assays enable monitoring of the transcriptional activation of genes of interest. These reporter genes could be either enzymes such as luciferases or fluorophores like GFP and generate bioluminescent or fluorescent signals after transcription activation of the gene of interest. In both cases reporter genes were introduced into cells by transfecting plasmids encoding the reporter gene under control of a promoter of interest. Those plasmids were introduced into the genome of cells randomly or with the help of recombinases that require a certain recognition site already integrated into the genome. However the efficiency of integration was low and furthermore the size of cloned promoters was restricted to 5 kb. To solve these problems, genome editing has been developed. The TALEN (transcription activator-like effector nucleases) and Cas9 technology revolutionized targeting and manipulation of genomic loci for integration that allow the expression of recombinant proteins without harmful effects on cell viability, that are referred to as “safe harbor loci” such as the human AAVS1 locus2. Reporter gene assays with tagged target genes allow to follow gene regulation in the natural genomic context.
In this presentation we present data from different genome editing technologies and discuss practicability and typical application scenarios.

**S03-03**

**CELLULAR AND MOLECULAR PROBING OF INTACT HUMAN ORGANS**

A. Ertürk

Helmholtz Center Munich, Institute of Tissue Engineering and Regenerative Medicine (iTERM), Munich, Germany

Tissue labeling and clearing technologies allow unraveled imaging of intact biological specimens at sub-cellular resolutions. However, so far, they could only be applied on the tissues of organisms with no more than several months of age such as mouse or human embryos but not decades-aged intact human organs. A major problem has been the sturdy nature of dermal and subcutaneous tissue. The accumulation of opaque and dense molecules such as lipofuscin and non-soluble collagen impede penetration of chemicals deep into human organs and, thereby block both clearing and labeling of human specimens larger than a few millimeters.

We introduce SHANEL for molecular and cellular profiling of intact human organs. SHANEL relies on a new tissue permeabilization chemistry that enables labeling and clearing of centimeters size human organs. We used SHANEL to generate the first intact transparent adult human brain and kidney, and perform 3D histology using antibodies and dyes in centimeters depth, a ~2-3 orders of magnitude scaling up. We revealed structural details of the sclera, iris and suspensory ligament in the human eye, and the vessels and glomeruli in the human kidney. We also applied SHANEL on transgenic pig organs to map complex structures of EGFP expressing beta cells in >10 cm size pancreas. Thus, SHANEL could accelerate large scale mapping/phenotyping projects such as the Human Protein Atlas, Human Brain Mapping and Brain Initiative. In addition, as the first technology to generate cellular details of intact human organs, it could facilitate tissue engineering studies, especially by 3D-bioprinting, which requires detailed blueprints (3D maps) of organs to be replicated.

Related references:


**S03-04**

Using single cell genomics to study cancer as a tissue – cell types and organ structure in malignancies

V. Zachariaidou1, N. Andrews1, J. Serviss1, H. Cheng1, E. Dzwonkowski2, M. Gerlinger1, M. Engel1

1Karolinska Institutet, Department of Oncology-Pathology, Stockholm, Sweden
2Karolinska Institutet, Department of Biosciences and Nutrition, Huddinge, Sweden

Single-cell genomics methods have greatly improved our ability to study minor cell populations in complex mixtures of cells such as primary tissue or infiltrating tumor cells. We can now routinely profile many thousands of cells by single-cell RNA-seq (scRNA-seq), enabling the study of very rare cell populations in detail. However, many scientific questions cannot be answered by RNA sequencing alone. The nature of scRNA-seq means that each cell is analyzed without context - while it is ideal for detailed cataloguing of cell types, the higher order structure of the organ is not retained. Also, cancers are frequently multiclonal and their expression profile can only be interpreted in the context of the genetic aberrations that the clone carries. In many cancers, it remains unclear to what extent there exists a hierarchy of stem-like to differentiated cells, as opposed to clonal selection of random genetic events during treatment, and if the mechanism of treatment failure is related to either of these.

We have developed methods to specifically address both these shortcomings of scRNA-seq. Direct Nuclear Tagmentation and RNA-sequencing (DNTR-seq) allows joint whole genome sequencing and mRNA sequencing in the same cell, with equivalent mRNA data quality to gold standard SmartSeq2. Compared to existing parallel DNA/mRNA-sequencing methods, DNTR-seq does not rely on initial preamplification of DNA, reducing bias in sequencing, and reduces the initial cost of isolation several fold. We successfully apply DNTR-seq to >1000 cells from patients with acute leukemia, and reveal features not inferred by either modality alone.

Cell Interaction by Multiplet sequencing (CIM-seq) is based on sequencing of cell multiplets from incompletely dissociated tissue, followed by computational deconvolution of each multiplet into its most likely cell type constituents. CIM-seq then establishes statistical significance of cell type connections in the tissue by determining which of the cell types are found in multiplets together more often than expected by random chance. Since the cell types are derived from scRNA-seq data, they can be defined at an arbitrary precision limited only by the size and accuracy of the dataset, and the classification can be refined without performing additional experiments. We apply this method to colorectal stem cell crypts and pancreatic liver metastases to define stem cell niches in healthy tissue and cancer.

Related references:


**Symposium 404**

**Oligodendrocyte precursor cells become heterogeneous with age: different functional cell states?**

Y. Kamen1,2, S. O. Spitzer1,2, S. Sitnikov1,2, K. A. Evans1, D. Kronenberg-Versteeg1, H. Gautier1, H. Pivovarova1, R. T. Karadottir1,2

1University of Cambridge, Wellcome – MRC Cambridge Stem Cell Institute, Cambridge, UK
2University of Cambridge, Department of Veterinary Medicine, Cambridge, UK

Myelin is essential for normal brain function, as it enables fast information transmission and trophic support for axons. Its importance is evidenced in diseases such as spinal cord injury and multiple sclerosis, where it is lost or damaged, as this leads to mental and/or physical disability.

During development, oligodendrocyte precursor cells (OPCs) differentiate into myelinating oligodendrocytes, which myelinate axons. In white matter diseases, adult OPCs, the main proliferative cell in the adult CNS, rapidly respond to demyelinating stimuli and differentiate to replace lost myelin sheaths to recover fast axonal transmission; however, this repair process often fails.

Increasing evidence show that neuronal activity and glutamate signalling can promote OPC proliferation, differentiation and myelination, both during development and after a demyelinating injury. OPCs have the capacity to sense neuronal activity as they receive synaptic inputs from neurons, express voltage-gated ion channels, and express neurotransmitter receptors (such as AMPA/kainate receptors, and NMDA receptors). However, not all OPCs express the same electrophysiological properties; thus this differentiation have a functional significance or is it solely a transitional stage prior to differentiation. Using whole-cell patch-clamp in NG2-1TFP transgenic mice, we show that OPCs are first born without any voltage-gated ion channels or glutamate receptors, and gradually acquire them at different rates. In the second postnatal week, OPCs diverge between and within regions, with a greater proportion of OPCs responding to NMDA application in heavily myelinated regions, whereas NMDARs disappear from unmyelinated regions first. Thus, unlike previously thought, OPCs are heterogeneous both temporally and between and within regions. In addition, by combining patch-clamp with flow cytometry and bulk RNAseq, we show that ion channel expression correlates with the proliferation or differentiation potential of OPCs, suggesting that this heterogeneity rather indicates different functional states. Finally, we find similar OPC states during remyelination. A number of cues, such as cytokines, growth factors, or GPCR5, are known to regulate ion channel expression, and may, therefore, regulate OPC state transitions both in healthy and lesional tissue. Thus, understanding both OPC functional states and state transitions may be fundamental to our understanding of myelination and myelin regeneration.

Related references:

Excitatory glutamatergic synapses between neurons and glioma cells drive brain tumour progression

V. Venkataramani1,2,3, D. Tanev1,2,3, C. Strahle1, A. Studier-Fischer1,2,3, L. Frankhauser1,2,3, T. Kessler1,2,3, F. Winkler1,2,3, T. Kunker1
1Heidelberg University, Functional Neuroanatomy, Heidelberg, Baden-Württemberg, Germany
2University Hospital Heidelberg, Neurology Clinic and National Center for Tumor Diseases, Heidelberg, Baden-Württemberg, Germany
3German 1 Cancer Research Center (DKFZ), Clinical Cooperation Unit Neurooncology, Heidelberg, Baden-Württemberg, Germany

Symposium 05: Physiology and Pathophysiology of the Lung

Hypoxic pulmonary vasoconstriction (HPV) is an essential mechanism of the lung adapting blood flow to alveolar ventilation. Under conditions of regional alveolar hypoxia, HPV reduces blood flow to poorly ventilated (and thus hypoxic) lung regions and shifts blood flow to well ventilated areas. HPV thus optimizes pulmonary gas exchange. A disturbance of HPV, as it can occur in pneumonia or during anesthesia can lead to life-threatening hypoxemia. If alveolar hypoxia, however, is generalized (occurring e.g. at high altitude), HPV leads to pulmonary hypertension (PH) by generalized pulmonary arterial vasoconstriction.

HPV is also known as the von Euler-Liljestrand-mechanism, as they first recognized the meaning of HPV in 1946. Nevertheless, the underlying molecular mechanism is not fully resolved yet. Pulmonary arterial smooth muscle cells (PASMC) of the lung’s resistance vessels were identified as the main site of HPV: PASMC, even if isolated, depolarize, increase their intracellular calcium upon hypoxia and contract. Voltage-gated potassium (Kv) channels and, at least in mice, transient receptor potential channels (TRPC) have been shown to be essential for acute HPV, involving translocation of TRPC6 by an interaction with cystic fibrosis transmembrane conductance regulator channels. Research is complicated by the fact that acute HPV, occurring within seconds and lasting up to minutes and sustained HPV (lasting minutes-hours) are at least in part regulated by different mechanisms. - A third phase of HPV contributes to development of PH if hypoxia lasts days-years. In addition, an essential contribution of lung vascular endothelial cells to HPV has been shown. Reactive oxygen species have been suggested as mediators of HPV, but there is a debate whether they decrease or increase in PASMC upon hypoxia.

On the O2-sensing level recent evidence favors the concept that mitochondria in the PASMC sense the decrease in pO2. In this context it has been shown that a specific subunit of mitochondrial complex IV (Cox4i2) is essential for acute and sustained HPV and we suggest that a Cox4i2-dependent mechanism drives an increase of O2-release from mitochondrial complex III upon hypoxia. O2- then, after  dissimilation to H2O2, triggers a closure of Kv channels and thus contraction of PASMC. Developing the mechanism underlying HPV is a prerequisite for the development of strategies to treat a disturbed HPV and subsequent hypoxemia as well as acute hypoxia-induced PH.

Symposium 05-02

Basement membrane determines function of pulmonary vascular cells

G. Kwapiszewska
Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria

Vascular alterations can present in lung diseases such as chronic obstructive lung disease (COPD) and lung fibrosis (LF). These alterations manifest as remodeling and even obliteration of pulmonary arteries or as a change in vessel distribution. Vascular remodeling is caused by dysfunction of endothelial cells (EC), proliferation of smooth muscle cells (SMC) and deposition of extracellular matrix (ECM) within and around the vessel wall. When pronounced, vascular remodeling leads to pulmonary hypertension (PH), which when detected in COPD or LF patients, worsens their outcome.

Basement membrane (BM) is a specialized ECM which underlies endothelial cells. An intact BM provides structure, support and compartmentalization and is essential for proper cellular function. BM integrity can be disturbed by increased proteolysis by matrix metalloproteases that liberate bioactive fragments, termed matrikines. These fragments include newly unmasked protein domains whose biological activity can influence cellular processes such as cell survival, migration, proliferation, and angiogenesis. However, little is known about the involvement of the BM in PH. Recently, using transcriptome analysis, we have identified a dysregulation of BM genes in the vasculature of PH patients. We found not only elevated expression of Col18 in pulmonary arteries but also increased serum levels of endostatin, a matrikine-derived from this collagen, in IPAH patients. Endostatin levels were correlating with worsen outcome of these patients. Therefore, delineating of the composition of BM in diverse form of PH can not only lead to better understanding of the vascular remodeling but can potentially lead to discovery of new biomarkers such as endostatin.
Collective epithelial cell migration in lung injury, repair, and regeneration
J. A. Park
Harvard T. H. Chan School of Public Health, Boston, US

The airway epithelium plays a central role as a biochemical and physical barrier against external stimuli. However, excessive stimulation causes injury of the airway epithelium. Therefore, rapid repair and regeneration of the injured airway epithelium are key for maintaining proper lung function. Under chronic disease conditions, including COPD and asthma, aberrant epithelial repair and regeneration further propagate disease progress. Moreover, recent studies suggest that abnormality of the airway epithelial cells as a core factor contributing to asthma.

During asthma exacerbations, bronchoconstriction causes buckling of the airway, which imposes mechanical compression on airway epithelial cells. In our previous studies using in vitro mechanical compression that mimics the mechanical environment of bronchoconstriction, we have shown that mechanical compression of well-differentiated human bronchial epithelial (HBE) cells leads to the recapitulation of key features airway remodeling, including collagen deposition, goblet cell hyperplasia, and contraction and proliferation of airway smooth muscle cells. Mechanical compression also induces secretion of asthma-associated mediators, including maspin, YKL-40, and tissue-factor contained in extracellular vesicles. Our RNA-seq analysis further reveals that compression of HBE cells is sufficient to induce inflammatory, late repair, and fibrotic signaling pathways which recapitulate the profile of asthmatic changes before compression. Our work and that of Grange et al. have established that the mechanical effect of bronchoconstriction is sufficient to drive airway remodeling. Moreover, mechanical compression causes a transition of the confluent layer of the HBE cells from a solid-like to a fluid-like phase, as evidenced by the onset of collective cell migration and cell shape elongation. These events mark so-called the unjamming transition (UJT) marked by collective cell migration and cell shape elongation.

In summary, our recent findings suggest that collective epithelial cell migration might be needed for effective epithelial repair and regeneration but become maladaptive under certain disease conditions.

Endothelial cells, the hidden svengali of lung vascular remodeling
N. Yabata, A. Tabuchi, J. Matuszak, W. M. Kübler
Charité – Universitätsmedizin Berlin, Institute of Physiology, Berlin, Germany

Pulmonary hypertension (PH) is a fatal disease caused by medial hypertrophy of pulmonary arteries and muscularization of previously non- or sparsely muscularized precapillary arteries. While PH research has therefore largely focused on pulmonary artery smooth muscle cell (PASMC) hypertrophy and proliferation, recent evidence suggests a critical role for endothelial apoptosis and subsequent proliferation of apoptotic-resistant endothelial cells as an initial trigger of the disease process.

What makes an endothelial cell apoptosis-resistant or –sensitive, and how these processes contribute to or even drive smooth muscle cell hyperplasia and hypertrophy remains, however, unknown.

The pulmonary vascular endothelium is highly heterogeneous, with pulmonary artery endothelial cells (PAECs) outlining vessels > 40 μm in diameter and pulmonary microvascular endothelial cells (PMVECs) differing markedly in their genetic, anatomic, and functional profile. Using a combination of in vitro and in vivo techniques, we identified distinct differences between PMVECs and PAECs which not only explain the co-emergence of endothelial cell apoptosis and apoptosis-resistance cells, but also provide the basis for the novel paradigm that distal vessel muscularization is primarily driven by the endothelium. While PH-inducing conditions such as hypoxia or growth factors induce endothelial autophagy in both PMVECs and PAECs, the resulting autophagic stress leads to vastly different cellular responses in that it causes PAECs to undergo apoptosis, whereas PMVECs fixate as apoptosis-resistant cells.

Moreover, mechanical compression causes a transition of the confluent layer of the HBE cells from a solid-like to a fluid-like phase, as evidenced by the onset of collective cell migration and cell shape elongation. These events mark so-called the unjamming transition (UJT) marked by collective cell migration and cell shape elongation. The UJT is therefore conserved across various diverse biological contexts, both in vitro and in vivo.

In summary, our recent findings suggest that collective epithelial cell migration might be needed for effective epithelial repair and regeneration but become maladaptive under certain disease conditions.

Novel mediators and therapeutic targets of lung fibrosis
O. Eickelberg
University of Colorado Anschutz Medical Campus, Pulmonary Sciences and Critical Care Medicine, Aurora, US

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal chronic lung disease, affecting over 5 million people worldwide. To date, there are no therapies that effectively stop progression or reverse the disease. IPF is characterized by altered cellular composition and dysfunction of epithelial-mesenchymal interaction in the peripheral lung, leading to excessive accumulation of extracellular matrix (ECM) and progressive scarring. The IPF lung is characterized by a heterogeneous distribution of normal or mildly affected regions, alternating with regions of significant fibrosis containing septal thickening, honeycombing, aberrant epithelial reprogramming, and fibroblastic foci. Since homeostasis and regeneration of the human lung after injury is controlled by delicate interplay between the ECM and multiple resident cell populations, it is imperative to define the sequential contributions of enhanced ECM secretion and crosslinking on cellular functions. Hence, we will summarize recent findings on protein changes during fibrosis and highlight novel strategies for the identification of precise therapeutic angles for IPF.

Pharmacological Gq protein inhibition in the lung
D. Wenzel
University of Bonn, Institute of Physiology, Bonn, Germany

G protein-coupled receptors (GPCRs) regulate many (patho)physiological processes in various organ systems; therefore, GPCRs are central targets for pharmacological therapies. Because different GPCRs converge on a limited number of G proteins direct modulation of G proteins is a very promising option for effective drug therapy. In particular, Gq protein modulation could improve time regulation of airways and arteries because a variety of constractors such as angiotensin, endothelin and angiotsensin are known to act via Gq. However, the limited availability of pharmacological G protein modulators as well as potential adverse effects prevented the application of this strategy up to now.

We could isolate FR900359, a cyclic desamidepeptide, from the leaves of the plant Aristolochia clematitis and found that it is a strong and specific pharmacological Gq protein inhibitor in heterologous expression systems. Moreover, we could demonstrate that the compound specifically inhibits Gq proteins in human airway and pulmonary arterial smooth muscle cells and it efficiently relaxes airways and pulmonary arteries of different species in isometric force measurements. The local pulmonary application of FR900359 as an aerosol or via intratracheal application also reduced airway resistance and pulmonary blood pressure in mice in vivo. This was achieved without systemic side effects because high tissue concentrations were only found in the lung but not in other organs as determined by LC-MS measurements.

The relaxant effect of FR900359 was also noticed in murine disease models of ovalbumin-induced asthma and hypoxia-dependent pulmonary hypertension. Additionally, the repetitive application of FR900359 also prevented the development of asthma and pulmonary hypertension. This was reflected by diminished airway hyperresponsiveness and pulmonary blood pressure but also effects on remodeling.

Thus, pharmacological Gq protein inhibition strongly diminishes airway and pulmonary arterial tone and may be a promising strategy for the treatment of chronic lung diseases.
S 06-01
Native PMCA – Proteomic surprises with old pumps for local calcium signalling in neurons
B. Fahlke
University of Freiburg, Institute of Physiology, Freiburg, Germany

Plasma membrane Ca²⁺-ATPases (PMCA), a family of P-type ATPases, extrude Ca²⁺ ions from the cytosol to the extracellular space and are considered key regulators of Ca²⁺-signalling. Using functional proteomics we have recently found that native PMCAs are heteromeric complexes assembled from two pore-forming PMCA1-4 subunits and two of the single-span membrane proteins either neuroplastin or basigin. Complex formation of neuroplastin/basigin with PMCA1-4 occurs in the endoplasmic reticulum and is obligatory for stability of the PMCA proteins and for delivery of PMCA complexes to the surface membrane. Knockout and (over)-expression of both neuroplastin and basigin profoundly affect the time-course of PMCA-mediated Ca²⁺-transport, as well as the submembranous Ca²⁺-concentration under steady-state conditions.

I will present our recent advances on PMCA-neuroplastin complexes and discuss the significance of neuroplastin and basigin as obligatory auxiliary subunits of native PMCA proteins and their impact on the time course of activity-dependent changes in intracellular Ca²⁺-concentration.

S 06-02
Dopaminergic modulation of dendritic synaptic integration in striatal spiny neurons
J. L. Piskin
Stony Brook University Renaissance School of Medicine, Neurobiology & Behavior, Stony Brook, US

The striatum is the major input nucleus of the basal ganglia, a group of interconnected subcortical nuclei that guide action selection and motor control. Striatal spiny projection neurons (SPNs) are key circuit components of the striatum, and integrate highly convergent synaptic inputs from diverse cortical and subcortical brain regions. SPNs are situated in striosome (patch) and matrix compartments of the dorsal striatum, allowing compartmentalized control of discrete aspects of behavior. Despite the significance of such organization, it is still unclear how compartment-specific striatal output is dynamically achieved. In my seminar I will discuss work we have done to elucidate the mechanisms guiding synaptic integration in striosome and matrix SPNs. I will present data that show dopamine signaling through D1 receptors oppositely modulates compartment-specific responses to convergent glutamatergic synaptic stimulation in striosome vs matrix SPNs. Activation of postsynaptic D1 dopamine receptors promotes the generation of long lasting synaptically-evoked dendritic plateau potentials in matrix SPNs, but opposes it in striosomes. The direction of dopaminergic modulation is determined by compartment-specific differences in dendritic L-type voltage gated calcium channel (L-VGCC) availability, and manipulating L-VGCC engagement can reverse the compartment-specific responses to D1 receptor activation. These results suggest a novel role for dopamine in shaping the balance of compartment-specific striatal output.

S 06-03
The schizophrenia-risk gene CALN1 encodes a CaM-like transmembrane EF-hand Ca²⁺ sensor
M. R. Kreutz
Leibniz Institute for Neurobiology, Magdeburg, Germany

Calneuron-1 is a transmembrane Calmodulin (CaM)-like Ca²⁺-sensor whose gene in humans has been associated with schizophrenia by yet unknown mechanisms. In this study we show that Calneuron-1 serves as an accessory subunit of muscarinic M1 receptors (M1R). Early after biogenesis it tightly associates with M1R and traffics with the receptor to the plasma membrane where it then regulates G-protein coupling and displaces Goα11 from the third intracellular loop at elevated (Ca²⁺) and thereby disrupts downstream G-protein signaling. Collectively the data suggest that Calneuron-1 is an accessory subunit of M1R that regulates G-protein coupling. In a cellular context the association of Calneuron with M1R modulates M1R-driven long-term depression (LTD) in the medial prefrontal cortex (mPFC) that is relevant for psychosis related sensory gating. Multiple studies suggest that cholinergic signaling is disrupted in schizophrenia patients and in animal models of schizophrenia. M1R-related changes in cholinergic signaling lead to impairments in muscarinic LTD and likely contribute to the underlying pathophysiology in PFC function. Accordingly, alterations in mPFC Calneuron-1 protein levels in schizophrenia might interfere with M1R function and thereby elicit schizophrenia-related behavior.

S 06-04
New modulators of DREAM/KChIP3 NCS protein-protein interactions
J. R. Naranjo
1Centro Nacional de Biotecnología, CSIC, Madrid, Spain
2CIBERNED, Madrid, Spain

Spatial and temporal changes in intracellular free Ca²⁺ regulate multiple physiological processes, from fertilization or muscle contraction to neuronal activity. Neuronal calcium sensor (NCS) proteins participate in the precise detection and regulation of free intracellular Ca²⁺ in cells in general and in neurons in particular and their activity is crucial for keeping normal cell function. DREAM/KChIP-3 is a multifunctional neuronal calcium sensor with specific functions in different subcellular compartments. In the nucleus, the Ca²⁺-free form of DREAM binds tightly to DNA and controls expression of several genes related to Ca²⁺ homeostasis, neuronal excitability and neuronal survival. As an auxiliary protein in the plasma membrane, DREAM interacts with and regulates gating of Kv4 potassium channels, voltage-dependent Ca²⁺ channels, and N-methyl-D-aspartate (NMDA) receptors. Calcium binding to functional EF-hand motifs in the DREAM protein triggers a conformational change that prevents binding to DNA and modifies its affinity for some interacting proteins e.g. CREM, CREB and GRK2, but not for others e.g. Kv4 potassium channels. Importantly, changes in the protein conformation of DREAM and other members of the neuronal calcium sensor superfamily are also induced upon binding of small molecules, like arachidonic acid, glidensand some diaryl-urea derivatives, which modifies their protein-protein interacting properties. Thus, binding of these molecules to proteins of this family will markedly change some of their biological properties. To improve affinity and gain specificity, we have designed new series of DREAM binding molecules and we have analyzed their effect in DREAM-mediated protein-protein interactions.

S 07-01
Dysregulated elf2-dependent Translation in Neurodegenerative Disease
E. Klann
New York University, Center for Neural Science, New York, US

A requirement for de novo protein synthesis is one of the hallmarks of long-lasting synaptic plasticity and long-term memory. Numerous studies, including several from our laboratory, have identified signaling cascades that couple cell surface receptors to the translation regulatory machinery during the formation of long-lasting synaptic plasticity and the consolidation of long-term memory. Interestingly, mutations in negative upstream regulators and downstream effectors of translation initiation are associated with several types of brain disorders, including intellectual disability and autism spectrum disorder.

Moreover, dysregulated control of translation initiation has been observed in postmortem brains in humans with neurodevelopmental and neurodegenerative disorders, as well as in mouse models of these disorders. For example, PERK-dependent phosphorylation of serine 51 on the alpha subunit of elf2, which inhibits general translation, is upregulated in a number of neurodegenerative diseases, including Alzheimer’s disease. I will discuss our studies of the regulation of PERK-elf2alpha translational control in the normal mouse brain and in mouse models of Alzheimer’s disease. I also will discuss studies on cell type-specific disruption of PERK-elf2alpha translational control in dopaminergic neurons and its impact on motor behavior and cognition. These studies have revealed interesting links between translation initiation, motor behavior, and cognition, and may also provide insight into the molecular basis for synaptic, motor, and cognitive dysfunction in neurodegenerative diseases.

S 07-02
Harnessing human brain cell models with robotics and deep learning to discover causes and treatments for neurodegenerative disease
S. Finkbeiner
GladeStone Institutes, UCSF, San Francisco, US

Despite numerous clinical trials, it remains the case in 2019 that there are no disease-modifying therapies for any of the major adult-onset neurodegenerative diseases such as Alzheimer’s disease (AD), Frontotemporal dementia (FTD), Parkinson’s disease (PD), Huntington’s disease (HD) or amyotrophic lateral sclerosis (ALS). The preclinical pipeline to identify causes and find treatments that translate into effective therapies has largely failed to serve its purpose. In this talk we will describe our work to address this major unmet medical need. In an effort to create more relevant disease models, we developed human patient-derived neuronal models of AD, FTD, PD, HD and ALS. To exploit these models, we...
invented an automated imaging platform, called robotic microscopy (RM), capable of high throughput longitudinal single cell analysis. RM and longitudinal single cell analysis is 2–3 orders of magnitude at detecting phenotypic differences than conventional snap shot HTS approaches. Moreover, multivariate longitudinal single cell data can be integrated using Bayesian analysis methods to develop models that predict fate at a single cell level and used to create blueprints for interventions. Recently, artificial intelligence / deep learning methods have been developed to uncover insights in imaging data that are not evident to the human eye. Observational studies from different models suggested that deficits in protein homeostasis were predictors of whether and when cells would undergo neurodegeneration. Based on these observations, we developed a high throughput assay for autophagy flux based on optical pulse labeling to search for compounds that could stimulate autophagy. We developed nanomolar potent orally available compounds that stimulate autophagy in neurons in vitro and in vivo, promote the clearance of tau, synuclein, TDP43, and huntingtin, and mitigate cytopathology and neurodegeneration phenotypes in our patient-derived models of HD and ALS. We propose that deficits in the ALP may be a common thread that cuts across multiple neurodegenerative diseases and could be a useful therapeutic target.

S 07-03
Synaptic mechanisms of neurodegeneration and neuroprotection in ALS
F. Roselli
DZNE, Darmstadt, Germany

According to the Excitotoxicity Hypothesis, excessive excitation, through synaptic or extrasynaptic receptors and together with intrinsic hypersensitivity of motorneurons, may drive motoneuropathy degeneration in Amyotrophic Lateral Sclerosis (ALS) by cause of calcium overload, ER stress and mitochondrial dysfunction. However, in vivo electrophysiological measurements have revealed that MN are actually hypoactive, and chemogenetic approaches have demonstrated that restoration of excitability is indeed neuroprotective. We have now proved if synaptic inputs may be the source of loss of excitation in MN and we have sought to link the loss of synaptic excitation to MN vulnerability. Using in vivo single-MN electrophysiology and selective input stimulation, we reveal that response of the synapses impinging on MN are reduced in presynaptic SOD1(G93A) mice. Indeed, propriospinal synapses display disrupted clustering several post-synaptic proteins (Homer, Shank and AMPAR subunits) but intact presynaptic terminals. We have used multiplexed PSAMOREADD chemogenetics approaches to determine that acute and chronic activation of PKA is sufficient to restore the synaptic content of AMPAR and, through the modulation of MN firing, to provide beneficial effects on multiple disease-related pathways. Furthermore, we highlight the existence of a positive feedback loop in which increased excitability contributes to restoring synaptic structure and reducing disease burden. Thus, we show that dysfunction of excitatory synapses is an early but reversible event in ALS and influences disease pathogenesis by modulating MN firing.

S 07-04
High-resolution imaging of human synapses in health and disease
C. Heuer1,2, C. Smith1, T. Gillingwater2, I. Deary1, S. Abraham1, S. T. Spire-Jones1,2
1University of Dundee, Division of Systems Medicine, Dundee, UK
2University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, UK
3University of Edinburgh, Centre for Clinical Brain Sciences, Edinburgh, UK
4University of Edinburgh, School of Philosophy, Psychology and Language Sciences, Edinburgh, UK
5University of Edinburgh, UKDRI, Edinburgh, UK

We have more synapses in our brain, than stars in our galaxy. These tiny structures are critical for normal brain function and we know that in diseases such as dementia, loss of these connections associates strongly with cognitive decline. Furthermore, synaptic loss is an early feature of most neurodegenerative diseases, therefore it is important to understand where and why these synapses are being lost in both normal and pathologic brain aging. However, synaptic biology is still in its infancy due to their small size and how high-resolution imaging techniques are required. We have optimised the high-resolution technique array tomography for human post-mortem tissue, allowing us to study human brain changes at the synaptic level. Briefly, fresh human brain is dissected into small cortical blocks and tightly fixed in paraformaldehyde, then dehydrated in ethanol before embedding in resin. The tissue blocks are cut at 70nm in serial ribbons and stained for synaptics proteins using conventional immunofluorescence. Images are collected along the ribbon and reconstructed into three dimensions.

In this presentation, I will discuss the characterisation of the first post-mortem donation from a healthy ageing cohort established in Edinburgh. The aim of the Lothian Birth Cohort 1936 is to discover what influences cognitive decline in later life and the team collects data ranging from longitudinal pre-mortem cognitive function to post-mortem brain characterisation, at the synaptic level. I will also discuss some of the Alzheimer’s projects underway in Edinburgh, looking at pathological protein accumulation at the human synapse. Using array tomography, we have been investigating the accumulation of amyloid and tau at synapses as a potential driver of synaptic breakdown in AD.

Finally, much of my work focuses on synaptic pathology in Amyotrophic Lateral Sclerosis (ALS). I have utilised the array tomography technique to study synaptic integrity in ALS and the accumulation of pathological proteins at the synapse. I will present data showing that synaptic loss in ALS is associated with cognitive decline and that the major pathological protein pTDP-43 is found at the synapse. Taken together, this presentation will summarise the use of array tomography as a tool for human synaptic analysis in health and disease.

S 07-05
Hypothalamic in neurodegeneration
D. Yilmazer-Hanke
Ulm University, Section Clinical Neuroanatomy, Neurology, Ulm, Germany

The hypothalamus is a brain region with numerous subregions and nuclei that regulate bodily functions and homeostasis as well as complex behavioral stereotypes in conjunction with endocrine, metabolic and autonomic responses. These complex hypothalamic behavioral patterns are under the control of higher limbic brain areas and are part of a larger neuronal network that extends to downstream brainstem areas. Behavioral patterns encoded by hypothalamic networks include food intake, sleep and circadian rhythms, sexual function and reproduction, mood, adaptation to stress, and coping with threats resulting in aggressive or defensive behaviors and fight or flight responses. Alterations in the structure and function of specific hypothalamic nuclei are found in neurodegenerative conditions such as Alzheimer’s, Parkinson’s, Pick’s and argyrophagic grain disease (AGD) and may also play an important role in amyotrophic lateral sclerosis (ALS). Here, neurochemical and cytoskeletal changes in hypothalamic neurons producing various hormones and neuropeptides are reviewed in various neurodegenerative diseases in humans and in mouse models. Because weight loss and autonomic dysfunction are common non-motor symptoms in ALS, hypothalamic regions regulating these functions are studied to test the hypothesis that disturbances in hypothalamic circuits may serve as a previously unappreciated facilitator of neurodegeneration. These regions are in favor of a contribution to synaptic pathology and the resulting metabolic abnormalities and disturbances in central homeostatic systems to the development of neurodegenerative changes. They set the stage for reappraisal of hypothalamic function and dysfunction in a larger group of diseases of the human brain including ALS.

Symposium 08: Novel developments in phosphate homeostasis – health and disease

S 08-01
Np2a inhibition in chronic kidney disease
L. Thomas1, J. Xue1, S. K. Murali2, R. A. Fenton2, J. A. Dominguez Rieg3, T. Rieg3
1University of South Florida, Molecular Pharmacology and Physiology, Tampa, USA
2Aarhus University, Department of Biomedical, Aarhus, Denmark

Treatment of hyperphosphatemia, which is common in the later stages of chronic kidney disease (CKD), is limited to dietary phosphate (Pi) restriction and oral phosphate binders. The sodium-phosphate cotransporter Np2a mediates a large proportion of renal Pi reabsorption, but no renal Pi transport protein is currently a pharmacological target. Recently, we identified a biodegradable and orally available selective Np2a inhibitor (Np2a-i, PF-08682006) has been described to reduce Pi uptake in HEK cells transfected with mouse or rat Np2a; however, in vivo studies have so far been lacking. We used C57BL/6J mice to study the effect of Np2a-i (oral gavage, 1% of body weight) or vehicle. Np2a-i dose-response (0.3-300 mg/kg) relationships of Pi excretion were assessed in metabolic cages for 3 hours. Np2a-i inhibition caused a dose-dependent increase in urinary Pi excretion from 27±6 nmol min⁻¹ in response to vehicle to a maximum of 150±14 nmol min⁻¹ at 300 mg/kg, ED50 = 21 mg/kg. Plasma Pi (0.5±0.1 mmol L⁻¹, P<0.05) and PTH (103±12 µg mL⁻¹, P<0.05) significantly decreased after 3 hours, with both returning to near baseline levels after 24 hours. Vehicle application did not affect plasma Pi (0.8±0.1 mmol L⁻¹, NS) or PTH levels (123±11 µg mL⁻¹, NS) at 3 or 24 hours (not shown). In order to determine if Np2a-i inhibition is useful in CKD, we employed a Ni nephrectomy (Nx) model. Mice with 5/6 Nx showed a higher urinary Pi excretion in response to vehicle compared to Sham mice (15±2 versus 15±2 mmol min⁻¹, P<0.05). Increasing doses of Np2a-i in these mice also showed a dependence for urinary Pi excretion; however, the maximum effect was reduced compared to Sham mice (116±8 versus 157±7 mmol min⁻¹, P<0.05). A dose of 100 mg/kg significantly reduced plasma Pi (Sham: -8±0.1; 5/6Nx: -9±0.5; P<0.05) and PTH (Sham: -63±22; 5/6Nx: -78±6; P<0.05) in both groups after 30 mins; however, the effect on plasma Pi was less pronounced in 5/6 Nx mice compared to Sham (P<0.05). Vehicle application did not affect plasma Pi or PTH in either group or timepoint (not shown). In summary, Np2a-i inhibition might be a useful treatment for conditions with impaired Pi homeostasis with or without impaired kidney function.
Medial vascular calcification is defined as the deposition of calcium-phosphate in the medial layer of the arteries. This arterial mineralization presumably causes increased vascular stiffness, impaired autoregulation of organ perfusion and modulation of atherosclerotic plaque stability. Medial vascular calcification is associated with an increased risk of cardiovascular events and mortality. Under physiological conditions, ectopic calcium-phosphate crystallization is prevented by calcification inhibitors. Increased phosphate levels may exceed the capabilities of this anti-calcific defense. Thus, phosphate has been recognized as a key factor augmenting vascular calcification and the most extensive vascular calcification is observed in hyperphosphatemia during chronic kidney disease. However, this vascular calcification is not simply a passive precipitation of calcium and phosphate, but was recognized as a highly regulated and active process. This process is, at least in large parts, mediated by vascular smooth muscle cells (VSMCs). Phosphate exposure is able to induce an osteo-chondrogenic transdifferentiation of VSMCs. These transdifferentiated VSMCs impair the physiological calcification mechanisms, cause a generalized subtype inflammation, degrade and remodel the extracellular matrix and release calcifying extracellular vesicles. This results in deposition of hydroxyapatite and an active mineralization of vascular tissue and Recent observations elucidate some key signaling pathways that mediate the toxic effects of phosphate on VSMCs. Identifying these key mechanisms regulating the phosphate-induced VSMC-transdifferentiation may open new therapeutic opportunities to ameliorate progression of vascular calcification.

FGF23 and cardiovascular disease

M. Leifheit-Nestler
Hannover Medical School, Department of Pediatric Kidney, Liver and Metabolic Diseases, Pediatric Research Center, Hannover, Germany

Left ventricular hypertrophy (LVH), myocardial fibrosis, and vascular calcification are the major cardiovascular pathologies in the general population and in patients with chronic kidney disease. The causes of cardiovascular disease pathologies are multifactorial. However, for about ten years, the bone-derived phosphaturic hormone fibroblast growth factor (FGF) 23 has been discussed as a new mediator for the progression of LVH. Reaching serum levels of up to 1,000-fold higher than in healthy individuals, FGF23 and associated alterations in mineral metabolism, including hyperphosphatemia, hypercalcemia, secondary hyperparathyroidism, and vitamin D and klotho deficiency, are associated with uremic cardiomyopathy, LVH, premature death, and all-cause mortality. Recent studies indicate that FGF23 is also expressed in the heart, and markedly enhanced in various clinical and experimental settings of cardiac remodeling and heart failure independent of preserved or reduced renal function. On a cellular level, FGF23 is expressed in cardiomyocytes and in other non-cardiomyocytes, including cardiac fibroblasts, vascular smooth muscle and endothelial cells in coronary arteries, and in inflammatory macrophages. In contrast to the established FGF23/FGFR1α/α-klotho signaling complex in the kidney which leads to the downregulation of sodium-phosphate co-transporters via induction of the MAPK pathway, FGF23/GFRα4 activates PLCγ in cardiomyocytes and induces hypertrophic cell growth using calcineurin/NFAT signaling in the absence of α-klotho. Furthermore, FGF23 increases intracellular calcium levels in cardiomyocytes in vitro and promotes contractility of murine cardiomyocytes and ventricular muscle strips ex vivo α-klotho independently. Current data also suggest that FGF23 can stimulate pro-fibrotic factors in myocytes to induce fibrosis-related pathways in fibroblasts and consequently cardiac fibrosis in a paracrine manner. In addition, chronic inflammation in cardiac fibroblasts was shown to induce the expression of FGF23 in the heart, suggesting a paracrine signaling mechanism of chronic inflammation inducing cardiac fibrosis. Moreover, recent investigations suggest that endogenous expression and secretion of FGF23 by infiltrating macrophages induce cardiovascular disease progression through induction of inflammation and finally pro-inflammatory cytokines promote the development of cardiac hypertrophy and fibrosis. However, hard evidence from experimental studies is lacking.

Fibroblast growth factor 23 and inflammation

D. Ertl-Schiegl
University of Zurich, Institute of Physiology, Zürich, Switzerland

Fibroblast growth factor 23 (FGF23) is a bone derived endocrine hormone that lowers plasma phosphate levels by increasing urinary phosphate excretion and by reducing calciotriol production in the kidney. In patients with chronic kidney disease (CKD), plasma FGF23 levels rise early during the course of CKD and are independently associated with mortality and morbidity of these patients. Similar in patients with acute kidney injury (AKI), plasma FGF23 levels rise rapidly after onset of AKI and are associated with AKI severity and progression. In both CKD and AKI mouse models, phosphate restriction is not enough to normalize plasma FGF23 levels which suggests that other mechanisms different from phosphate metabolism regulate FGF23 levels. CKD and AKI are affected by renal inflammation. Recently we have shown that the inflammatory cytokine tumor necrosis factor (TNF) increases FGF23 levels in wild type mice while TNF blockade in CKD as well as in non-renal inflammation mouse models reduces plasma FGF23 levels. Other inflammatory cytokines such as interleukin 1β (IL-1β) and IL-6 are also able to stimulate FGF23 expression. Interestingly, patients with autoinflammatory diseases such as inflammatory bowel disease, psoriasis, lupus nephritis and osteoarthritis have also elevated FGF23 levels. The function of FGF23 in the immune system is so far not well understood. It has been shown that FGF23 decreases calcitriol production in macrophages in vitro and impairs neutrophil infiltration into inflamed tissue in vivo. The implication of elevated FGF23 in the course of inflammation must be revealed in the future, just like the question if inflammation itself drives the rise of plasma FGF23 in CKD and AKI.

Symposium 09: Watching the brain compute: next-generation imaging technologies to dissect neural information processing in behaving animals

S 09-01
Two-Photon imaging of engram activity in the hippocampus during learning

M. Barthele
University Freiburg, Institute for Physiology I, Freiburg, Germany

The dentate gyrus (DG) is the entrance gate of the hippocampus and translates the rich input stream from the entorhinal cortex into sparse non-overflowing memories. The network mechanisms underlying sparse coding are however largely unknown. This talk aims to bridge between recent in vivo and in vitro studies to highlight new insights on the role of the various components of the DG network, in the sparse coding of information and the spatio-temporal emergence of DG population activity during learning. It will focus on synaptic mechanisms underlying information processing, 2-Photon imaging of single cells and populations of neurons in the DG of behaving mice. We will highlight recent studies on the functional role of granule cells and GABAergic interneurons in sparse representations in the DG circuitry. Thus, with this talk, we aim to improve our understanding on the synaptic, cellular, and network mechanisms underlying sparse activity in the DG and to provide new insights on the spatio-temporal representation of information on the level of single cells and cell assemblies during learning.

S 09-02
Stimulus and decision coding in layer 2/3 barrel cortex

C. Buetftering
University College London, Wolfson Institute for Biomedical Research, London, UK

Sensory information enables us to make informed choices that are critical for survival. Primary sensory cortices are thought to process incoming sensory information, while choice-related variables important for decision-making are assumed to be generated further downstream. Here, we used two-photon calcium imaging of neurons in layer 2/3 (L2/3) of mouse primary somatosensory cortex (S1) during a cued two-choice texture discrimination task to determine whether neurons in L2/3 of S1 can code for behaviourally-relevant decision variables. We found neurons carrying information about the stimulus irrespective of the behavioural outcome (‘stimulus neurons’) as well as neurons whose activity carried information about the choice to be made (‘decision neurons’). Choice-related activity in decision neurons is not driven by signals related to motor output, but instead follows stimulus presentation. Furthermore, ambiguous population coding of decision neurons predicts miss trials and an improvement in categorical coding in decision neurons coincides with learning the stimulus-choice association. Our identification of neurons encoding stimulus and behaviourally-relevant decision signals interleaved within the same circuit suggests a direct involvement of L2/3 of S1 in the decision-making process.
S 09-03
Neural coding principles of sensory perception and learning revealed by multiplane 2-photon imaging
M. M. Kohl1,2
1University of Glasgow, Institute of Neuroscience and Psychology, Glasgow, UK
2University of Oxford, Department of Physiology, Anatomy and Genetics, Oxford, UK

How neuronal activity in different layers of the neocortex contributes to sensory processing is a fundamental question in neuroscience. Neural network models highlight the importance of a quantitative understanding of the relationship between activity across cortical layers on a trial-by-trial rather than trial averaged basis (Minsky and Papert, 1969; Dayan and Abbott, 2001). Until recently, we have been lacking methods for recording neuronal responses with both the spatial and temporal resolution required to study the dynamics of visually identified neurons in different cortical layers in vivo. We combined a novel 2-photon calcium imaging strategy and the use of dimensionality reduction methods to address this need (Chong et al., 2018). Our method allows fast, quasi-simultaneous imaging (30 Hz) of neuronal activity in two planes within layers II, III, and IV of the visual somatosensory cortex in head-fixed mice running on a wheel. Animals were trained to perform a whisker-dependent discrimination task and expressed the genetically encoded calcium sensor GCaMP6s. The same neurons were imaged longitudinally throughout the training period, from the naive to the expert stage. When examining neuronal representations of task as well as non-task related variables, we categorised neurons into “functional clusters” according to their overall activity. Our data show that the stimulus could be decoded with higher accuracy from neurons imaged deep within layer III than from neurons in superficial layer II. Moreover, trial-by-trial fluctuations seemed to be explained by a slow remapping of sensory responses across neurons in the same imaging field. Finally, our data suggest that high noise correlations for neurons located at the interface between layer II and layer III are necessary during late but not early stages of sensory learning. Overall, we present a detailed analysis of neuronal dynamics during associative learning, which may anticipate new insights into the specific role of primary somatosensory cortex in perception and behaviour.

S 09-04
Disinhibitory amygdala microcircuits for aversive learning
S. Krabbe
Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Learning and memory are fundamental neuronal processes that are essential for adaptive behaviour in an ever-changing environment. Mechanisms of memory formation are critically shaped by dynamic changes in the balance of excitatory and inhibitory neuronal circuit elements. Although local inhibitory interneurons only represent a minority of the cells in cortical areas, they tightly regulate the activity and plasticity of excitatory projection neurons in a spatially and temporally precise manner. However, to date, little is known how distinct subtypes of interneurons contribute to sensory processing and memory formation. Using fear conditioning as a model for associative learning, we find that behaviourally relevant, salient stimuli cause learning by tapping into a local microcircuit consisting of precisely connected subtypes of inhibitory interneurons. By employing the calcium imaging and optogenetics, we demonstrate that vasoactive intestinal peptide (VIP) expressing interneurons in the basolateral amygdala are activated by aversive events and provide a mandatory disinhibitory signal for associative learning. Notably, VIP interneuron responses are highly plastic themselves, and are strongly modulated by outcome expectations during learning. Our findings indicate that VIP interneurons are a central component of a dynamic circuit motif mediating adaptive disinhibitory gating in order to specifically learn about unexpected, salient events, thereby ensuring appropriate behavioural adaptations.

S 09-05
"Recording and interpreting neuronal population codes in health and disease."
B. Grewe
ETH Zurich, Institute of Neuroinformatics, Zurich, Switzerland

In my talk I will present recent advances of miniaturized in vivo population calcium imaging in freely moving mice during "healthy" and abnormal states. I will thereby include work from several collaborations and showcase different deep brain endoscopic brain imaging approaches and related population metrics that were used to analyze and interpret longitudinal changes in complex population activity. These novel technologies, analysis methods and results set the stage for a systems level perspective on deep brain neuronal circuits and how these process and memorize information in health and disease.

Symposium 10: Attention, memory and gate control: Neural processing of sensory information and its psychiatric pathologies

S 10-01
Synaptic interactions in the thalamocortical system during sensory processing and behavior
A. Groh
Universität Heidelberg, Institut für Physiologie und Pathophysiologie, Heidelberg, Germany

The cerebral cortex – often considered the ‘cognitive’ headquarter of the brain – is thought to accommodate distinct cortical output pathways from the mouse barrel cortex. We will then focus on functional aspects of the long range interactions between the barrel cortex and the thalamus studied with deep brain electrophysiology techniques in combination with optogenetics. By measuring synaptic signals directly using patch clamp we found that some areas in the thalamus – the higher order thalamus – are dominated by cortical activity through sparse yet strong excitatory synaptic inputs. In contrast, we also found transiently inhibitory and weak sustained excitatory synaptic interactions between the cortex and the thalamus which impose an adaptive sensory filter onto touch signals sampled by the mouse’ whiskers. Lastly, we will discuss corticothalamic interactions during simple behaviors and their potential involvement in salience encoding.

S 10-02
Impact of visual landmarks on the spatial representations of the medial entorhinal cortex
K. Allen
Heidelberg University Hospital and DKFZ, Department of Clinical Neurobiology, Heidelberg, Germany

Grid cell networks in the medial entorhinal cortex (MEC) integrate information about running speed and heading to keep track of the current position of an animal in space. This integration is thought to depend on the activity of head-direction and speed cells, and to rely principally on self-motion cues (e.g., vestibular cues and proprioception). To what extent visual information influences the activity of grid cells and other spatially selective neurons of the MEC is less clear. In my talk, I want to highlight how the presence of visual landmarks affects spatial coding in the MEC. I will present evidence that the stability of grid cell fields is lost when mice navigate in complete darkness. Moreover, the firing rate of speed cells is also sharply reduced when visual landmarks are absent. Finally, I will show that head-direction cells in the MEC form two classes that differ in their theta rhythmicity and their responses to manipulations of visual landmarks. Together, these findings suggest that the function of MEC neurons in navigation extends well beyond the integration of self-motion cues.

S 10-03
Hippocampal synaptic plasticity as a window on brain changes in psychosis
D. Manahan-Vaughan
Ruhr University Bochum, Medical Faculty, Neuropsychology, Bochum, Germany

In psychosis, sensory information processing at the perceptual is impaired, as are the subsequent creation of a sensory representation and the resultant behavioural response. Whereas the primary sensory and prefrontal cortices are intrinsically involved in the generation of the percept, the hippocampus is crucial for the creation of spatial and associative representations, both of which critically depend on appropriate processing of sensory information. It is therefore likely, that the hippocampus is especially vulnerable to brain changes in psychosis. This talk will focus on insights as to changes in hippocampal function that may occur following the first episode of psychosis. Immediate and long-term changes to the hippocampus on a timescale of days through months will be described in an animal model. Following the emulation of a first-episode in this model, hippocampal function becomes profoundly and progressively impaired: reflected at the level of hippocampus-dependent learning and memory, synaptic plasticity, experience-dependent immediate early gene expression, neuronal oscillations and excitability, neurotransmitter receptor expression and hippocampus-prefrontal cortex information relay and modulation. These data support the hypothesis that the hippocampus is a primary locus and putative initiator of brain changes in psychosis and that the hippocampal MDMA receptor is at the centre of this process.
vasopressin (AVP). AVP stimulates vasopressin 2 receptors (V2R) at the basolateral membrane of cysts which results in
growth. In line with these findings, our recent data show that accumulating luminal ATP stimulates the purinergic receptor
P2Y2R which leads to Ca2+-activated chloride secretion and increase of intracellular Na+ concentration in
villi. Moreover, we show that ATP secretion from cyst cells is increased in chronic renal failure which may be
involved in the development of renal failure.

In conclusion, our findings indicate that ATP and P2Y2R activation contribute to cyst cell proliferation in ADPKD, which
may be relevant for the development of novel therapeutic strategies.

S 11-03
TRPP2 ion channels and morphogenesis - lessons from polycystic kidney disease
M. Kottgen
University Freiburg - Medical Center, Department of Medicine, Renal Division, Freiburg, Germany

Mutations in transient receptor potential channel polycystin-2 (TRPP2) cause autosomal dominant polycystic kidney disease (ADPKD). The disease is characterized by the progressive replacement of functional renal tubules with fluid-filled cysts leading to end-stage renal disease. TRPP2 is a C2+-permeable non-selective cation channel. To date, it is not known how TRPP2 channel activity translates into morphogenetic programs controlling the shape of epithelial tubes. We hypothesize that local Ca2+-signals are critical to regulate specific downstream factors in TRPP2 signaling micro-domains. The molecular composition of the TRPP2 complex at the core of these micro-domains is unknown. We have recently employed knock-out-controlled affinity purification of native TRPP2 protein complexes followed by liquid chromatography tandem mass spectrometry and have identified several novel proteins constituting the TRPP2 channel complex. Notably, several of these proteins are Ca2+-regulated and therefore plausible effector proteins downstream of TRPP2. Many of the proteins in the TRPP2 complex share a common theme: they are constituents or regulators of the actin-myosin system and their generation controls the morphology of cells and organs, which may link TRPP2 channel function with morphogenesis. In fact, we have studied selected members of the TRPP2 complex in cell culture and in zebrafish and found that their loss of function phenocopies TRPP2-deficiency by impairing morphogenesis. We therefore propose that TRPP2-mediated Ca2+-signals regulate the generation of subcellular forces through the actin-myosin network thereby controlling cellular morphology, the

Symposium 11: Ion channels and transporters in kidney disease

S 10-05
New insights into dopamine functions in fear extinction
T. Duvart
Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt, Germany

The ability to learn which stimuli predict danger is crucial for survival but it is equally important to adapt behavior when those stimuli no longer represent a threat. One classic example of this is fear extinction learning, during which the repeated presentation of a stimulus (conditioned stimulus, CS) that no longer predicts an aversive outcome (unconditioned stimulus, US) leads to a gradual decrease in learned fear responses. Deficits in this form of safety learning are hallmarks of anxiety disorders and thus understanding the neural basis of fear extinction has clinical significance. In order to initiate extinction learning, the absence of the expected aversive outcome must be detected and signaled to the brain regions mediating fear extinction. However, the neural substrates of such a signal are largely unknown. In this talk, I will show evidence demonstrating that dopamine neurons play a crucial role in detection and signaling of the unexpected US omission during fear extinction. Bidirectional optogenetic manipulations reveal that this dopamine signal is both necessary for, and sufficient to, accelerate, normal fear extinction learning. Together, these findings identify a neuronal signal that is necessary to initiate fear extinction and reveal a crucial role of DA neurons in this form of safety learning.

Symposium 11: Ion channels and transporters in kidney disease

S 11-01
The impact of chloride secretion on the progression of polycystic kidney disease
A. Kraus1, D. Faria1, G. Schley1, J. Schödel1, S. Grampp2, D. J. Peters2, R. Schreiber1, K. Kunzelmann1, K. U. Eckardt2
1Friedrich-Alexander-Universität Erlangen-Nürnberg, Dept. of Nephrology and Hypertension, Erlangen, Germany
2University of Regensburg, Dept. of Physiology, Regensburg, Germany

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by numerous bilateral renal cysts which
predominantly originate from principal cells of the collecting duct. Continuous cyst enlargement leads to compression of
adjacent intact nephrons which over time results in loss of renal function and often the need for renal replacement. Cyst
growth is driven by an increase in cell proliferation and fluid transport into the cysts lumen which depends on chloride
secretion at the apical membrane of cyst epithelial cells. Chloride conductance has been shown to be triggered by
Ca2+ which leads to activation of apically localized cystic fibrosis transmembrane conductance regulator (CFTR) chloride
channels. ADPKD patients are often characterized by urine concentrating defects and elevated levels of arginine
vasopressin (AVP). AVP stimulates vasopressin 2 receptors (V2R) at the basolateral membrane of cysts which results in
increase of cAMP. Recently, tolvaptan, a V2R antagonist, has been approved for the treatment of ADPKD. However, the
effects are limited and accompanied by significant side effects. We and others have shown that in addition to cAMP, also ATP- and Ca2+-activated chloride secretion contributes to cyst
growth. In line with these findings, our recent data show that accumulating luminal ATP stimulates the purinergic receptor
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Symposium 11: Ion channels and transporters in kidney disease

S 10-04
Filtering of self-generated sounds by auditory cortical neurons
T. Sigurdsson
Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt, Germany

Much of the brain’s sensory input, such as the sound of our own footsteps, is directly caused by our actions. This raises
the question of how such self-generated stimuli are distinguished from similar sensory input caused by external events (for
example the sound of someone else’s footsteps). It has long been suggested that the brain solves this problem using
“corollary discharge” signals that represent the expected sensory consequences of movement, and which are subtracted from
the actual sensory input. Consistent with this idea, electroencephalographic measurements in human subjects have
consistently shown that stimulus, including sounds, which are generated by the subject’s behavior elicit smaller neural
effects are limited and accompanied by significant side effects.

We and others have shown that in addition to cAMP, also ATP- and Ca2+-activated chloride secretion contributes to cyst
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In conclusion, our findings indicate that ATP and P2Y2R activation contribute to cyst cell proliferation in ADPKD, which
may be relevant for the development of novel therapeutic strategies.
hope that a better understanding of the TRPP2 signaling network will help to design rational therapies for autosomal dominant polycystic kidney disease.

S 11-04 Cystic Fibrosis in the kidney: a story of dysfunctional β-intercalated cells of the collecting duct

Aarhus University, Biomedicine, Aarhus, Denmark
University of Regensburg, Dept. of Physiology, Regensburg, Germany

Patients suffering from Cystic fibrosis (CF) are unresponsive to activate pancreatic HCO₃⁻ secretion after application of the hormone secretin. In addition, CF patients do not respond with an increase in urinary HCO₃⁻ excretion after stimulation with secretin.

Using different mouse models, we studied the mechanism of secretin-induced urinary HCO₃⁻ secretion. The secretin receptor is expressed in the cortical collecting duct (CD) and localizes to the basolateral membrane of β-intercalated cells (β-IC). CFTR is also found in the CD and localizes together with pendrin to the apical membrane of the β-IC. In vivo application of secretin (1 pM) induced a marked and dose-dependent activation urinary HCO₃⁻ excretion. The secretin effect was absent in mice lacking the HCO₃⁻ secretory transporter pendrin (SLC26A4).

In global and tubulocentric CFTR knock-out mice, secretin failed to elicit significant increases in urinary HCO₃⁻ excretion. In the isolated perfused CD, secretin (10 nM) acutely stimulated the transport rate of pendrin in single β-IC. CFTR KO CD baseline pendrin activity was significantly lower and could not be activated by secretin. These results define the molecular mechanism of secretin-induced urinary HCO₃⁻ excretion and demonstrate its absence in mice lacking functional CFTR and pendrin. The cellular mechanism recapitulates the well-described molecular mechanism of secretin-activated HCO₃⁻ secretion in the excocrine pancreas. We propose that secretin serves a role in the homeostatic control of plasma HCO₃⁻.

S 11-05 Evolution of epithelial sodium channels and transport

M. Althaus, I. Wichmann, J. S. Dulai, J. Marles-Wright, S. Maxeiner, P. P. Szczesniak, I. Manzini
Freie Universität, School of Natural and Environmental Sciences, Newcastle upon Tyne, UK
Josted-Liebig University, Institute for Animal Physiology, Giessen, Germany
Saarland University, Institute of Anatomy and Cell Biology, Homburg, Germany
Johann-Wolfgang-Goethe University, Department of Medicine, Frankfurt, Germany

The limited sodium availability of freshwater and terrestrial environments was a major physiological challenge during vertebrate evolution. The epithelial sodium channel (ENaC) is present in the apical membrane of sodium-absorbing vertebrate epithelia and evolved as part of a machinery for efficient sodium conservation. ENaC belongs to the degenerin/ENaC protein family and is the only member that opens without an external stimulus. We hypothesise that ENaC evolved from a proton-activated sodium channel as found in insectocysts of freshwater vertebrates and investigated whether such ancestral traits are present in ENaC isoforms of the aquatic frog Xenopus laevis. Using whole-cell and single-channel electrophysiology of Xenopus oocytes expressing ENaC isoforms assembled from αβγ- or δβγ-subunit combinations we demonstrate that Xenopus αβγ-ENaC is profoundly activated by extracellular acidification within biologically relevant ranges (pH 8.0 – pH 6.0). This effect was not observed on Xenopus αβγ-ENaC or human ENaC orthologues. We show that protons interfere with allosteric ENaC inhibition by extracellular sodium ions, thereby altering gating kinetics and increasing probability of channel opening. Based on homology modelling of ENaC structure and site-directed mutagenesis, we identify a region within the extracellular loop of the δ-subunit which contains several acidic amino acid residues that determine proton- sensitive ENaC activation and inhibition by extracellular sodium ions. We suggest that Xenopus δβγ-ENaC can serve as a model allowing the investigation of ENaC transformation from a proton-activated towards a constitutively active ion channel, which might have occurred during evolution of tetrapod vertebrates to enable bulk sodium absorption during water-to-land transition.

S 11-06 Modulatory effects of apical and basolateral proteases on renal ENaC function

M. Barteg, C. Körb-Rietscheck
Friedrich-Alexander-Universität Erlangen-Nürnberg, Institut für Zelluläre und Molekulare Physiologie, Erlangen, Germany

The epithelial sodium channel (ENaC) is a heteromeric channel composed of three subunits (α, β, γ). The channel is localized in the apical membrane of principal cells in the aldosterone sensitive distal nephron (ASDN) where it constitutes the rate limiting step for sodium reabsorption. Its appropriate regulation by aldosterone and calcium is essential for renal sodium homeostasis and the long term control of arterial blood pressure. A unique feature of ENaC regulation is its complex proteolytic processing at specific cleavage sites within the extracellular domains of the α- and γ-subunits. This is thought to contribute to ENaC transformation from a proton-activated towards a constitutively active ion channel, the mechanisms contributing to ENaC regulation by proteases and their interplay with the aldosterone-dependent regulation of ENaC are still incompletely understood. Moreover, the physiologically relevant proteases remain to be identified. Under pathophysiological conditions, aberrantly occurring urinary proteases may lead to abnormal ENaC activation which may contribute to sodium retention, e.g. in nephrotic syndrome.

In addition to the direct proteolytic processing of the channel, renal interstitial proteases may also modulate ENaC activation. In preliminary experiments we observed that selective activation of PAR2 caused a sustained stimulation of ENaC-mediated transepithelial sodium transport in cultured tubular epithelial cells. This effect was preserved in cells pre-stimulated with aldosterone. Thus, this mechanism could provide an aldosterone-independent way of stimulating ENaC-mediated sodium reabsorption in the ASDN. The underlying signalling and the physiological relevance of PAR2-dependent ENaC activation remain to be elucidated. It is tempting to speculate that in inflammatory renal disease locally released interstitial protease may stimulate ENaC by activating PAR2. In conclusion, tubular and interstitial proteases are likely to contribute to ENaC regulation under physiological and pathophysiological conditions in a highly complex manner.

S 11-07 Diseases of paracelllar pathways

M. Bleich
Christian-Albrechts-Universität zu Kiel, Physiologisches Institut, Kiel, Germany

Epithelial transport of ions, water and organic substrates requires the coordinated function of epithelial cells separating the luminal and basolateral compartment. This epithelial layer forms a barrier against uncontrolled diffusion or penetration. At the same time it provides a regulated and selective permeability through the cell membranes as well as in between the cells. The respective transport routes are transcellular and paracellular, the latter being formed by the proteins of bi- and tricellular tight junctions like claudin, tricellulin and angulin. Only the coordinated action of the transcellular pathway, which contains the primary energy consuming transport step, and the paracellular pathway, which uses this energy indirectly by voltage or chemical gradients, guarantees the most efficient way of epithelial transport. There are several diseases which comprise either the loss of salt, water, or substrates or which indirectly show symptoms of electrolyte derangement. The origin of these diseases has been partially traced back to defects in membrane transport proteins like NCC2 (Bartter Syndrome), NCC (Gitelman Syndrome) or ion channels like ROMK (Bartter Syndrom) or ENaC (Pseudohypoaldosteronism). Some diseases also originate in the limited energy supply by mitochondrial function (Fanconi Syndrome). Meanwhile there is more knowledge on the molecular composition and function of the paracellular pathway and a couple of diseases found a mechanistic explanation in the defect or malfunction of paracellular proteins. Animal models have shown the respective symptomatic signature of these diseases for the search in human databases. In future we expect to identify more patient groups with distinct "caudinopathies" or defects in tricellular junctions. Mechanistic understanding of pathophysiology and compensatory action of the body to maintain homeostasis will be key to develop the respective treatment strategies for these patients until save gene therapy will be available.

Symposium 12: New Insights to Dopamine Function

S 12-01 Heterogeneity of input activity to ventral midbrain neurons in vivo

G. Palestrini
UTSA, Neurosciences Institute, San Antonio, US

The firing pattern of midbrain dopamine neurons is controlled by afferents to encode reward prediction error and drive reward-related behavior. However, the underlying synaptic mechanisms driving the activity are unknown. We obtained whole-cell recordings of identified midbrain dopamine neurons to measure subthreshold activity in vivo. Similar to their activity in vitro, dopamine neurons in vivo fire action potentials in a single spike pattern that is insensitive to synaptic noise, but can be driven by changes in synaptic activity state to fire bursts or pauses in firing. We identify two types of bursts: one driven by a depolarized state in membrane potential, and another driven by a rebound from a hyperpolarized state. Rather than a single burst signature, dopamine neurons display different bursts elicited by separate classes of afferents.
The diversity of dopamine signals in macaque monkeys

M. Matsumoto 1,2
1University of Tsukuba, Faculty of Medicine, Tsukuba, Japan
2University of Tsukuba, Transborder Medical Research Center, Tsukuba, Japan

Dopamine neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are known to encode reward prediction error and have been implicated in learning and motivation to obtain rewards. On the other hand, our recent studies in macaque monkeys have shown that a subgroup of dopamine neurons in the SNc transmits a signal related to the “salience” of external events rather than reward prediction error. These neurons are activated not only by rewarding stimuli but also by aversive stimuli and cognitively demanding stimuli. In this talk, we will introduce our recent data suggesting that these salience-related neurons regulate a cognitive ability, called “response inhibition”, to inhibit planned or ongoing motor actions that would lead to unwanted outcomes. We recorded single-unit activity from dopamine neurons in the SNc and VTA and neurons in the caudate nucleus while monkeys performed a saccadic countermanding task. In this task, after the monkey fixated on a point that disappeared and a saccadic target was presented. In 70% of the trials, the monkey was required to make a saccadic eye movement to the target. In the remaining 30%, the fixation point reappeared as a “stop signal” after the onset of the saccadic target. The monkey was required to cancel the planned saccadic eye movement. We found that dopamine neurons in the SNc, but not in the VTA, exhibited a significant excitation to the stop signal. This excitatory response decreased when the monkey failed to cancel planned saccadic eye movements. We also found that caudate neurons, which receive dopaminergic projections mainly from the SNc, exhibited a significant excitation to the stop signal as well. Furthermore, injecting haloperidol, D2 antagonist, into the caudate nucleus impaired the performance of canceling planned saccadic eye movements. These results suggest that the nigrostriatal dopamine pathway, mainly transmitting salience signal, regulates saccadic response inhibition.

S 12-02

Understanding the pathophysiological timeline of disease is paramount to developing cures and preventative measures for PD. Specifically, identifying when in the disease course the various phenotypes begin and, how they interact with one another, will allow the scientific community to ascertain which mechanisms can be targeted and critically, when. Many useful therapeutics could currently have their validity missed due to administration at the wrong time. Thus generation of a timeline from model systems mimicking this human disease is of the utmost importance.

S 12-05

Understanding the pathophysiological timeline of disease is paramount to developing cures and preventative measures for PD. Specifically, identifying when in the disease course the various phenotypes begin and, how they interact with one another, will allow the scientific community to ascertain which mechanisms can be targeted and critically, when. Many useful therapeutics could currently have their validity missed due to administration at the wrong time. Thus generation of a timeline from model systems mimicking this human disease is of the utmost importance.

S 12-04

A novel mouse model of CACNA1D-associated autism spectrum disorder

N. J. Ortner1, N. T. Hofer1, M. Khariyonoval2, E. Paradiso2, F. Ferraguti3, N. Singewald3, E. Carbone3, N. T. Hofer1
1Medical University of Innsbruck, Department of Neurology, Innsbruck, Austria
2Medical University of Innsbruck, Department of Pharmacology, Innsbruck, Austria
3Medical University of Innsbruck, Department of Neurology, Innsbruck, Austria

Voltage-gated Cav1.3 L-type Ca2+-dependent gene expression. Heterozygous Cav1.3-deficiency does not induce a detectable brain pathology in human or mice. In contrast, eight patients harboring germline de novo mutations in the CACNA1D gene (coding for the Cav1.3 α1subunit) have been identified with neurodevelopmental disease including autism spectrum disorder (ASD). All of them induced pronounced channel gating changes with gain-of-function features when expressed in HEK293 cells. Therefore we predict a disease-causing enhanced Ca2+-influx through Cav1.3 mutant channels in vivo which raises the exciting opportunity of symptomatic treatment with clinically approved LTCC-inhibiting drugs.

1. Calcium release deficit
2. Calcium signalling protein decreases.
3. Electrophysiological mutation in control IPS derived neurons.
4. Mitochondrial stress shown by altered mitochondrial membrane potential.
Symposium 13: Current progress in the development of new models for investigating nociception and pain in human

A large fraction of chemicals inducing pain stimulate the cation channels TRPA1 and TRPV1. TRPA1 agonists tested previously in human pain models lack specificity at the chosen concentrations, thus the sensation generated by isolated TRPA1 activation in humans was unknown. Using intradermal injections of the potent TRPA1 agonist J7010 and the potent TRPA1 antagonist A-967079 in a double-blind cross-over study, it was shown that isolated TRPA1 activation in human subjects causes pain. This can be used to validate new TRPA1 antagonists. More importantly, quantitative inhibition by A-967079 allows to quantify the TRPA1-dependent component in physiology and pathophysiology. Absorption of J7010 at polypropylene surfaces has to be considered when this new pain model is used. Second, human tissue acidosis occurring in pathophysiology has been translated into an improved human pain model. With this we explored whether and to what extent TRPV1, TRPA1 and ASICs are involved in the sensation of tissue acidosis. Addressing potential interactions between ion channels, respective inhibitors (BCTC, A-967079, amiloride) were injected in the volar forearm skin in healthy volunteers in a pre-randomized, double-blind and balanced design. Confirming the primary study hypothesis, the combination of all antagonists reduced acid-induced pain at pH 6.0. BCTC, but not A-967079 or amiloride inhibited pH 6.0-induced pain in the presence or absence of A-967079.

Investigations of the two-pore domain potassium channels in the human heart could identify the TASK-1 channel as an important regulator of the atrial action potential. Expression of TASK-1 channel is restricted to the atria and is significantly enhanced in atrial fibrillation (AF) patients resulting in a shortened atrial action potential duration (APD). Inhibition of TASK-1 reverses AF-related APD shortening to values observed in patients with sinus rhythm (SR). Therefore, we investigated the antifibrillatory effect of TASK-1 inhibitors and a novel adenosine-associated virus (AAV) gene therapy for TASK-1 knockdown in a porcine model of AF, and consecutively tested a TASK-1 inhibitor in patients with AF. Furthermore, we established a porcine model of persistent AF. After pacemaker implantation, AF was induced by atrial burst pacing. AF induction was controlled by a biofeedback algorithm that inhibits pacing in case autonomous AF is detected. Catheter-based electrophysiological investigations were conducted prior to and following 14 days anti-TASK-1 treatment. Pigs in the gene therapy group received multilocal intra-atrial injections of AAVs. Pigs in two pharmaceutical treatment groups received one of two TASK-1 inhibitors, Doxapram or A293, twice per day. Rhythm status was continuously recorded by intracardiac long-term ECG monitors. Following the treatment, porcine cardiomyocytes were isolated and investigated by patch-clamp and multi-electrode experiments. Atrial electrical remodeling was characterized by analyses of ion channel expression at mRNA and protein levels. TASK-1 mRNA, protein and current were significantly increased in AF pigs compared to SR controls resulting in shortened atrial APDs. In all AF pigs treated with AAVs or TASK-1 inhibitors the AF burden was significantly reduced. In all treatment groups, TASK-1 currents and atrial APDs recorded in porcine cardiomyocytes were reduced to values similar as in SR animals. A293 and Doxapram could be successfully applied for cardioversion in pigs with persistent AF. On average, Doxapram inhibited AF most effectively. TASK-1 inhibition significantly suppressed AF episodes and normalized cellular electrophysiological characteristics in a porcine model of AF. Therefore, TASK-1 inhibition represents a promising new strategy for AF treatment. In January 2019, the DOCTOS (Doxapram conversion to sinus rhythm) trial was started at the University Hospital Heidelberg to evaluate Doxapram as anti-arrhythmic drug.

The molecular basis for an allosteric inhibition of K+-flux gating in TASK channels

The presentation will review nociceptive function via the analysis of human perception. Various stimulation models in humans, which are either punctate, focal or wide area pressure stimuli will be discussed and arguments presented that delineate the type of tissue (superficial vs. deep) and type of small caliber afferent (C vs. Aδ-fiber, thermal vs. mechanical), which dominates a particular subtype of perception. Selective conduction or excitation blockade techniques (nerve pressure; excitochemical chemical) and sensitization methods will be presented that carve specific functional subgroups. Differences in the perceived stimulus qualities shall also be briefly highlighted. Finally, the functional importance of different subsets of nociceptors in sensitization of human pain pathways will be identified by contrasting their role in human models of primary and secondary hyperalgesia.

Symposium 14: Structure, pharmacology and therapeutic potential of K+ channels

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preferentially bind to a conserved binding site in the central cavity. Thus, there is a high medical need to identify novel drug binding sites located outside the conserved lipophilic cavity. We identified an unusual voltage-dependent block of TASK-1 by the local anesthetic bupivacaine and subsequently mapped the binding site in the side fenestrations of the channel underneath the second pore helices using a functional alanine mutagenesis screen approach in combination with in silico drug dockings and molecular dynamics. We found the drug positioned so far laterally in the fenestrations, that pore occlusion can be excluded as a block mechanism. Instead bupivacaine located in the side fenestration interferes with the voltage-dependent K⁺ flux gating, a mechanism describing Kᵦ₃⁺ channels to operate as K⁺₂P⁺⁺ channels, in a way that K⁺ ions can sense the voltage, causing voltage dependent K⁺ effluxes by removing a C-terminal like inactivation of the selectivity filter. Thus, in the presence of the drug, the increased outward currents that are physiologically present upon depolarization are impaired, resulting in a lack of outward rectification and a preferential inhibition of outward currents. In conclusion, this newly identified binding site and allosteric mechanism of Kᵦ₃⁺ flux gating inhibition described here may allow the rational drug design of potent and Kᵦ₃⁺ subfamily specific blockers with specific kinetic features of inhibition.

The author has objected to a publication of the abstract.

S 14-04
High Throughput Screening to identify novel TASK1 blockers
T. Müller, M. Hahn, M. Pätzl, M. Delbeck, B. Bastling, H. Meier, S. Mündt
BAYER AG, PH-RD, Wuppertal, North Rhine-Westphalia, Germany

The brain controls adiposity via central and peripheral neural circuits. We used molecular genetic tools such as optogenetics to probe the connection between peripheral sympathetic neurons and adipocytes. Further, we found this neuro-adipose junction to drive lipolysis via norepinephrine (NE) signaling (1) and that the SNS is necessary and sufficient for fat mass reduction (1,2). As obesity is a chronic inflammatory state, we set to define neuroimmune mechanisms that link inflammation to SNS neurons (3). We report the discovery of Sympathetic neuron-associated Macrophages (SAMs) that directly regulate the extracellular availability of norepinephrine (NE). We identified the molecular mechanism by which SAMs import and metabolize norepinephrine (NE). Abrogation of the mechanism for the uptake of NE outside the brain is sufficient to promote weight loss. Thus, we chemically modified an amphetamine, which targets the NE transporter, such that it does not cross the BBB. The anti-obesity effect of this novel drug will be discussed.

Acknowledgements: Howard Hughes Medical Institute, The Welcome Trust, Human Frontiers Science Fund, European Molecular Biology Organization, Fundação para Ciência e Tecnologia.

Symposium 15: Brain-body interface: hypothalamic control of innate behaviours and homeostasis

S 15-01
Deconstruction of neuron ensemble dynamics across behavioral states with combinatorial molecular identity
S. Stensman, S. Xu, H. Yang
Janelia Research Campus, HHMI, Reston, US

The brain controls adiposity via central and peripheral neural circuits. We used molecular genetic tools such as optogenetics to probe the connection between peripheral sympathetic neurons and adipocytes. Further, we found this neuro-adipose junction to drive lipolysis via norepinephrine (NE) signaling (1) and that the SNS is necessary and sufficient for fat mass reduction (1,2). As obesity is a chronic inflammatory state, we set to define neuroimmune mechanisms that link inflammation to SNS neurons (3). We report the discovery of Sympathetic neuron-associated Macrophages (SAMs) that directly regulate the extracellular availability of norepinephrine (NE). We identified the molecular mechanism by which SAMs import and metabolize norepinephrine (NE). Abrogation of the mechanism for the uptake of NE by SAMs increases NE availability, which in turn promotes thermogenesis and browning, and long-term amelioration of obesity independently of food intake (3). These results suggest that blockade of NE uptake outside the brain is sufficient to promote weight loss. Thus, we chemically modified an amphetamine, which targets the NE transporter, such that it does not cross the BBB. The anti-obesity effect of this novel drug will be discussed.

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Symposium 15: Brain-body interface: hypothalamic control of innate behaviours and homeostasis

S 15-02
Hypothalamic control of sleep and napping
S. A. Reiner
University of Zürich, Institute of Pharmacology and Toxicology, Zürich, Switzerland

Humans and rodent species divide their daily activity into an initial burst of high activity, followed by a period of reduced alertness or sleep – a siesta – then a subsequent bout of wakefulness. Across and within these different states, different aspects of behavior and perception are tightly regulated, many in surprising ways. For example, previous studies in humans and mice suggest that a mid-day siesta is dependent on sleep pressure. Recently, we have shown that siesta sleep is in fact driven by a specific population of neurons within the suprachiasmatic nuclei (SCN) - the circadian ‘master clock’ - that are active when most SCN neurons are silent. Using optogenetic and chemogenetic approaches, we showed that silencing this population of SCN neurons that express the neuropeptide VIP delays the daily siesta, while changing the timing of VIP+ SCN neuron signaling creates a siesta at will. Thus, a daily siesta is a “hard-wired” property encoded by the biological clock.

In a second and unexpectedly direct level of control, we find that both clock time and sleep state influence downstream cortical function at a cellular level. Thus, while a circadian clock directs successive “waves” of functionally relevant transcripts to cortical synapses in anticipation of activity and rest, actual sleep-wake state determines the translation of these transcripts and acutely regulates intracellular phosphorylation cascades, resulting in time- and sleep-dependent regulation of ion channel abundance and function. Together, these cascades intersect to produce a detailed timescourse of cellular activity optimized for its functional needs.

Symposium 15: Brain-body interface: hypothalamic control of innate behaviours and homeostasis

S 15-03
Hypothalamic temperature sensing in thermoregulation
G. Amara
Heidelberg University, Pharmacology Department, Heidelberg, Germany

Body temperature homeostasis relies, among other things, on the activity of specific groups of hypothalamic neurons that receive thermal information from peripherally-located thermoreceptors. Additionally, changes in hypothalamic temperature can trigger thermoregulatory responses as well, suggesting that the brain is also endowed with thermoreceptive cells. However, the physiological relevance of central thermosensation in body temperature regulation and the molecular mechanisms underlying temperature detection in the hypothalmsus are not fully understood. In this talk, I will introduce a technique we recently developed in the lab that allows us to produce controlled modifications of brain temperature in freely moving mice. Additionally, I will discuss the contribution of peripheral and hypothalamic thermoreceptors in body temperature homeostasis and present mechanistic insights into the temperature detection machinery of preoptic warm-sensitive neurons, a neuronal population that has long been proposed to work as hypothalamic thermoreceptors.

Symposium 15: Brain-body interface: hypothalamic control of innate behaviours and homeostasis

S 15-04
Sympathetic Neuroimmunity for Obesity
A. Domingos
University of Oxford, oxford, UK

The brain controls adiposity via central and peripheral neural circuits. We used molecular genetic tools such as optogenetics to probe the connection between peripheral sympathetic neurons and adipocytes. Further, we found this neuro-adipose junction to drive lipolysis via norepinephrine (NE) signaling (1) and that the SNS is necessary and sufficient for fat mass reduction (1,2). As obesity is a chronic inflammatory state, we set to define neuroimmune mechanisms that link inflammation to SNS neurons (3). We report the discovery of Sympathetic neuron-associated Macrophages (SAMs) that directly regulate the extracellular availability of norepinephrine (NE). We identified the molecular mechanism by which SAMs import and metabolize norepinephrine (NE). Abrogation of the mechanism for the uptake of NE by SAMs increases NE availability, which in turn promotes thermogenesis and browning, and long-term amelioration of obesity independently of food intake (3). These results suggested that blockade of NE uptake outside the brain is sufficient to promote weight loss. Thus, we chemically modified an amphetamine, which targets the NE transporter, such that it does not cross the BBB. The anti-obesity effect of this novel drug will be discussed.

Acknowledgements: Howard Hughes Medical Institute, The Welcome Trust, Human Frontiers Science Fund, European Molecular Biology Organization, Fundação para Ciência e Tecnologia.

Symposium 32
References:

Symposium 16: Novel mechanistic insight linking cardiovascular/metabolic disease and aging

S 16-01
Non-coding RNAs in the cardiovascular system
R. P. Brandes
Goethe-Universität Frankfurt, Frankfurt, Germany

Epigenetic mechanisms are though to stabilize the cellular activity state and to provide an improved adaptation to environmental conditions and cellular needs. The majority of the human genome is non-coding and it is progressively becoming clear, that non-coding RNAs have important epigenetic functions. As illustrated for several IncRNAs expressed in endothelial cells and studied by the authors in the presentation, IncRNAs can recruit chromatin remodeling complexes, decry genetic suppressors or guide them to specific sequences in the RNAs. Through this, non-coding RNAs fine tune gene expression and allow endothelial program execution. Given the large number and the high tissue-specificity of non-coding RNAs, altering their expression might be a novel approach in the treatment and prevention of cardiovascular disease.

S 16-02
Role of perivascular adipose tissue in cardiac immune response
S. Steffens
LMU University Munich, Institute for Cardiovascular Prevention, München, Germany

Past research investigating the underlying inflammatory mechanisms of cardiac repair has mainly focused on cells within the heart. The potential relevance of the perivascular adipose tissue, in particular the adipose tissue surrounding coronary arteries and myocardium, in the context of MI and thereof resulting heart failure, has been largely neglected so far. Adipose tissue is the largest endocrine organ in the human body and has a role in the development of cardiovascular disorders such as insulin resistance, cardiovascular disease, type 2 diabetes and many other complications. Perivascular adipose tissue is a more general term and comprises, amongst others, the epicardial adipose tissue (EAT) surrounding coronary arteries as well as pericardial adipose tissue (PAT) overlaying the pericardium. In humans, the EAT thickness correlates with acute coronary events and its echocardiographic assessment has been proposed as a diagnostic tool for acute coronary risk stratification. Moreover, studies measuring inflammatory cytokines and immune cell subsets in EAT biopsies of patients with coronary artery disease confirmed increased levels of inflammatory markers. However, the causal relationship between perivascular adipose tissue inflammation and outcome after an acute ischemic cardiac event is still largely unknown. Perivascular adipose tissue in mice and humans contains clusters of immune cells, which have been designated as fat-associated lymphoid clusters (FALCs). We recently found that the murine PAT, which contains a high density of these FALCs, is a relevant site for lymphocyte proliferation after MI. This immune cell activation in response to MI seems to have a crucial impact on cardiac healing after MI.

S 16-03
Role of Arginase in Cardiovascular and Metabolic Inflammaging
Z. Yang
University of Fribourg, Department of Endocrinology, Metabolism, and Cardiovascular System, Platform of Integrative Physiology, Faculty and Science and Medicine, Fribourg, Switzerland

Oxidative stress and inflammation are essential and common mechanisms of cardiovascular disease, metabolic disease and organismal aging. Studies in recent years provide evidence that increased activity of arginase, particularly, the extrathoracic mitochondrial urea hydrolyase type-II, Arginase-II (Arg-II) which metabolizes L-arginine to urea and L-citrulline, plays an important role in phenotypic changes in vascular endothelial cells, smooth muscle cells, and macrophages through regulation of oxidative stress and inflammation. The phenotypic changes in these cells lead to cardiovascular disease, adipose tissue inflammation, NAFLD, type-2 diabetes, cellular senescence, and ultimately acceleration of organismal aging. The underlying molecular mechanisms of Arg-II in accelerating inflammation cytokine release, oxidative stress, and cellular senescence, such as interaction with mTORC1/S6K1 and other signaling pathways will be addressed. Regulatory mechanisms of Arg-II in the disease conditions and perspectives of targeting Arg-II as therapeutic options in aging, and age-associated chronic diseases will be discussed.
Symposium 17: Ion Channels in Immunity

S 17-01
Identification of novel ion channels regulating T cell-mediated immunity
S. Feske
New York University School of Medicine, Pathology / Medicine, New York, US

Ion channels and transporters (ICTs) control ion fluxes across lipid membranes and play pivotal roles in a multitude of cell functions. While ICTs have been extensively investigated in excitable cells, there is a surprising lack of knowledge with respect to their function in immune cells and immunity. Of the more than 500 known ICTs only 10-15 are well established to play a role in immune responses. This includes Ca$^{2+}$ channels such as CRAC (encoded by ORAI and STIM genes), TRPM2 and TRPM7, the Na$^+$ channel TRPM4, the Mg$^{2+}$ transporter MAGT1, the K$^+$ channels Kv1.3 and Kc3.1, the Cl$^-$ channel LRRC8A and the Zn$^{2+}$ transporter ZIP7. Overall, our knowledge of ICTs in immune function is very limited. In order to fill this gap, our lab has developed in vivo shRNA screening approaches to identify novel ion channels and regulators that are required for T cell-mediated immune responses to viral infection, tumors and in autoimmune diseases. This talk will provide an overview of ICTs regulating immune function, immune ion channelopathies and discuss new insights into the role of ion channels in T cell mediated immune function.

S 17-02
Melastatin-like Transient-Receptor-Potential Channel-Kinase-Function – from Cellular Signaling to Immune System Homeostasis
S. Zierler
LMU Munich, Walther Straub Institute of Pharmacology and Toxicology, Munich, Germany

The dual-function transient-receptor-potential-potential protein, TRPM7, comprises a Ca$^{2+}$- and Mg$^{2+}$-permeable channel as well as a serine/threonine kinase. It has been shown to be essential for immune cell proliferation and development. However, the precise mechanisms by which TRPM7 regulates the immune system remain elusive. Using different mouse models, we decipher an essential role for this unique channel-kinase in cellular signaling and in vivo immune reactions. Exploring a role of TRPM7-kinase in an established immune system homeostasis, we found that its genetic disruption in mice prompts altered susceptibility to allergic reactions. Our findings highlight a regulatory function of TRPM7-kinase activity on mast cell degranulation and immunological reactivity [1]. Previously it was shown that conditional knock out of Trpm7 in the T cell lineage disrupts thymopoiesis, and results in altered cytokine and chemokine expression profiles. Using a homozygous kinase-dead mouse model with a single point mutation at the active site of the kinase, Trpm7$^{p539A}$, we demonstrated that TRPM7-kinase activity is not essential for thymopoiesis. Importantly, we found that the enzymatic activity of TRPM7 is required for gut intraepithelial T cell homeostasis. Kinase-dead mice lack intraepithelial T cells in the gut due to an intrinsic defect in CD103 transcriptional regulation. TRPM7-kinase activity controls TGF-β-induced CD103 expression as well as proinflammatory Tc17 cell differentiation, but is dispensable for anti-inflammatory, regulatory T cell (Treg) differentiation. Notably, we identified the C-terminal SK5-region of SKM22 as a novel substrate of the TRPM7-kinase. Moreover, we found that TRPM7-kinase activity promotes gut colonization by T cells in acute graft-versus-host-disease, with its disruption preventing disease development [2]. Thus, our results unveil a fundamental role of the TRPM7-kinase in immune cell function and suggest a therapeutic potential of kinase inhibitors in averting allergic reactions and acute graft-versus-host-disease. Restoration of immune homeostasis via TRPM7-kinase blockade might also be beneficial for the treatment of autoimmune diseases.

KV1.3 in neutrophil function - An embryonic-derived peptide reveals a crucial role of KV1.3 during neutrophil recruitment

A. B. Parekh
Oxford University, Centre of Integrative Physiology, Department of Physiology, Anatomy and Genetics, United Kingdom

During pregnancy, the maternal immune system plays a dual role. On the one hand, it remains competent to successfully protect the fetus and the mother from bacterial and viral infections. On the other hand, it requires mechanisms preventing to recognize fetal tissue as a 'semi-allogenic transplant' and to initialize immune responses against the unborn. Preimplantation factor (PIF) is a 15 amino acids small peptide, secreted by trophoblast cells and present in maternal circulation throughout pregnancy. PIF is associated with immune cells and was shown to alleviate the course of several autoimmune diseases, therefore predicted to have immune modulatory properties. However, mechanisms how PIF modulates immune cell functions are poorly understood.

To investigate the influence of PIF on acute inflammatory processes in vivo, intravital microscopy of postcapillary venules in TNF-α stimulated mouse cremaster muscles was carried out, reflecting the role of PIF in maternal serum. Administration of PIF prior to induction of inflammation impaired leukocyte recruitment by significantly decreasing intravascular adhesion and by significantly reducing the number of extravasated neutrophils into inflamed tissue. With the help of electrophysiological assays and the use of knockout mice, we were able to demonstrate that PIF inhibits the voltage gated potassium channel Kv1.3 on neutrophils, thereby affecting sustained store operated Ca²⁺ entry. Reduced intracellular Ca²⁺ levels impaired neutrophil spreading and adhesion strengthening, resulting in an increased susceptibility to physiological shear forces.

Findings from a LPS-dependent acute lung injury model corroborated the influence of PIF on neutrophil recruitment and identified PIF as a potential therapeutic agent for acute and chronic inflammatory diseases. Taken together, we identified Kv1.3 as an important regulator during neutrophil recruitment and revealed a mechanism how maternal immune cells are modulated by extra-embryonic tissue. In addition, our work highlights the potential of PIF as an anti-inflammatory drug to treat diseases, characterized by excessive infiltration of neutrophils and finally contributes to a better understanding of the feto-maternal crosstalk during pregnancy.

Senescence induction by the ATP-gated P2X7 receptor limits T cell effector response in the tumor microenvironment

F. Grassi
Institute for Research in Biomedicine, Bellinzona, Switzerland

Peculiar features of the tumor microenvironment condition the function of infiltrating immune system cells and eventually protect the malignant tissue from eradication. Extracellular adenosine triphosphate (eATP) is a signaling molecule, which variably affects directly or after hydrolysis to adenosine, all cells of the immune system. Whereas eATP is virtually absent in the interstitium of normal tissues, it can be present in the hundreds micromolar range in tumors, a concentration compatible with activation of the ATP-gated ionotropic P2X7 receptor. Here we show that P2X7 activity in tumor-infiltrating T effector/memory (TEM) cells limits cell proliferation and tumor suppression. Deletion of P2X7, encoding for P2X7, promotes a transcriptional signature that correlates with enhanced cytotoxic T cell response in human solid tumors. Conversely, P2X7 stimulation leads to cell cycle arrest and cellular senescence of TEM cells. Pharmacological antagonism of P2X7 or hypomorphic polymorphism of human P2X7 enhance TEM cells proliferation. These results uncover a purinergic checkpoint that limits the TEM cells response within the tumor microenvironment and can be targeted to improve the efficacy of cancer immunotherapy strategies.
WORKSHOPS

Workshop: Animal Welfare in Physiology & Life science

WS-01 Increasing transparency in biomedical research – the Animal Study Registry

B. Bert¹, C. Heinl¹, J. Chmielow ska¹, B. Gruene¹, M. Greiner², G. Schönfelder¹

¹German Federal Institute for Risk Assessment, German Centre for the Protection of Laboratory Animals (Bf3R), Berlin, Germany
²University of Veterinary Medicine, Foundation, Institute for Food Quality and Safety, Hannover, Germany

The reproducibility of results gained from animal experiments and their extrapolation to humans are intensively discussed. Reporting bias, HARKing (Hypothesizing After the Results are Known) and p-hacking have been identified as major factors contributing to the reproducibility crisis. Hence, greater transparency of animal experimentation is requested by the public as well as by the scientific community. As a potential countermeasure to reduce selective reporting, the German Centre for the Protection of the Laboratory Animals (Bf3R) has launched a new preregistration platform for animal studies, the Animal Study Registry (www.animalstudyregistry.org).

The registry is free of charge and is designed for exploratory and confirmatory studies in the field of fundamental and preclinical research. The registration form serves as a check list for researchers, helping them to plan their study thoroughly by asking detailed questions concerning study design, methods, statistics, and animal characteristics. With the registration in Animal Study Registry the study receives a digital object identifier (DOI) which marks the study as intellectual property of the researcher. During the embargo period the visibility of the study is restricted to the title, a short summary, and name of the institution (see Fig. 1). The Bf3R is part of the Federal Institute for Risk Assessment, a scientifically independent federal institution, which guarantees continuity and data security.

Registering a study in the Animal Study Registry is a possibility for researcher to show their commitment to transparency and data quality to editors, third party donors, and the general public.

WS-02 Biology-inspired Microphysiological Systems to advance Medicines

E. - M. Dehne, U. Marx

TissUse GmbH, Berlin, Germany

Microphysiological systems – microfluidic devices that aspire to emulate human biology in vitro – have proven to be a powerful tool in basic research and the drug development process. Their ability to host three-dimensional organ models in a controlled microenvironment under constant media perfusion enables them to create and maintain homeostasis. Their increased physiological relevance enhances the translatability of assay readouts and results to the human situation. By providing preclinical assays with great predictive power a global paradigm shift in drug development is envisaged. Various dedicated research programs in Europe and Asia and an extraordinary US governmental initiative have led recently to the first industrially relevant achievements of human single- and multi-organ microphysiological systems. The new opportunities addressed. Furthermore, latest result of our microfluidic chip platform interconnecting different organ equivalents will be presented. Several combinations of organs have been performed (e.g. a combination of liver equivalents with skin, intestine, pancreatic islets or neuronal tissues). In addition, a four-organ-chip for ADME profiling will be presented. In this system, a human primary intestinal model and a skin biopsy have been integrated on standard cell culture inserts. A fluid flow connected these barrier models with liver spheroids and a kidney model segregating the media flow through the organs from fluids excreted by the kidney. Finally, a roadmap into the future is outlined, to allow for more predictive and regulatory-accepted drug testing on a global scale.

WS-03 Transparency and and proactive communication of an emotional topic - insights from 3 years of "Tierversuche verstehen"

R. M. Stilling

Informationsinitiative, Tierversuche verstehen, Münster, Germany

The current success of biomedical research critically relies on the use of animals – to study fundamental biological processes or as models for human disease. At the same, time animal welfare in research facilities has made significant progress in the last decades. However, animal-based research has a notoriously bad reputation throughout society and is facing the imminent threat of increasing political pressure to be curtailed and then stopped prematurely. We propose that, to a large extent, this situation is due to a lack of transparency and openness to communicate about the necessity, the regulatory framework, and significance of animal research despite the obligation to justify the use of taxpayers’ funds. In this talk I will highlight best-practice measures to increase pro-active communication about animal research at the international, national and local level and will share first-hand experiences with this approach.

Finally, I will showcase the German initiative “Tierversuche verstehen” that was launched in 2016 to comprehensively and transparently inform the public about all aspects of experimental methods using animals in research.

Fig. 1 Scheme of the registration process.

A registered study will be under an embargo period of maximum five years. During this period, studies are only visible with limited contents. Once registered, the study receives a digital object identifier (DOI). Comments to the original study plan can be added at any time.

Tierversuche verstehen

"Tierversuche verstehen" ist eine Informationsinitiative der Wissenschaft, koordiniert durch die Allianz der Wissenschaftsorganisationen. Sie informiert umfassend, aktuell und faktenbasiert über Tierversuche an öffentlich geförderten Forschungseinrichtungen.

Logo "Tierversuche verstehen"
Conclusions

Phosphorylation by PKA inhibits the migration enhancing effect of Connexin 43

K. Pöpperle, U. Pohl, P. Kamerlisch

Ludwig-Maximilians-Universität München, Walter-Brenner-Centre of Experimental Medicine, Institute for Cardiovascular Physiology, Planegg-Martinsried, Germany

Question: Connexion 43 (Cx43) enhances cell migration and filopodia formation in a channel-independent manner via its cytoplasmic C-terminal tail (aa257-382). Since this part has multiple phosphorylation sites we analyzed here whether phosphorylation by PKA affects Cx43 mediated increase in cell migration and filopodia formation.

Methods: We mutagenized the phosphorylation site of Cx43 (S364A, S369G and S373G) by site-directed mutagenesis. Cell migration of HeLa cells expressing Cx43, mutated Cx43 (Cx43-PKA) or transfected with the empty vector as control (CTL) was analyzed in a wound assay under control conditions (10% NBCS) and in the presence of forskolin (10 µM FSK) to activate or PKI (30 µM) to inhibit PKA. Directionality of migrating cells was determined by immunofluorescence staining of the Golgí apparatus. Filopodia were quantified after staining the F-actin with AF546-labeled phalloidin. Immunoprecipitation and Western blot analyses were used to study interaction with PKA and its effector protein VASP as well as PKA-dependent phosphorylation of VASP (Ser157). Cx43 peptides containing the PKA phosphorylation site were used to analyze the effect in endogenously Cx43 expressing endothelial cells (HMEC).

Results: Cell migration of HeLa cells expressing Cx43-PKA was significantly enhanced compared to HeLa-Cx43 cells (mean accumulated distance: Cx43: 321 ± 23 µm; Cx43-PKA: 483 ± 44 µm), but directionality was decreased. Likewise, migration of Cx43-expressing cells was enhanced by PKA inhibition. Stimulation with FSK reduced migration and filopodia formation of Cx43-expressing cells. In contrast migration of Cx43-PKA or CTL cells remained unaffected by treatment with PKI or FSK. Activation of PKA by FSK increased binding of PKA and the PKA effector VASP to Cx43 and was associated with augmented phosphorylation of VASP, which is involved in the control of cell polarity and directed migration. A Cx43-peptide containing the PKA phosphorylation site fused to Tat for cellular entry prevented the FSK-induced decrease in migration of endothelial cells.

Conclusions: Our results demonstrate that PKA-dependent phosphorylation of Cx43 modulates cell motility in a channel-independent manner and plays a pivotal role in regulating directed cell migration. A PKA-dependent phosphorylation has also been shown for other connexins, however, whether this phosphorylation has similar effects on cell migration has to be further investigated.

Conclusions

These observations show that TRPP2 is required for final maturation and intercalation of cells after tight junction formation. Given that this effect seemed to reflect impaired contractile actomyosin activity, it is tempting to speculate that epithelial intercalation may reflect a physiological milestone to initiate terminal differentiation and facilitate epithelial coordination.

ORAL SESSIONS

Oral Session 01: Unusual Ion Channels

OS 01-01

Cell-free Expression and Electrophysiological Characterization of TREK Two-Pore Domain Potassium (K2P) Channels in Artificial Membranes

W. Musienkzi, T. Baukrowitz

Christian-Albrechts-Universität zu Kiel, Physiologisches Institut, Kiel, Germany

Potassium channels comprise about 80 genes in the human genome and enable basic physiological processes like action potential generation, muscle contraction and secretory processes. The superfamily of K2P channels feature an extracellular cap structure and four transmembrane helices with two pore domains in tandem. Therefore only two subunits combine to form the fourfold symmetry of the potassium selective pore. K2P channels are modulated by many physiological stimuli such as pH, lipids, temperature or phosphorylation, thus integrating extra- and intracellular signalling pathways. K2P channels have been characterized in native cells and heterologous expression systems by the Patch-Clamp technique. However, important problems can be explored particularly well in the absence of cellular environment in artificial membranes. Reconstituted protein production of human K2P channels was so far only feasible after removal of cytoplasmic N- and C-termini. Single channel properties of truncated channels were altered, and regulation mechanisms effective at the C-terminus cannot be studied. Hence, this thesis establishes cell-free expression as an alternative scheme for production of full-length K2P channels and their electrophysiological characterisation in artificial membranes of known composition. The translation reaction was performed in presence of different unilamellar liposomes, into which K2P channels were directly incorporated. Membrane localisation in liposomes was assessed by confocal fluorescence microscopy. The channels showed a preferential orientation in the liposomal membrane that indicated a cotranslational insertion mechanism. The function of TREK1 K2P channels was demonstrated by electrophysiological means in liposomes and planar artificial membranes. Finally, single hTREK2- and hTREK2-GFP channels were electrophysiologically characterised in vertical artificial membranes. Single hTREK2 channels showed an inwardly rectifying current-voltage relationship and a single channel conductance of 106 pS at -40 mV and 69 pS at +40 mV. This is consistent with characteristics reported for wildtype channels. The open probability of hTREK2 channels in artificial membranes was as high as 0.5 and indicated a destabilisation of the low activity ‘down’ state by the branched Lipid DPVPC. Cell-free synthesis of K2P channels can thus provide an opportunity to further investigate open questions, particularly of lipid regulation, in a well-defined environment.

OS 01-02

TRPP2-dependent cellular intercalation during epithelial maturation

A. Hofherr, C. Damke, J. Jahn, T. Busch, M. Köttgen

Medical Center – University of Freiburg, Renal Division, Department of Medicine, Freiburg im Breisgau, Germany

Question

Loss of transient receptor potential channel polycystin-2 (TRPP2) causes autosomal dominant polycystic kidney disease (ADPKD) in humans, a disorder characterized by progressive cystic transformation of tubular epithelia. Loss-of-function studies in animals have shown that TRPP2 controls epithelial morphogenesis in the kidney and other organs. However, the cellular mechanism connecting TRPP2 to epithelial morphogenesis has remained elusive.

Methods

Here we studied the cell biological function of TRPP2 in the context of precisely engineered genotypes modeling ADPKD in Madin-Darby canine kidney (MDCK) cells. TRPP2-dependent epithelial maturation in wild-type and mutant MDCK cell lines was followed from solitary cells to confluent epithelium using scanning electron microscopy, transcriptional electrical resistance measurements and immunofluorescence-based light microscopy. To scale and objectively the observed difference in cell shape, we developed a blinded, unbiased workflow of large-area light microscopy, fully automated machine learning-based image segmentation and high-throughput mathematical image analysis.

Results

Our analyses show that overall development and epithelial structure were similar in wild-type and ADPKD cells. Irrespective of genotype, all cells developed into sculpted shapes within tight, continuous epithelial monolayers. Cellular intercalation, however, was strongly dependent on TRPP2 and the actin cytoskeleton, as assessed by ZO1-mediated visualization of apical cell shape. In marked contrast to wild-type apical MDCK cells, which – after tight junction formation – develop intercalated cell boundaries, mutant cells lacking TRPP2 or cells with mutations disconnecting TRPP2 from the actin cytoskeleton showed straight boundaries without intercalation.

Conclusions

Modulation of proton currents by zinc reveals important insights in the inhibition mechanism on the voltage-gated proton channel

G. Chaves1, S. Bengt-Glumk7, A. Arne Fransen5, I. Mahorikys5, B. Mussel1

1PMD Nürnberg, Institute of Physiology and Pathophysiology, Nürnberg, Germany
2Forschungszentrum Jülich, Institute of complex systems 4 Cellular biophysics, Jülich, Germany

Zinc is the main physiological inhibitor of the voltage-gated proton channel, H1. Studies in several species as Homo sapiens, Rattus norvegicus, Mus musculus, Crystallinus crucianus, Rana esculenta, Helix aspersa, Ciona intestinalis, Coccodrillus palliaceus, Elmina huysyi and Helimusa viridis and Lingulodinium polyedrum show proton current reduction when Zn2+ is applied extracellularly. We have investigated the effect of zinc on a novel member among the H1 family: NpH-1 from Nicolera phytophila[1]. Meanwhile mammalian channels have an externally accessible histidine on the S3- S4 loop, NpH-1 has an acidic residue (Asp145) instead. The low pK of the aspartate (~ 3.7) allows it to be deprotonated in a pH as low as 4. When Zn2+ was applied externally on concentrations up to 1 mM, the channel showed affectation already at pH 6 and appeared insensitive at pH 5. Neutralization of Asp145 decreases slightly the activation time constant (taup) but not the positive shift of the conductance-voltage curve (Δ Vg). However, Asp to His mutation drastically boost the electrostatic effect of Zn2+ inhibition on the voltage sensor. Both Δ Vg and taup are increased, reaching values comparable to human pi and rat channels[2]. The results reveal a minor role of Asp145 on Zn2+ coordination and contradicts the hypothesis of the importance for zinc inhibition of an acidic residue at this position[3]. Our Hodgkin and Huxley model of NpH-1 proposes that Zn2+ and CDP2 modulate the rate of opening during the inhibition process, promoting the channel close state. From the results of another current analysis of the NpH-1 WT and mutant channels opposes the idea of Zn2+ as voltage-dependent blocker and points rather to an allosteric mechanism. Gating charge calculations and high resolution native gel electrophoresis (nCNE) confirms the dimeric nature of NpH-1, which opens the possibility of Zn2+ coordination in between the channel subunits.
Our data suggest that Cx43 expression is strongly modulated by miR-1 in iPSC-CMs. Furthermore, our findings demonstrate that metabolic stress leads to reduced Cx43 expression by enhancing miR-1 activity. Reduction of miR-1 activity via anti-miR-1 significantly improved intercellular communication. In conclusion, we propose miR-1 as a novel tool for improving intercellular coupling of iPSC-CMs to meet the functionality of native cardiomyocytes.

Pathophysiological events of the respiratory airway epithelium such as inflammation exacerbate adenosine release and gap junction hemichannel function. We used the Calu-3 cell line as a model to analyse possible interplay between connexin (Cx) channels and the adenosine signalling of human respiratory airway epithelial cells. The expressed Cx isoforms and adenine receptor subtypes were identified by quantitative RT-PCR analysis. The function of the different adenosine receptor subtypes and the induced signalling pathways were analysed using a combination of pharmacological agents and specific sIRNAS. Application of 5’-N-ethyloxycarbonyladenosine (NECA) increased the dye uptake rate in Calu-3 cells. Gap junction hemichannel activity was studied by dye uptake experiments. The pannexins and gap junction hemichannels inhibitor carbonic anhydrase (CBA) was not able to suppress the dye uptake rate at pannexin specific concentrations (<100 μM). At high CBX concentrations that also affect gap junction hemichannels suppressed the dye uptake in Calu-3 cells. The NECA related increase of dye uptake rate depended on stimulated cAMP synthesis and subsequent activation of the protein kinase A (PKA) as showed by quantification of cAMP levels and inhibition of adenyl cyclase and PKA. Further pharmacological experiments as well as knockdown experiments with specific sIRNAS showed the predominance of A2B adenosine receptor subtype for the increase of the dye uptake rate. The NECA related increase of the dye uptake correlated with a downregulation of Cx43 mRNA expression and an upregulation of Cx26 expression and protein synthesis and was inhibited by application of Cx26-sIRNA. Of note, a sIRNA related knockdown of Cx43 upregulated the expression of Cx26 mRNA and enhanced the dye uptake rate. The Calu-3 cell model shows that stimulation of A2B adenosine receptor activates synthesis of cAMP, which upregulates expression and synthesis of Cx26 and downregulates the expression of Cx43. The increased Cx26 production leads to an enhancement of gap junction hemichannel activity in the cell membrane. The report identifies a mechanism that integrates adenosine signalling and gap junction hemichannel activity and shows how adenosine signalling and Cx channels may act together and participate in promotion of persistent inflammation, which is observed in several chronic diseases of the respiratory airway.
surfaces. Hla monomers initially bind to the plasma membrane of host cells, where they form heptameric pores that are 50nm in diameter and function as oxygen sensors.

The sensitivity to the toxin-mediated effects differs in different cell lines: Human airway epithelial cells (16HBE14o- and A549) survive exposure to different toxin concentrations while HeLa cells are more sensitive to Hla than 59 cells. To understand the differences in toxin sensitivities we investigated possible factors including the densities of potential Hla-monomer receptors on the plasma membranes of these cells, the lipid composition of the plasma membranes, which has a decisive role in Hla-internalization and in pore formation, and the elimination of the Hla pores from the plasma membrane by endocytosis, which may be a defence mechanism of host cells against S. aureus in vivo.

**Oral Session 02: O2 Sensing and Hypoxia**

**OS 02-01**

ArhGAP29 fine-tunes RhoA activity and myofibroblast differentiation in hypoxia


1 University Medical Center, Georg-August University, Göttingen, Institute of Cardiovascular Physiology, Göttingen, Germany
2 King’s College London, Randall Centre of Cell and Molecular Biophysics, London, UK
3 University of Bristol, School of Cellular and Molecular Medicine, Bristol, UK
4 University Medical Center, Georg-August University Göttingen, Institute of Pharmacology and Toxicology, Göttingen, Germany

**Abstract**

Fibroblasts show a high range of phenotypic plasticity including the transdifferentiating into myofibroblasts. Myofibroblasts are responsible for the generation of the contraction forces that are important for wound healing and scar formation. Overactive myofibroblasts on the other hand are involved in abnormal scarring. Cell-stretching and extracellular signals such as transforming growth factor-β can induce the fibroblastic myofibroblastic proliferation and chemokine-dependent or hypoxic environments such as reduced tissue oxygenation have an inhibitory effect. We investigated the effects of hypoxia on fibroblastic properties and linked this to RhoA activity. Hypoxia resulted in a decreased transdifferentiation of primary fibroblasts to mesenchymal-positive myofibroblasts. This was accompanied by alterations in cell contractility, actin reorganization, and RhoA activity. We identified a hypoxia-inducible induction of ArhGAP29, which down-regulates RhoA activity. This is critically involved in fine-tuning the hypoxia-induced RhoA response and thus reduces the activity of the RhoA-actin-MRTF-A (myocardin-related transcription factor-A) pathway and the differentiation of myofibroblasts in hypoxia. This novel link between the regulation of RhoA signaling and hypoxia is likely to be important for ischemia-induced tissue remodeling and the fibrotic response.

**OS 02-02**

Adaptation of the oxygen sensing system in murine liver and kidney during intrauterine and perinatal development

C. L. J. Jacoba, K. M. Kirschnieb, C. Dames, L. C. Sciesieliskia

1 Charité-Universitätsmedizin Berlin, Klinik für Neonatologie, Berlin, Germany
2 Charité-Universitätsmedizin Berlin, Institut für Physiologische Physiologie, Berlin, Germany

**Abstract**

After birth, arterial oxygen tension (PaO2) abruptly rises. In premature birth, this is considered to be a trigger for impaired vasculosclerosis and erythropoiesis. To ensure adequate oxygen supply throughout intrauterine development, the oxygen sensing system should already adapt to the constantly changing oxygen supply and demand in utero. The aim of the study was to describe the basal mRNA expression levels of the oxygen sensing system during mouse embryonic, fetal, neonatal, adult development, in particular the hypoxia inducible factor (HIF)-regulating oxygen sensors prolyl hydroxylase 2 and 3 (Egln1, Egln3) and factor inhibiting HIF (FIH). The activity of the oxygen sensing system was evaluated by analyzing corresponding expression levels of Hif target genes such as erythropoietin (Epo), vascular endothelial growth factor A (Vegf) and glucose transporter 1 (Glut1).

**Methods**

Basal mRNA expression levels of Egln1, Egln3, HIFα, Epo, Vegf and Glut1 in the liver and kidney of C57BL/6J mice (embryonic/fetal period: Theiler Stage 17-26; postnatal (P) period: P1/3/7/14/30; adult) were measured by RT-qPCR. To localize specific mRNAs in tissue samples, an in situ hybridization technique RNAscope was used.

**Results**

In the liver, the expression levels of the oxygen sensors Egln1 and Egln3 were re-adjusted, especially around the fetal period. In the kidney, Egln3 expression was upregulated in early development, whereas Egln1 expression stayed nearly constant. Fih expression did not significantly change during development. Expression of the Hif target gene Vegfa rose continuously during the fetal and postnatal period, whereas Glut1 expression did not substantially change during development. Hepatic Epo expression decreased stepwise until stage P1, however, rose up to fetal level again (P14) before Epo mRNA became undetectable in adult liver. Renal Epo expression continuously increased during neonatal development, until it reached adult levels at stage P14.

**Conclusions:** Our developmental data describe a spatio-temporal adaptation of the mRNA expression of the two main oxygen sensors Egln1 and Egln3. Interestingly, HIFα is regulated in neither liver nor kidney. Stepwise switch-off in hepatic Epo expression and the tissue-specific upregulation renal Epo after birth seem to occur independently from the expression of oxygen sensors and might be mediated by additional factors apart from the oxygen sensing system.

**OS 02-03**

The role of the factor-inhibiting HIF (FIH) in a mouse model of colitis-induced colorectal cancer

V. Schütz, K. Prost-Fingerle, A. Bicker, J. Fandrey, S. Winning

1 University of Duisburg-Essen, Institute of Physiology, Essen, Germany
2 Johannes Gutenberg-University, Institute of Organismic and Molecular Evolution, Mainz, Germany

**Abstract**

Hypoxia is a characteristic of inflammation as well as of solid tumors and enforces a gene expression response controlled by transcription factors called hypoxia-inducible factors (HIFs). HIF is regulated by prolyl hydroxylases and the asparaginyl hydroxylase Fh. Colorectal cancer is the fourth most common cause of cancer mortality worldwide and can be promoted by inflammatory bowel diseases as chronic colitis. HIF is suggested to be a tumor suppressor in colorectal cancer development by repressing the HIF-1α pathway. On the other side, epithelial HIF stabilization leads to a less severe inflammation in an acute colitis mouse model. In this work, the role of epithelial FIH in a mouse model of colitis-induced colorectal cancer is studied.

We induced chronic intestinal inflammation and colon cancer by using the oncogene azoxymethane (AOM, 10 mg/kg body weight, i.p.) and dextran sodium sulfate (DSS, 1.5% in the drinking water) administration to mice with Fhfl/fl and FiH-/- (KO). Mice were treated with AOM twice and with two 5-day/one 4-day phases of DSS with regeneration times of two weeks. As an alternative experiment, the pan-hydroxylase inhibitor dimethylglyoxaline (DMOG) is administered during DSS-treatment. The disease activity index (DAI) comprising weight loss, stool consistency, and fecal (pellet) bleeding was recorded and samples for molecular biological analyses were taken. To study the interaction and localization of FIH and its target HIF we used co-immunoprecipitation (co-IP, in vitro) and FRET measurements (in vivo: cell culture).

We observed a strong tumor development in the mice treated with AOM and DSS compared to control animals. Tumor occurrence did not significantly change between WT and KO mice. However, the WT mice showed a higher DAI compared to the KO mice in the last DSS phase with DMOG treatment. Additionally, AOM/DSS/DMSO-treated KO mice showed a higher infiltration of macrophages immunohistochemically detected by F4/80 expression than KO mice of the same experimental group. RNAseq analysis identified an enrichment of immune response-associated GO terms and changes in gene expression of metabolic processes. Thus, FIH knockout in colon epithelial cells seems to influence the inflammatory and metabolic status in chronic colitis which is possibly enhanced by temporary chemical pan-hydroxylase inhibition.

**OS 02-04**

Activation of AMP-activated protein kinase under hypoxia

F. Dezignier, H. Pfannkuche, G. Gäbel

University of Leipzig, Institute of Veterinary Physiology, Leipzig, Germany

**Abstract**

The intestinal epithelium exists at a thin line between physiological and pathological hypoxia while still maintaining a high metabolism. Thus, the enterocytes seem to command elaborate adaptation mechanisms reacting quickly to changes in oxygenation. However, the major regulator of adaptation to hypoxia, hypoxia inducible factor (HIF), orchestrates mainly changes on the transcriptional level while we observe functional adaptations within minutes, indicating the involvement of other, quicker mechanisms on protein level. We wondered if AMP-activated protein kinase (AMPK), a sensor protein controlling energy consuming cellular processes, might be one of the rapid acting mechanisms. Therefore, we investigated if AMPK is activated under hypoxia in intestinal epithelial cells.

**Methods**

We cultivated CaCo-2 cells in T25 flasks under conventional conditions (37°C in an incubator with humidified atmosphere, DMEM with 10% FCS, 3 mM L-glutamine and 100 U/ml pen/strep, 21% oxygen) until confluency. Then, cells were incubated either under 1% oxygen or kept at 21% oxygen (control) for 0.5, 1, 3, 6 and 24 h. Additionally, cells were pre-treated with 3 mM dimethylglyoxaline (DMOG), an inhibitor of prolyl-hydroxylases (PHDs) that control HIF activation oxygen-dependently (i.e. an inhibition of the PHDs leads to an activation of HIF). Subsequently, total protein was extracted from the cells, separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Total amounts of AMP-K and its target acetyl-CoA carboxylase (ACC) as well as their phosphorylated forms pAMPK and pACC were quantified by Western Blot.

**Results**
After 0.5 h, both 1% oxygen and DMOG incubation resulted in a significantly increased phosphorylation of AMPKα and ACC. EphB2-dependent signaling promotes neuronal excitotoxicity in the acute phase of ischemic stroke. 0.24
HIF-2α mediates hypoxia-induced expression of the Wilms tumor gene WT1 in Kelly neuroblastoma cells via an intrinsic hypoxia responsive element. 0.21
Neuroblastoma (NB) is a malignant childhood tumor arising from the peripheral sympathetic nervous system. The MYCN amplification is a strong predictor of poor prognosis, which also correlates with high levels of the Wilms tumor transcription factor WT1 in NB. The hypoxic tumor microenvironment promotes an undifferentiated phenotype and rapid tissue progression. To obtain novel insights into the molecular mechanisms underlying NB growth, we analyzed WT1 expression in human NB cell lines with (IMR-32, SK-N-SE (2), Kelly) and without (SK-N-SU, SH-EP1, SH-SY-5Y) the MYCN amplification. Experiments were also performed in hypoxia (1% O₂), and with addition of the HIF-stabilizer dimethylxalylglycine (DMOG). At 21% O₂, we found four cell lines with robust levels of WT1, however, no correlation between WT1 and MYCN amplification. Kelly cells had significantly higher WT1 protein and mRNA levels at 1% O₂, while presenting an almost undetectable amount at 21% O₂ (p<0.01, n=6). Silencing of HIF-2α, but not HIF-1α, by RNA interference significantly reduced WT1 protein and mRNA levels at 1% O₂ and in the presence of DMOG (1% 21% O₂). Similar results were obtained by disrupting the coding regions of either HIF-1α or HIF-2α with CRISPR/Cas 9 technology in Kelly cells. To further analyze the underlying molecular mechanisms, chromatin immunoprecipitation and electrophoretic mobility shift assays were performed on hypoxic Kelly cells (1% O₂; 24 h). HIF-2α bound to a putative hypoxia-responsive element (HRE) in intron 3 of the WT1 gene but not to another HIF consensus motif in the first intron. The element conferred oxygen sensitivity to otherwise hypoxia-resistant WT7 and SV40 promoter reporter constructs. CRISPR/Cas 9 mediated knockout of this genomic HIF binding site abolished WT1 protein and mRNA expression in hypoxic Kelly cells. Furthermore, we were able to demonstrate long-range interaction between the intrinsic HRE and the WT1 promoter by chromosome confirmation capture assays.
These findings demonstrate that binding of HIF-2α to an oxygen-sensitive enhancer in intron 3 stimulates transcription of the WT1 gene in NB cells by hypoxia-independent chromatin looping. This regulatory mechanism might offer novel insights into the pathophysiology of NB and possibly serve as a future therapeutic target. Acknowledgment: This work was supported by a Promotionsstipendium from the Berlin Institute of Health to C.C. and a grant from the Wilhelm Sander-Stiftung.

OS 02-05

OS 02-06

Deubiquitinase OTUB1 links cellular oxygen-dependent signalling by FIH with systemic energy metabolism. C. Pickel1, A. Ruiz-Serrano1, J. Günter1,2, R. H. Wenger1,2, C. C. Scholz1,2
1University of Zurich, Institute of Physiology, Zurich, Switzerland
2National Centre of Competence in Research “Kidney.CH”, Zurich, Switzerland
The author has objected to a publication of the abstract.

OS 02-07

EphB2-dependent signaling promotes neuronal excitotoxicity in the acute phase of ischemic stroke. R. Kunze1, A. - S. Ernst1, L. - I. Böhler1, A. M. Hagenston2, H. Bading2, H. H. Martin3, T. Korf3
1Heidelberg University, Institute of Physiology and Pathophysiology, Department of Cardiovascular Physiology, Heidelberg, Germany
2Heidelberg University, Department of Neurobiology, Interdisciplinary Center for Neurosciences, Heidelberg, Germany
Question: Local cerebral hypoperfusion causes ischemic stroke while driving a complex cascade of detrimental events occurring within different temporal and spatial frames. This ischemic cascade encompasses initial bioenergetic failure and ionic imbalance, followed by glutamate excitotoxicity via N-methyl-D-aspartate receptors (NMDARs), oxidative stress, dysfunction of the blood-brain barrier, and inflammatory responses, which altogether lead to the progressive death of neurons, glial and endothelial cells. Despite the relevance of these pathophysiological mechanisms for disease progression and outcome, molecular determinants controlling the onset of these processes are only partially understood. In this context, our study intended to investigate the functional role of EphB2, a receptor tyrosine kinase that is crucial for synaptic function and binds to membrane-associated ephrin-B ligands.

OS 02-08

Cargo specific sorting of Clathrin coated vesicles requires T-plastin and is oxygen dependent. G. J. van Belle1, D. Heidenreich1, M. W. Paul1, A. Zieseniss1, D. M. Katschinski1
1Georg-August University Göttingen, Institute of Cardiovascular Physiology, Göttingen, Germany
2Erasmus University Medical Center, Department of Molecular Genetics, Rotterdam, Netherlands
An essential feature of all eukaryotic life is the trafficking of vesicles between the endomembrane compartments and the plasma membrane. When environmental challenges arise, cells must rapidly adapt to these changed circumstances. This for instance happens when cells encounter a reduction of oxygen. Using immunofluorescent staining techniques we found that after 6 hours at 1% O₂, less than 2% of endocytosed vesicles (CCVs) are present in the cells and that the location of these CCVs is more near to the centroid of the cell. To look more into the dynamics of this process, we performed live cell imaging of cells expressing a GFP tagged Clathrin, tracking CCVs for 60 min in normoxia and hypoxia. We saw an increase in mean square displacement (MSD) of CCVs in normoxia, but not in hypoxia. When stimulating WT and T-plastin KO MDA-MB-231 cells with fluorescently tagged EGF and fluorescently tagged transferrin, we observed differential effects on the MSDs of loaded and unloaded CCVs. WT cells showed for both cargos a difference between the MSDs of loaded and unloaded CCVs. In T-plastin KO cells this difference between the MSDs was abolished and the overall MSDs were higher. Moreover, hypoxia markedly lowered the MSD of all CCVs when cells were stimulated with transferrin, but not with EGF. These findings suggest a tightly regulated cargo sorting mechanism that is dependent on oxygen and on T-plastin.
In conclusion, using Crispr/Cas9 cell lines has many advantages, although some simple rules for the generation and the application of these cell lines should be taken into account.

OS 03-03
Following the path of Aquaporin-2 to establish a personalized human collecting duct model in vitro
K. Haricharan1, D. Han1, S. Dieckel2, E. Klussmann2, K. Schmidt-Ott2, A. Kurz1
1Charité Universitätsmedizin, Berlin, Germany
2Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany
3Berlin Institute of Health, Stem Cell Core facility, Berlin, Germany

Kidney collecting duct principal cells participate in autosomal-dominant polycystic kidney disease (ADPKD), the most frequent genetic cause of end-stage renal disease. In ADPKD, defective trafficking of the water channel aquaporin 2 (AQP2) and aberrant lumens expansion in the collecting duct are observed. Targeting arginine-vasopressin (AVP)-induced AQP2 trafficking has recently shown promise in clinical trials of ADPKD. In an effort to develop an individualized human experimental model of the collecting duct, we derived principal cells from human induced pluripotent stem cells (hiPSCs) cells using our unique 14-day protocol via generation of ureteric bud cells. Tubular-epithelial cells derived from hiPSC were sorted using an AQP2-GFP reporter cell line developed using CRISPR-Cas technology, to obtain a homogeneous population of principal cells that can be expanded. Additionally, we designed an AQP2 reporter construct, which carries a HA-tag that allows the visualization of AQP2 in response to hormonal or chemical stimuli. We demonstrate the molecular and functional characterization of AQP2 localization and dynamics in principal cells by means of the tagged protein. The tendency of IPS-derived principal cells to assemble into three-dimensional cell clusters when placed in Matrigel cultures and form cysts consisting of polarized cells surrounding a fluid-filled lumen is being characterized in healthy and mutated cell lines. As a first step towards individualizing treatment decisions, IPS-derived collecting duct principal cells from patients with ADPKD can be generated and evaluated with regard to their response to pharmacological therapy. Improving our knowledge of the function and development of the collecting duct system will pave way to the discovery of new therapeutic strategies for treating kidney disease.

OS 03-04
Small Molecules to Enable, Facilitate and Improve iPSC Generation and Cell Reprogramming
K. Chang1, R. A. Gama Brambila1, Y. Dabin1, R. Mrowka1, S. Wu1
1Universität Heidelberg, Institut für Pharmazie und Molekulare Biotechnologie, Heidelberg, Germany
2Universitätsklinikum Jena, Experimentelle Nephrologie, Jena, Germany

Access to patient specific differentiated cell types is essential for the continued development and commercialization of stem cell therapies and regenerative medicine applications. The generation of transgenic animals is one of the areas that has been a major benefit from this fast and efficient technology. In addition, Crispr/Cas9 can also be adopted for the easy and fast generation of sequence-specific mutated cell lines. These mutated cell lines can be used in many applications. During the last years, we applied the Crispr/Cas9 technology for the generation of gene knockout cell lines. These cell lines have served us as useful tools in several projects. Our recent application of Crispr/Cas9 gene knockout lines in our lab is the verification of antibodies. But they can also be taken to analyse the function of proteins. We have used the mouse mesonephron derived M15 cell line in combination with Crispr/Cas9 to generate cells deficient for the transcription factors Wt1 and Gat4. These mutant cell lines have been established to analyse the influence of Wt1 and Gat4 on the transcription by using RNAseq. Furthermore, a Cdbdp2 knockout cell line was generated to analyse the effect of retrolateral transduction of CDBDP2 wildtype versus CDBDP2 mutant on alternative RNA splicing. Knockout cell lines of the transcription factors Hif1a or Hif2a have been established to identify which of them interacts and activates a hypoxia responsive element located in the Wt1 gene and which of them mediates the hypoxia-dependent expression of Wt1 in the Kidney neuronblastoma cell line. In addition to generating gene knockout cell lines, Crispr/Cas9 is also a powerful method to mutate transcription factor binding sites in the genome. We have used it in the Kidney cell line to disrupt the hypoxia responsive element in the Wt1 gene to prove its importance for hypoxia-dependent Wt1 expression.

In conclusion, using Crispr/Cas9 cell lines has many advantages, although some simple rules for the generation and the application of these cell lines should be taken into account.
We have systematically tested these diverse ion channels using automated patch clamp. Firstly, we have recorded NMDA correct abnormal ion transports in airway epithelial cells. Hence, we developed chitosan nanocapsules loaded with capsaicin to enhance mucus solubility and to downregulate sodium hyperabsorption, as it has been shown that the vanilloid reduces P2XαENaC expression. Furthermore, we have recorded Nδ currents and ligand-gated currents mediated by GABAA, AMPA and NMDA receptors from these neurons using the automated patch clamp approach. Our results demonstrate that pain pathways can be successfully studied on automated patch clamp systems, facilitating the discovery of novel pain therapeutics.

OS 03-06
High-throughput microcircuit analysis of individual human brains through next-generation multineuron patch-clamp

Y. Peng1, F. X. Mittermaier1, H. Planert1, U. C. Schneider1, H. Alle1, J. R. P. Geiger1
1Charité-Universitätsmedizin Berlin, Institut für Neuropsychologie, Berlin, Germany
2Charité-Universitätsmedizin Berlin, Klinik für Neurologie, Berlin, Germany
3Charité-Universitätsmedizin Berlin, Klinik für Neurochirurgie, Berlin, Germany

Comparing neuronal microcircuits across different brain regions, species and individuals can reveal common and divergent principles of network computation. Simultaneous patch-clamp recordings from multiple neurons offer the highest temporal and subthreshold resolution to analyse local synaptic connectivity. However, its establishment is technically complex and the experimental performance is limited by high failure rates, long experimental times and small sample sizes. We introduce an in-vitro multipatch setup with an automated pipette pressure and cleaning system facilitating recordings of up to 10 neurons simultaneously and sequential patching of additional neurons. We present hardware and software solutions that increase the usability and speed and data throughput of multipatch experiments which allowed probing of 150 synaptic connections between 17 neurons in one human cortical slice and screening of over 600 connections in tissue from a single patient. This method will facilitate the systematic analysis of microcircuits and allow unprecedented comparisons at the level of individuals (Peng et al. 2019, bioRxiv 639329).

OS 03-07
Analyzing capsaicin-loaded chitosan nanocapsules coated with wCfTR-mRNA as a potential treatment of abnormal ion transports in cystic fibrosis

A. K. Kolonko1, N. Banger-Ruland1, F. M. Goycoolea1, W. - M. Weber1
1University of Münster, Institute of Animal Physiology, Münster, Germany
2University of Leeds, School of Food Science and Nutrition, Leeds, UK

Cystic fibrosis (CF) is the most common lethal genetic disorder in the Caucasian population affecting approximately 1 in 2500 newborns. The underlying problem of the disease is an imbalanced homeostasis of ion and water transports in secretory epithelia causing problems in multiple organs, especially in the lung. This imbalance is caused by impaired chloride secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) and associated sodium hyperabsorption via the epithelial sodium channel (ENaC). In this study we aim at establishing a double tracked strategy targeting both proteins to correct abnormal ion transports in airway epithelial cells. Hence, we developed chitosan nanocapsules loaded with capsaicin to enhance mucus solubility and to downregulate sodium hyperabsorption, as it has been shown that the vanilloid reduces P2XαENaC expression. Furthermore, we have covered the capsules with wCfTR-mRNA, based on previous studies of our group, to restore chloride secretion. Physicochemical characterization of the capsules showed that addition of capsaicin increased the hydrodynamic diameter by ~60 nm indicating successful encapsulation. Adsorption of mRNA to the surface did not have a dominant-negative effect even if both wild type and mutant channels are trafficked to the membrane. Recent molecular studies showed that Na+ channels form dimers linked by a protein called 14-3-3 and patch clamp experiments indicate that this link causes coupled gating and synchronization of openings and closings. This leads to the hypotheses that Na+ channel function is modulated by allosteric interactions between channels and that these interactions may contribute to the negative dominance of certain mutations. Methods: A modelling paradigm was developed in which pairs of interacting Na+ channels are considered as the functional units underlying the Na+ current. Markovian models of two channels are combined together and allosteric interactions are incorporated by modifying the free energies of the combined states and/or barriers between states, which preserves microscopic reversibility. Systematic simulations permit to investigate how specific interactions affect the behaviour of the Na+ current at the single channel and ensemble average levels. Results: Simulations using 2-state models (C-C, closed-open) revealed that increasing the free energy of the compound states CO/COC (one channel closed, the other open) synchronizes the openings and closings. This energy can be regarded as potential energy accumulated in the linker protein. Simulations using two 3-state models (closed-open-closed) revealed that synchronization of both openings and closings must also involve interactions between further compound states. Using two 6-state Na+ channel models, previously reported experimental results could be replicated mainly by increasing the free energies of the CO/COC states and lowering the energy barriers between the CO/COC and CO/O states. The Na+ channel model was then modified to simulate a negative dominant Na+ mutation (LS259R). Simulations of homodimers vs. heterodimers in the presence vs. absence of interactions showed that the latter impairs the opening of the wild-type channel and thus contribute to negative dominance.

Conclusions: This new modeling framework is an invaluable tool to understand the interactions between ion channels and to identify interaction mechanisms based on experimental observations. Allosteric interactions between cardiac Na+ channels may contribute to the negative dominance of certain mutations.

OS 04-01
Oral Session 04: Circuit and Behavioural Neuroscience

OS 04-01
Essential role of Trp5c5 cation channel in hypothalamic dopamine neurons

T. Blum1, A. Moreno-Pérez1, M. Pyrski1, B. Bute1, A. Artiuc1, P. Weissgerber1, M. Freichel1, F. Zufall1, T. Leinders-Zufall1
1Saarland University, Center for Integrative Physiology and Molecular Medicine, Homburg (Saar), Germany
2Saarland University, Department of Pharmacology and Toxicology, Homburg (Saar), Germany
3University of Heidelberg, Institute of Pharmacology, Heidelberg, Germany
4University of Applied Sciences Kaiserslautern, Immunology Section, Faculty of Computer Science and Microsystems Engineering, Zweibrücken, Germany

Dopamine neurons of the hypothalamic arcuate nucleus (ARC) tonically inhibit the release of the protein hormone prolactin from lactotrophic cells in the anterior pituitary gland and thus play a central role in prolactin homeostasis of the body. Prolactin, in turn, orchestrates numerous important biological functions such as maternal behavior, reproduction, and sexual arousal.
Here, we identify the canonical transient receptor potential (TRPC) channel Trpc5 as an essential requirement for normal function of dopamine ARC neurons and prolactin homeostasis. By analyzing female mice carrying targeted mutations in the Trpc5 gene including a conditional Trpc5 deletion under control of the tyrosine hydroxylase promoter, we show that Trpc5 is required for maintaining highly stereotyped infraslaw membrane potential oscillations of dopamine ARC neurons. Trpc5 is also required for eliciting prolactin-evoked tonic plateau potentials in these neurons that are part of a regulatory feedback circuit. Trpc5 mutant females show severe prolactin deficiency or hypoprolactinemia that is associated with irregular reproductive cyclicity, gonadotropin imbalance, and impaired reproductive capabilities. Thus, Trpc5 is a major determinant of hypothalamic prolactin regulation and defines the functional properties of dopaminergic ARC neurons that play a central role in prolactin homeostasis. These results reveal a previously unknown role for the cation channel Trpc5 in prolactin homeostasis of female mice and provide new strategies to explore the genetic basis of reproductive disorders and other malfunctions associated with defective prolactin regulation in humans.

OS 04-02
Vasopressin cells in the rodent olfactory bulb resemble non-bursting superficial tufted cells and are primarily inhibited upon olfactory nerve stimulation

M. Lukas1, H. Suyama2, V. Egger3
1University of Regensburg, Neurobiology/Neurophysiology/Zoology, Regensburg, Germany
2University of Regensburg, Regensburg, Germany

Efficient sensing of conspecific odor signatures is essential for most rodent social behavior. Although olfactory bulb vasopressin was shown to be a potent facilitator of social odor processing, little is known on the cellular substrate of the intrinsic vasopressin system, except that glutamatergic vasopressin cells (VPCs) in the olfactory bulb are located at the medial border of the granule layer and resemble interneurons with dendritic tufts.

Methods:
To characterize VPCs in detail, we combined various electrophysiological, neuroanatomical and two-photon Ca2+ imaging techniques in acute bulb slices from juvenile transgenic rats with eGFP-labelled VPCs.

Results:
VPCs showed regular non-bursting firing patterns, and displayed slower membrane time constants and higher input resistances versus other glutamatergic tufted cell types. VPC axons spread deeply into the external plexiform and superficial granule cell layer. Axonal projections fell into two subclasses, with either denser local columnar collaterals or longer-ranging single projections running laterally within the internal plexiform layer and deeper within the granule cell layer. VPCs always featured lateral dendrites and a tortuous apical dendrite that innervated a single glomerulus with a homogenously branching tuft. These tufts lacked Ca2+ transients in response to single somatically-evoked action potentials and showed a moderate Ca2+ increase upon prolonged action potential trains.

Notably, electrical olfactory nerve stimulation did not result in synaptic excitation of VPCs, but triggered substantial GABA receptor-mediated IPSPs that masked excitatory barriages with yet longer latency. Exogenous vasopressin application reduced those IPSPs, as well as olfactory-nerve evoked EPSPs recorded from external tufted cells.

Conclusion:
VPCs can be classified as non-bursting, vertical superficial tufted cells. While we also identify several targets of vasopressin action, we find that stimulation of the sensory inputs to the bulb results primarily in vasopressin cell inhibition, implying that excitation of the bulb vasopressin system requires additional still unknown excitatory or disinhibitory inputs which might confer social specificity. These insights may complement the knowledge on vasopressinergic modulation of social input in limbic brain structures.

OS 04-03
Impairment of spike timing-dependent plasticity in the hippocampal CA1 area of an APP/PS1 Alzheimer’s disease mouse model

M. Garad1, E. Edelmann1,2, V. Lessmann1
1Otto-von-Guericke University, Institute of Physiology, Magdeburg, Germany
2Center for Behavioral Brain Sciences, Magdeburg, Germany

Spike timing-dependent plasticity (STDP) consists of precisely timed coincident firing of action potentials in pre- and postsynaptic neurons in forward or backward sequence that induce bidirectional and timing-dependent changes in synaptic efficacy, termed spike-temporal-long-term potentiation (t-LTP) and —depression (t-LTD), respectively. Furthermore, STDP is a well-recognized model to study cellular and molecular processes underlying learning and memory.

Using whole cell patch clamp recordings at Schaffer collateral (SC)-CA1 glutamatergic synapses in acute hippocampal slices, we investigated intrinsic excitability as well as basal electrical properties of CA1 pyramidal neurons, and STDP at SC-CA1 synapses in an APP/PS1 mouse model of Alzheimer’s disease (AD, 6 months old). APP/PS1 mice showed unaltered intrinsic excitability and basal electrical properties of CA1 cells compared to wild-type (WT) littermates. Moreover, AD mice displayed t-LTP induced by a canonical STDP paradigm (i.e. pairing of 1 presynaptic with 1 postsynaptic action potential (AP)) under conditions where the location of amyloid β (Aβ) plaques in the recorded slices was not tested. Since we argued that the proximity of Aβ plaques might be decisive to observe t-LTP deficits, we next focused on t-LTP in CA1 neurons in the vicinity of Aβ plaques that were visualized by staining with the dye Methoxy-X04. Using this procedure we found that the density of Aβ plaques in CA1 is low in 6 months old APP/PS1 mice. Therefore, in blind conditions where Aβ plaques were not stained, recorded CA1 cells were mostly far away from plaques. However, Aβ plaques stained slices we observed that in CA1 neurons with their soma <200 μm away from the border of the nearest plaque, t-LTP induced by burst
stimulation (i.e. pairing of 1 presynaptic with 4 postsynaptic APs) was impaired in APP/PS1 mice, while L-LTP was unaltered in CA1 neurons ≥20 µm away from Aβ plaques. To investigate whether synaptic signal integration at SC-CA1 synapses was also affected in APP/PS1 mice, we stimulated SCs at different frequencies at half maximal stimulation intensity and analyzed the number of postsynaptic APs elicited in CA1 neurons. Here, APP/PS1 mice exhibited clear deficits in the efficacy to fire APs compared to WT littermates. Our data reveal the existence of an AP plaques distance-dependent impairment of burst induced L-LTP at SC-CA1 synapses in APP/PS1 mice.

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OS 04-04

From local to global signalling in rat olfactory bulb granule cell dendrites: Dendritic integration of simultaneous inputs
M. Möller, V. Egebo
Regensburg University, Neuropsychology, Regensburg, Germany

The inhibitory axonless olfactory bulb granule cells (GCs) form reciprocal dendro-dendritic synapses with mitral and tufted cells, the principal neurons of the olfactory bulb, via large spines. GC dendrites are excitable in multiple ways: Synaptic inputs to individual GC spines can generate Na+ spikes within the spine head, and stronger activation results in globally propagating signals that encompass both low-threshold Ca2+ spikes (LTS) and Na+ spikes. To investigate dendritic integration we implemented a holographic two-photon uncaging system which allows simultaneous multispine photostimulation in 3D. We uncage glutamate at up to 12 spines on GC dendrites in acute juvenile rat brain slices while recording the membrane potential from the soma and two-photon Ca2+ imaging within one focal plane. Although GC resting potentials are hyperpolarized, the threshold for global GC Na+ spiking is attained at similar numbers of simultaneously activated spines (8.9 ± 1.6, n = 35 spiking GC) as in cortical pyramidal cells, whereas activation of 5.3 ± 2.1 spines suffices to elicit Ca2+ signals in dendrites remote from the stimulated spines, possibly corresponding to the LTS (n = 49 GCs). This putative LTS decreases with distance from the spine set (n = 12 GCs). In a subset of 6 GCs Na+ spikes but also Ca2+ signals were observed.

In the subthreshold regime, EPSPs summate on average linearly at the soma, with occasional supra- and sublinear summation. Ca2+ signals in individual spines (n = 35) increase in a spike-like manner upon occurrence of the LTS. The amplitude of the LTS increases with the stimulated spines across the dendritic tree influences LTS generation but not Na+ spikes, and the spine neck length has no influence on either global signal. Pharmacological manipulations reveal that NMDA receptors play a crucial role for electrical integration, generation of the LTS and summation of Ca2+ (25 µM APV, n = 8) whereas voltage-gated Na+ channels exert little global influence (500 nM TTX, n = 8). In conclusion, GCs turn out to be yet more excitable than previously thought, and postsynaptic spine Ca2+ entry is modulated by the overall level of excitation. Therefore, rather low numbers of coincident inputs (1) can inform an individual GC spine - who according to our previous findings can operate as independent mini-neuron - on the general activation state of their ‘mother’ GC and (2) are likely to provide lateral inhibition across principal neurons. Funding: BMBF (01GQ1552)

OS 04-05

Network-specific synchronization of electrical slow-wave oscillations regulates sleep need in Drosophila
D. Raccuglia1, S. Huang1, A. Ender1, M. Heim1, D. Laber1, A. Liootta2, S. Sigrist1, J. Geiger1, D. Oswald1
1Charité, Institut für Neurophysiologie, Berlin, Germany
2Charité, NWFZ, Berlin, Germany
3FU Berlin, Biologie, Berlin, Germany
4DZNE, Berlin, Germany

Slow-wave rhythms characteristic of deep sleep oscillate in the delta band (0.5 - 4 Hz) and can be found across various brain regions in vertebrates. Across systems it is however unclear how oscillations arise and whether they are the causal unitary functional behavior. Here, for the first time in any invertebrate, we discover sleep-relevant delta oscillations in Drosophila. We find that slow-wave oscillations in the sleep-regulating R2 network increase with sleep need. Optical multi-unit voltage recordings reveal that single R2 neurons get synchronized by sensory and circadian input pathways. We show that this synchronization depends on NMDA receptor (NMDARs) coincidence detector function and on an interplay of cholinergic and glutamatergic inputs setting a resonance frequency. Genetically targeting the coincidence detector function of NMDARs in R2, and thus the uncovered mechanism underlying synchronization, abolished network-specific slow-wave oscillations. It also disrupted sleep and facilitated light-induced waking, directly establishing a causal role for slow-wave oscillations in regulating sleep and sensory gating. We therefore propose that the synchronization-based increase in oscillatory power likely represents an evolutionarily conserved, potentially ‘optimal’, strategy for constructing sleep-regulating sensory gates.

OS 04-06

Cav3.2 T-Type Voltage-Gated Ca2+ Channels Modulate Hippocampal Theta-Alpha Activity Related to Memory Formation
M. I. Arshaad1, M. E. Siwek, J. Daubner, K. Broich, C. Henseler, A. Papazoglou, M. Weiergräber1
Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM), Experimental Neuropsychopharmacology, Bonn, Germany

Voltage-gated Ca2+ channels (VGCCs) are key elements in the regulation of hippocampal function and memory formation. Low voltage-activated Cav3.2 T-type channels, besides other high voltage-activated L- and Non-L-type Ca2+ channels, were reported to contribute to hippocampal long-term potentiation and synaptic facilitation. Cav3.2 VGCCs are widely distributed in the hippocampal formation and ablation of Cav3.2 Ca2+ channels have been attributed to impairment of long-term potentiation, learning of spatial cues and retrieval of context-associated fear memory. In this study, we focused on the role of Cav3.2 Ca2+ channels on hippocampal electrophysiological behavior.

Cav3.2 Ca2+ knock-out mice were studied and compared with wild-type littermates. We analyzed hippocampal frequency characteristics in mice lacking the Cav3.2 VGCC using implantable video-EEG radiotelemetry. Radiotelemetry provides monitoring and collecting electrophysiological data from conscious, freely moving laboratory animals under basal physiological state or experimental injection regimes. Continuous spontaneous long recordings (24 hours, 12 hours dark cycle & 12 hours light cycle) from the hippocampal CA1 region were carried out in both controls and Cav3.2 knockout mice. In addition, hippocampal theta oscillations were induced pharmacologically by administration of urethane (900 mg/kg i.p.). Our results demonstrate that Cav3.2 ablation results in complex alterations, i.e., an increase in the theta (4-8 Hz) and theta-alpha (4-12 Hz) range, particularly during the inactive phase in both the light and dark cycle. Similarly, the urethane injection revealed enhanced pharmacologically induced hippocampal type II theta oscillations. These findings point to a functional contribution of Cav3.2 VGCC in type II theta oscillations in the murine hippocampus. However, in vivo electrophysiology and behavioral studies suggest impaired memory function in Cav3.2 knock-out mice. Therefore, the paradoxical increase in theta-alpha activity might be dysfunctional. Nevertheless, this is a behavior which is also observed in various Alzheimer mouse models integrating the role of Cav3.2 VGCC in memory contribution.

OS 04-07

The role of the neurotransmitter circuitry in innate behaviours
H. E., van den Munkhof1,2, F. J. de los Santos1-4, A. Petzold1-2, T. Kostikova2-4
1Max Planck Institute for Metabolism Research, Neuronal circuits and behaviour, Köln, Germany
2University of Cologne, Institute of Vegetative Physiology, Medical Faculty, Köln, Germany

Innate motivated behaviours such as feeding and social interaction are crucial for survival and reproduction. As dysregulation of these behaviours can have detrimental consequences and lead to psychiatric disorders, including eating disorders and autism, it is known that evolutionarily conserved brain areas including the lateral hypothalamus and the ventral tegmental area, with connections to the lateral septum, limbic and cortical areas, are implicated. Yet it remains unclear how exactly genetically identified subpopulations within this circuitry direct specific reward-related behaviours. One of these subpopulations produces the neuromodulator neurotensin (Nts). It has been shown that Nts is involved in motivation behaviour and reward, as central administration suppresses food intake, while in the ventral midbrain it readily self-administered and administration increases locomotion. We record and manipulate the activity of Nts neurons in the LH and VTA using calcium imaging, chemogenetics (DREADDs) and optogenetics, while mice engage in a range of motivated behaviours such as feeding and social interaction. We apply MoSeq, a recently developed unsupervised machine learning algorithm, to automatically classify mouse behaviour based on depth images, thus enabling unbiased identification of behavioural module sequences with sub-second precision. We found that activity of Nts neurons selectively changes during expression of different motivated behaviours. Furthermore, chemogenetic activation of Nts neurons in the LH altered social interaction and preference for food. Subsecond analysis of behaviour using MoSeq, upon a region-specific chemogenetic activation of Nts neurons, revealed changes in multiple behavioural modules on a subsecond scale. These results suggest that Nts neurons selectively regulate innate behaviours, with differential effects depending on the brain area. We gratefully acknowledge funding by the ERC Consolidator Grant 2017 (HypFeedNet, TK), the Max Planck Society (TK) and the DFG (233866666/GRK1960, FS).
Tuning of the respiratory rhythm by optogenetic activation of inhibitory neurons in vivo

S. Hülsmann
Universitätsgesellschaft Göttingen, Klinik für Anästhesiologie, Göttingen, Germany

The respiratory rhythm is generated by specialized neurons in the medulla oblongata. While excitatory pre-Bötzinger Complex (preBötC) neurons have been confirmed to be indispensable, the role of inhibitory neurons is still under debate. Here we used transgenic mice that express channelrhodopsin in inhibitory neurons to address this question in vivo. To activated inhibitory neurons in the preBötC in vivo, double transgenic mice (Tg[Ttx2a5a-kre]121Veul, 129S-GiROSA25SNSG2[Os2d][Tte][Kre]171Veul) were anesthetized (Ketamin, Medetomidine, Lidocaine) and placed in a stereotactic frame. A 200 µm optical fiber was implanted with the tip targeted to the preBötC. When stimulated with constant light (470-473 nm) or pulses (10 ms) at rates larger than the 10 Hz the breathing was significantly depressed. However, at lower rates (3-10 Hz) an increase of the respiratory rate and entrainment was observed. To further analysis the mechanism of the entrainment, light pulses were applied in different phases of the respiratory cycle. While stimulation during expiration reduced the respiratory rate, stimulation of inhibitory neurons during inspiration resulted in a shortening of the expiratory interval, an earlier termination of the ongoing breath and an increase in respiratory rate. In conclusion, our data strongly supports the concept that inhibitory neurons are capable to control the respiratory cycle by initiation phase transition due to phasic inhibition of the population of excitatory rhythm generating neurons.

Oral Session 05: Cardiac Physiology

OS 05-01
p38 MAPKα is a Key Regulator in Cardiac Metabolism

L. Kaltheus, K. Bottermann, V. Oenarto, L. Leitner, T. Harris, A. Gödecker
1Heinrich-Heine University, Institute of Cardiovascular Physiology, Düsseldorf, Germany
2Heinrich-Heine University, Department of Molecular Physiology, Düsseldorf, Germany
3University of Virginia, Department of Pharmacology, Charlottesville, USA

Question The heart utilizes different substrates to maintain a high rate of ATP production to ensure a sufficient cardiac performance even under conditions of changing nutrient supply. Throughout the progression of heart failure (HF) the ability for the heart to switch substrates may impaired even before overt cardiac dysfunction. This loss of metabolic flexibility and insulin sensitivity may promote HF. To elucidate associated pathomechanisms, we investigated the influence of p38 MAPKα signaling on metabolic remodeling during the early phase of HF development.

Methods and Results Cardiomyocyte specific, tamoxifen inducible p38 MAPKα KO mice (KO) were generated and treated with angiotensin II (AngII) as stress stimulus. 48h after the onset of AngII treatment echocardiography revealed a massive left ventricular dilatation and an impaired heart function in KO hearts (ejection fraction [%]: KO 29±7.4, Ctrl 49±12). Substrate utilization measured by NMR spectroscopy with 1C-labeled substrates showed that KO hearts failed to upregulate glucose utilization after insulin stimulus already under baseline conditions. This insulin resistance in KO hearts was associated with a decreased GLUT4 protein amount (app. 50%) and a reduced GLUT4 translocation after insulin stimulation (app. 75% less GLUT4 in plasma membrane). However, AKT mediated insulin signaling was not compromised. Surprisingly, AngII led to increased insulin plasma levels in KO mice, which enhanced cardiac AKT phosphorylation. Activated AKT reduced activating AMPKα2 and increased inhibitory AMPKα1,2 phosphorylation shifting AMPK to its inactive form which may contribute to reduced energy supply under AngII treatment. The insulin mediated AMPK inhibition was not found in cardiomyocyte specific AKT KO hearts underlining the importance of AKT in AMPK inhibition. We suggest that the co-transcription factor PGC1-α contributes to the underlying mechanism of the "metabolic phenotype" of p38 MAPKα KO mice. PGC1-α is downregulated KO hearts after AngII treatment and causal network analysis of transcriptomic data identified PGC1-α as the top candidate responsible for the observed difference between Ctrl and KO hearts.

Conclusion p38 MAPKα KO mice develop hyperinsulinemia, cardiac insulin resistance, and an extensive metabolic depression contributing to the progression of HF. Thus, p38 MAPKα signaling is a key regulator in cardiac metabolic adaption to elevated workload potentially mediated via PGC1-α.

OS 05-02
Del-1 as an anti-inflammatory approach to prevent cardiovascular damage during hypertension

I. Kopaliani, T. Failier, A. Neuwirth, I. Kourtzelis, P. Subramanian, G. Hajishengallis, T. Reinhart
1Technische Universität Dresden, Physiologie, Dresden, Germany
2Technische Universität Dresden, Institute for Clinical Chemistry and Laboratory Medicine, Dresden, Germany
3University of Pennsylvania, Penn Dental Medicine, Department of Microbiology, Philadelphia, US

Hypertension-related cardiovascular diseases cause the greatest number of deaths worldwide. Inflammation is implicated to mediate cardiovascular damage during hypertension. Therapeutic strategies used so far do not specifically target inflammation. This urges research for novel anti-inflammatory approaches.

We investigated the therapeutic role of endogenous anti-inflammatory factor Del-1 in hypertension-related cardiovascular remodeling. Efficacy of Del-1 was tested in a therapeutic study by injecting recombinant Del-1 in wild type mice induced with hypertension (infusion of angiotensin II using minipumps). Cardiac remodeling was assessed with optical and histological stainings, whereas inflammatory cell count in cardiovascular tissue with flow cytometry. Systolic blood pressure (SBP) was measured with the tail-cuff method.

After angiotensin II infusion, untreated mice developed cardiovascular remodeling expressed by cardiac hypertrophy and fibrosis, as well as acute medial hyperplasia and adventitial fibrosis. Del-1 treated mice were protected from remodeling. Compared to untreated mice they had less cardiac and aortic hypertrophy, along with less fibrosis in heart and aorta. Increase in SBP after one week of angiotensin II was similar in untreated and Del-1 treated mice. However, unlike in untreated mice, SBP did not further increase in Del-1 treated mice, resulting in a ~12 mm Hg difference. This was due to an almost complete prevention of the tissue remodeling response. Untreated mice had increased inflammation in heart and aorta, whereas Del-1 treated mice had less counts of CD45+ leukocytes, TCR-β+ T-cells and CD45+HL17+ double positive cells. MMP2 enzyme activities and active TGF-β1 levels were increased in heart and aorta of untreated mice, whereas Del-1 treated mice had less MMP2 activity and TGF-β1 levels both in heart and aorta.

We demonstrate that Del-1 is a potent anti-inflammatory factor limiting cardiovascular inflammation, MMP2 activity and active TGF-β1 levels in heart and aorta, thus, protecting from hypertension-related cardiovascular damage. These results indicate a novel anti-inflammatory approach in hypertension.

OS 05-03
Human iPS cell-derived engineered heart tissue does not induce ventricular arrhythmias in a guinea pig cryo-injury model

1Universitätsklinikum Hamburg Eppendorf, Institut für Experimentelle Pharmakologie und Toxikologie, Hamburg, Germany
2Universitätsklinikum Hamburg Eppendorf, Institut für Medizinische Biometrie und Epidemiologie, Hamburg, Germany
3Universitätsklinikum Hamburg Eppendorf, Institut für Zelluläre Genetik und Regenerative Medizin, Hamburg, Germany
4Deutsches Zentrum für Herz kreislauforschung (DZHK), Partner Site Hamburg, Kiel, Lübeck, Hamburg, Germany

We hypothesized that human iPS-derived engineered heart tissue (hEHT) has been used to remuscularize injured hearts in a guinea pig infarction model. The beneficial effects on cardiac remodeling have repeatedly been demonstrated. In contrast, only limited and inconsistent data is available addressing the arrhythmogenic potential of hEHTs transplanted into cardiac damaged myocardium. Here, we investigated that human iPS-derived EHFT increases the incidence of ventricular arrhythmias in a guinea pig cryo-injury model. Methods: hEHT were created from human iPS-derived cardiomyocytes and endothelial cells. Left-ventricular cryo-injury was induced in guinea pigs (n = 37) and telemetry sensors for continuous ECG monitoring were implanted. Implanted cell-free constructs were transplanted into the surviving animals 7 days following the cryo-injury (n = 15 HEFT and n = 9 control animals) and ECGs were recorded over the following 28 days. Statistical analyses were performed, as appropriate, using univariate student’s t-test, ANOVA followed by multiplicity corrected Tukey post hoc tests, mixed effects logistic regressions and mixed effects negative binomial regressions. Results: Infarct sizes were comparable in control and HEFT animals. 8 control and 10 HEFT animals survived the study and delivered ECGs of sufficient quality. Human muscle tissue was observed in the scar area of HEFT transplanted animals demonstrating graft survival. After implantation of HEFT or cell-free constructs, ventricular arrhythmias (premature ventricular contractions, coupled beats and non-sustained ventricular tachycardia) were observed in animals of both groups. Neither the fraction of animals with the respective arrhythmias, the time course of ventricular arrhythmias nor the total burden of arrhythmias (regardless of their subtypes) were statistically significantly different between the groups. Also, the rate of arrhythmic events was comparable. Finally, no clinically relevant sustained ventricular tachycardia or ventricular fibrillation were detected.
Conclusions: The current work used human iPSC-derived EHT transplanted on a guinea pig cryo-injury model to remuscularize the injured hearts. This approach did not affect the incidence of ventricular arrhythmias. Thus, the study provides evidence that transplanted iPSC-derived cardiomyocytes in HEHTs do not increase the arrhythmic burden, thereby supporting further development of this therapeutic strategy for myocardial regeneration.

OS 05-04
Deletion of the deubiquitinate OTUB1 affects viability, cardiac function and energy metabolism in mice
A. Ruiz-Serralles1, J. Günter1, S. Constantin2, C. Boyle1, S. Pfandstein1, G. Pellegrini1, I. Monné Rodriguez1, T. A. Lutz2, F. Paneni2, R. H. Wengel1, C. C. Scholz1
1University of Zurich, Institute of Physiology, Zurich, Switzerland
2University of Zurich, Center of Molecular Cardiology, Schlieren, Switzerland
3University of Zurich, Vetsuisse Faculty, Institute of Veterinary Physiology, Zurich, Switzerland
4University of Zurich, Vetsuisse Faculty, Institute for Veterinary Pathology, Laboratory for Animal Model Pathology, Zurich, Switzerland

The author has objected to a publication of the abstract.

OS 05-05
Transplantation of Cx43 expressing fibroblasts: An option for post-infarct arrhythmia prevention?
M. Schiffer1, E. Carl1, K. Wagner1, A. Engelbrecht1, D. Dürr1, A. Welz1, J. M. De la Fuente1, A. Pfeiffer1, B. Fleischmann1, W. Röll1
1University Hospital Bonn, Department of Cardiac Surgery, Bonn, Germany
2University of Zaragoza, Institute of Material Science of Aragoza, Zaragoza, Spain
3University Hospital Bonn, Department of Pharmacology and Toxicology, Bonn, Germany
4University Hospital Bonn, Department of Physiology 1, Bonn, Germany

Question: Myocardial infarction may result in a reduced ejection fraction (EF) and post-infarct ventricular tachycardia (VT). Dead cardiomyocytes are substituted by (myo-) fibroblasts during scar formation and therefore the content of the gap junction protein Connexin 43 (Cx43) is massively reduced. This impairs electrical conduction throughout the lesion critically and VT can originate by re-entry mechanisms. In the present study Cx43 (over-) expressing and magnetic nanoparticle (MNP) loaded cardiac fibroblasts were transplanted under a magnetic field into freshly infarcted myocardium.

Methods: Murine embryonic cardiac fibroblasts (eCF) were isolated from E13.5 hearts and cultured for 7 days before ventricle transduction with either Cx43-ires-eGFP or IRES-eGFP construct. One day prior to intramyocardial transplantation, eCF were loaded with fluorescently labeled MPs. Thereafter, 200,000 cells were injected into cryopreserved hearts of adult female CD1 wildtype mice with or without application of a magnetic field during and 10 minutes after injection. Heart function was analyzed 14 days post infarction by echocardiography and right heart catheter measurements. Cell engraftment and Cx43 expression was examined by western blotting and immunohistochemistry.

Results: The used MNP, tagged with a TAMRA fluorochrome, showed low toxicity (cell loss of 20% at 25 pg/cell, MTT Assay) and high magnetic cell retention rate in vitro. Overnight incubation with lentivirus resulted in a transduction efficacy of ca. 25% for the two used lentiviral constructs and significantly increased protein expression of eGFP and Cx43 was shown by western blotting. Two weeks post transplantation, engrafted eGFP positive eCF were found in a majority of hearts. Functional analysis resulted in significantly increased anterior wall thickening but not significantly increased global left ventricular function when MNP loaded, Cx43 transduced eCF were transplanted with application of a magnetic field. Electrophysiologically testing revealed reduced VT incidence after transplantation of Cx43 expressing eCF (+magnet: VT incidence 40%, n=15; -magnet: VT incidence 50%, n=14) compared to control mice (injection of eGFP expressing eCF, +magnet, VT incidence 89%, n=19).

Conclusion: Cellular cardiomyoplasty using Cx43 expressing cardiac fibroblasts results in significant reduction of post-infarct arrhythmias and local stabilization of the scar area but cannot augment cardiac pump function substantially.

OS 05-06
Preferential oxidation of elastic titin domains occurs in volume-overloaded hearts and stabilizes the unfolded state of the domains as a prerequisite for their phosphorylation
C. M. Loecher1, M. Breitkreuz2, Y. Li3, K. Toischer3, L. Leichert1, N. Hamdan1, W. A. Linke1
1Universität Münster, Institute for Physiology II, Münster, Germany
2Ruhr University Bochum, Bochum, Germany
3Universitätsmedizin Göttingen, Herzzentrum, Abt. Kardiologie und Pneumologie, Göttingen, Germany
4Ruhr University Bochum, Institute for Biochemistry and Pathobiology, Bochum, Germany

Background: Sarcomeric titin stiffness largely determines myocardial passive stiffness. The mechanical function of titin is modulated by oxidation and phosphorylation. In vitro, titin oxidation is promoted by unfolding of titin immunoglobulin-like (Ig) domains, which in turn cannot properly refold when S-glutathionylated. However, it is unknown whether titin oxidation occurs in vivo and if there is differential oxidation of sarcomeric I-band (extensible) and A-band (inextensible) titin. Therefore, we measured in vivo titin oxidation as a function of stretch and oxidative stress. Further, interactions between titin oxidation and phosphorylation have not previously been considered, and therefore were investigated in vitro.

Methods and Results: Titin oxidation was studied in the aorto-caval shunt mouse heart, which is under chronic volume overload (preload-increase) and develops oxidative stress. Titin oxidation was quantified by isotope-coded affinity tag labeling followed by mass spectrometry. Oxidative stress significantly increased the ratio of oxidized (GSSG) to reduced (GSH) glutathione. Hundreds of cysteines in titin became more oxidized under preload-increase conditions and increased the proportion of cysteines oxidized in I-band titin compared to A-band titin. Several Ig domains from elastic titin, found to be preferentially oxidized, were then recombinantly expressed. Thermal unfolding of these Ig domains, followed by S-glutathionylation, consistently resulted in increased aggregation. Mutant constructs of Ig2 showed that the substitution of one of two cysteines with an alamine prevented the enhanced oxidation-induced aggregation. Unfolding of Ig2 was also required for CaMKIIδ-mediated phosphorylation to occur. The unfolding of Ig2 together with S-glutathionylation, enhanced phosphorylation by CaMKII further. Force measurements on skinned human cardiomyocytes showed that S-glutathionylation during stretch reduced passive tension.

Conclusion: Titin oxidation occurs in vivo and elastic titin becomes more oxidized than A-band titin after stretch. This effect is due to increased Ig-domain unfolding in the stretched state and results in a reduction of passive tension. Increased S-glutathionylation of Ig domains stabilizes their unfolded state and promotes their CaMKIIδ-mediated phosphorylation. Understanding the mechanisms of oxidative stress-induced titin modifications may help design strategies for the treatment of heart failure.

OS 05-07
p22phox promotes obesity-induced cardiac dysfunction
M. E. Klieg1, L. Lü1, C. Wolf1, D. Kraćun1, A. Görlach1
1German Heart Center Munich at the Technical University Munich, Experimental and Molecular Pediatric Cardiology, Munich, Germany
2German Heart Center Munich at the Technical University Munich, Dept. of Pediatric Cardiology and Congenital Heart Diseases, Munich, Germany

Obesity is a major burden worldwide that is strongly associated with metabolic and cardiovascular disorders. Complications associated with cardiovascular dysfunction are the leading causes of morbidity and mortality in individuals with diet-induced obesity and diabetes. However, mechanisms implicated in obesity-induced cardiovascular disorders are still limited. An increased load of reactive oxygen species (ROS) has been associated with high fat intake and obesity. As NADPH oxidases have been identified as important sources of ROS in the cardiovascular system we hypothesized that these enzymes might contribute to obesity-induced cardiac dysfunction.

To this end, white type mice fed a high fat diet (60% calories from fat) for 20 weeks developed a significant increase in body mass index as well as glucose intolerance. Concomitant with an increase in left ventricular pressure, left ventricular hypertrophy and left ventricular remodeling, echocardiography indicated cardiac dysfunction with perturbed fractional shortening in high fat diet fed mice. Moreover, increased ROS levels were associated with elevated expression of the NAPDH oxidase subunit p22phox in high fat diet fed mice. Importantly, mice lacking a functional NADPH oxidase due to a mutation in the cyclo gene encoding p22phox were fully protected against high fat diet-induced hypertension and cardiac dysfunction.

At the cellular level, high fat diet promoted ER stress and upregulation of the ATF4-mediated unfolded protein response (UPR) in hearts from white type mice, but not in p22phox-deficient hearts. Similar responses were observed in isolated cardiomyocytes. These data show that p22phox-containing NADPH oxidases promote high fat diet-induced cardiac dysfunction and remodeling by triggering cardiac ER stress and the unfolded protein response. Thus, p22phox-containing NADPH oxidases might be interesting therapeutic targets to combat obesity-induced cardiac dysfunction and other cardiovascular pathologies.
Ventricular dysfunction precedes atrial conduction defects in JDP2 overexpression mice

OS 05-08

G. Euler1, M. Parahuleva2, J. Bornbaum3, J. Heger4, J. Kockskämper4, R. Schulz1

1Justus-Liebig-Universität, Physiologisches Institut, Gießen, Germany
2UGM, Kardiologie, Marburg, Germany
3Philippus-Universität, Institut für Pharmakologie und Klinische Pharmazie, Marburg, Germany

Question: Cardiac-specific JDP2 (Jun Dimerization Protein 2) overexpression in mice provokes ventricular dysfunction, atrial dilatation and atrial fibrillation. In this study we investigated, if ventricular dysfunction is predisposed to atrial fibrillation. Therefore, we analyzed ventricular function, ECG, mRNA- and protein-expression in ventricular and atrial tissues after 1 and 5 weeks of JDP2 overexpression.

Methods: JDP2 expression was under control of a Tet-off system. Therefore, JDP2 overexpression was started by withdrawal of doxycycline in 4-week-old mice. After 1 or 5 weeks, cardiac function was determined by echocardiography and ECG. mRNA expression was analyzed by real-time RT-PCR and protein expression in western blots.

Results: After 1 or 5 weeks, JDP2 mRNA levels were increased in ventricular and atrial tissues of JDP2 mice. Already after 1 week ventricular dysfunction emerged in JDP2 mice. Ejection fraction decreased from 64.6 ± 10.4 % in WT to 58.8 ± 9.3 % in JDP2 mice, and cardiac output from 23.0 ± 4.7 ml/min in WT to 19.4 ± 3.3 ml/min in JDP2 mice (n=11-16, p<0.05). In ventricular tissues, elastin mRNA expression increased, and the calcium handling protein SERCA decreased within one week of JDP2 overexpression (n=6, p<0.05 vs. WT). After 5 weeks of JDP2 overexpression ventricular dysfunction became even stronger with a cardiac output of 13.6 ± 2.5 ml/min (n=11, p<0.05 vs. WT). Still reduction in SERCA protein was observed, and increased fibrosis was detected. In atrial tissue, besides the 3.6 times increase of JDP2 mRNA, no changes could be detected within one week, and ECG recordings over 30 minutes did not show any abnormalities. Atrial dilatation became evident after 5 weeks of JDP2 overexpression. ECG-recordings revealed prolonged PR-intervals (34.6 ± 3.9 ms in JDP2-mice vs. 30.2 ± 7.0 ms in WT) and broadened QRS-complexes (15.8 ± 2.8 ms in JDP2 mice vs. 13.9 ± 1.7 ms in WT, n=10-12, p<0.05). mRNA of the calcium-handling proteins PLB, NCX and SERCA, and of the fibrillar marker genes collagen 1, fibronectin and elastin were dramatically reduced in atrial tissue of JDP2 mice (n=6, p<0.05 vs. WT), and may be functionally involved in atrial conduction defects.

Conclusion: Enhanced expression of JDP2 provokes ventricular dysfunction and fibrosis within one week, whereas changes in the atrial tissue and conduction defects occur later and seem to be a secondary effect that is provoked by the pre-existing ventricular dysfunction.

Molecular mechanisms in ion channels

OS 06-01

Increased phosphorylation leads to hypersensitivity of iPSC-derived nociceptors from chronic pain patients

C. Kerth, P. Hautvast, J. Körner, A. Lampert, J. Meents

University Hospital RWTH Aachen, Physiology, Aachen, Germany

The chronic pain syndrome inherited erythromelalgia (IEM) is attributed to mutations in the voltage-gated sodium channel (Nav) 1.7. In the past, we have investigated induced pluripotent stem cell (iPSC)-derived nociceptors from IE patients with the Nav1.7/II48ST mutation. These nociceptors display a hyperpolarized Nav channel activation and enhanced action potential characteristics. These features may well explain the pain phenotype described by the patients. As the II48ST mutation creates a novel phosphorylation site for thrombin, we tested whether phosphorylation might play a role in the observed shift in Nav1.7 voltage dependence of activation. Indeed we found in heterologous expression systems that non-specific kinase inhibitors reduce the shift in activation. In addition, phosphomimetics supported a role of phosphorylation in the enhanced activation of Nav1.7. Testing more specifically for protein kinase involvement, we observed that protein kinase C but not protein kinase A is likely to be responsible for the phosphorylation of the T465 residue in Nav1.7. In ongoing experiments, we are investigating whether clinically approved inhibitors of protein kinase C can be used in iPSC-derived nociceptors of IE patients to return action potential firing behavior to normal levels. These results may lead to the discovery of a novel and personalized treatment option for these particular IE patients. We therefore believe that our findings may open new avenues for pharmaceutical intervention in pain syndromes.

Activation of HCN2 channels by N6-modified cAMP-derivatives

OS 06-04

T. Leybold1, M. Bonus2, F. Spiegelhalter3, H. Gohlke1, T. Schwabe1, F. Schwede4, J. Kusch3

1Universitätsklinikum Jena, Institut für Physiologie II, Jena, Germany
2Heinrich-Heine-Universität Düsseldorf, Institut für Pharmazeutische und Medizinische Chemie, Düsseldorf, Germany
3BIOLOG Life Science Institute, Bremen, Germany

The second messenger cAMP is involved in a wide variety of cellular processes, which are mediated by three main types of proteins: protein kinase A (PKA), exchange protein directly activated by cAMP (Epac), and CN-modulated channels (CNG, HCN channels). Discrimination between the different cAMP signaling pathways requires the selective activation of only one type of protein. So far, cAMP modifications at position N6 of the adenosine ring (PKA) and position 2′-OH of the ribose (Epac) have been used to produce selective compounds. However, CN-modulated ion channels were usually outside the scope of most studies. Due to the wide distribution of those channels in various organs, possible channel cross-activation by PKA- or Epac-selective agonists is an important question.

Herein, we study the agonistic effects of three PKA-selective cAMP derivatives on HCN2 pacemaker channels: N6-Phe-cAMP, N6-Bn-cAMP, N6-Bz-cAMP. HCN2 channels were expressed in Xenopus laevis oocytes. Currents were measured in inside-out macropatches. Functional parameters as efficacy, apparent affinity, activation kinetics, and voltage of half maximum activation were compared. Binding modes of N6-modified cAMP derivatives were predicted with the Glide XP methodology implemented in the Schrödinger Suite. We show that all three compounds are able to activate hHCN2 channels, however with different apparent affinities. N6-modifications caused either a ten-fold higher (N6-Phe-cAMP, N6-Bn-cAMP, N6-Phe-cAMP)
cAMP or a tenfold lower sensitivity (NP-Rnz-cAMP). Docking experiments suggested that the sensitivity increase is caused by additional cation-π interactions with an arginine, while the decrease is caused by a steric clash between the conformationally restricted benzoyl substituent and the same arginine, leading to a different binding mode.

We conclude that some of the PKA-specific activators are also effective activators of HCN2 channels. Hence, when studying PKA-mediated cAMP-signaling with cAMP derivatives in native cells, activation of HCN channels should be considered.

OS 06-05

KCNQ1 an unexpected mediator of cold avoidance
A. K. Kiper1, S. Wegner1, A. Kadala1, M. A. R. Bertoune2, F. Touska1, V. Matschke1, E. Wrobel1, A. - K. Strell1, S. Rinné1, G. Seebohm1, J. Völkl1, F. Lang1, K. Zimmermann1, N. Decher1

1Philipps-Universität Marburg, Vegetative Physiologie / Institut für die Physiologie und Pathophysiologie, Marburg, Germany
2Friedrich-Alexander Universität Erlangen-Nürnberg, Klinik für Andrologie, Nürnberg, Germany

KCNQ1 channels have been implicated in the function of somatosensory nociceptors, pancreatic β-cells, vascular smooth muscle cells, epithelial cells in the ciliary body and choroid plexus, among others. These channels can be activated by heat and the endogenous steroid pregnenolone sulfate, are regulated by PIP2 levels in the plasma membrane and are inhibited by Gβγ subunits, liberated after activation of GPCR receptors. They are typically portrayed as Ca2+-permeable non-selective cation channels, but it has long been recognized that TRPM3 proteins are subject to extensive alternative splicing. Most of the sites of alternative splicing are located in the cytosolic N-terminal region, but alternative splicing also affects the pore region and the cytosolic C-term (reviewed in Obwierkiewicz & Philipp, Handb Exp Pharmacol, 2014, 222:427-59). The functional consequences of these alternative splicing events have been explored for only three of the (at least) seven published sites of alternative splicing. Interestingly, these three splice events have profound consequences for the functional properties of the channels. Splicing at exon 24 (which encodes part of the pore loop) dramatically alters ion selectivity of the channel, while the splice event at exon 13 abolishes channel activity for unknown reasons when the 18 amino acids encoded by this exon are not present. Recently, we showed that splicing out the 10 amino acids encoded by exon 17 abolishes the sensitivity to Gβγ, and hence to GPCR activation.

Using Ca2+-imaging and whole-cell patch-clamp electrophysiology, we investigated whether these three splice events (exon 13, 17 and 24), which so far only have been tested in isolation, interact with alternative splicing at the first exon (either exon 1 or exon 2 are incorporated in the mature mRNA) or with the splice event at the C-term (which shortens the C-terminus by almost 400 amino acids). Our results clearly indicate that neither the N- nor the C-terminal splice event influences the known functional channel properties. In ongoing experiments, we also asked whether other splice events, specifically those at exon 8 and 15, interact with the functional consequences of splice events at exons 13, 17 and 24. The results of these experiments will help to define the (patho-) physiological implications of alternative splicing for the functional properties of TRPM3 channels in processes such as pain perception or insulin secretion.

OS 06-06

Versatile modulation of Kv7-mediated K+ currents through (silent) modifier Kv channel subunits V. Renigunta1, M. Lindner1, D. Oliver2, M. G. Leitner3

1Medical University of Innsbruck, Division of Physiology, Innsbruck, Austria
2Philipps-Universität Marburg, of Physiology, Marburg, Germany
3University of Oxford, Nuffield Department of Clinical Neurosciences, Oxford, UK

Functional voltage-gated potassium (Kv) channels are composed as homotetramers of four individual subunits, but may also be heterotetramers formed by members within the same family. Of the twelve Kv channel families (Kv1-Kv12), members of four families (K5, K6, K8, K9) are particular exceptions in two respects: Firstly, they do not form functional homotetrameric channels, i.e. when expressed alone, these channels are electrically silent (termed K5). Secondly, these subunits assemble with co-expressed K2 subunits into heteromeric K2/K5/K7/K9 complex with unique properties and are thus physiologically-important modifiers of K2 channels. This K2-K5 interaction may constitute the only conclusive example yet known for heterotetramerization with isoforms of distinct Kv families.

Phenotypical analyses indicated somewhat closer relationship of K7 channels to K5 and K2 than to the other K families. We thus hypothesized that K5 may also constitute modifiers of K7 family members, and analyzed properties of recombinant K7 channels co-expressed with K5. We found that in Chinese hamster ovary (CHO) cells co-expression of certain K5, K6, K8 or K9 subunits significantly affected the voltage dependence of K7.7 and K7.4 subunits as well as voltage-dependent steady-state currents through these K7 isoforms. In line with the observed changes of whole cell current amplitudes, co-expression of certain K5 modulated membrane expression levels of K7.2 in Xenopus laevis oocytes. Proximity ligation assays (PLA) and co-immunoprecipitation (Co-IP) indicated that K5 and K7 subunits were located in very close proximity and closely interacted in the employed expression systems. To further analyze this unknown interaction, we generated K5 knock-out mice. Careful analysis of neurons natively co-expressing K5 and with K7 subunits will unravel potential (neuro)physiological relevance of K5-K7 heteromeric channels in-vivo and in-vitro.

Taken together, our results demonstrated that silent K5 subunits modified the properties of neuronal K7 channel subunits and provided strong evidence for functional K5 channel heteromers formed by members of distinct K-family.

OS 06-07

Functional consequences of alternative splicing for the properties of TRPM3 channels S. Dembla1, R. Enzerth1, M. Behrendt2, S. E. Philipp1, J. Obwierkiewicz1

1Philipps-Universität Marburg, Institut für Physiologie und Pathophysiologie, Marburg, Germany
2Universität Heidelberg, Experimentelle Schmerzforschung, Medizinische Fakultät Mannheim, Mannheim, Germany
3Universität des Saarlandes, Experimentelle und Klinische Pharmakologie und Toxikologie, Homburg, Germany

TRPM3 proteins form ion channels that have been implicated in the function of somatosensory nociceptors, pancreatic β-cells, vascular smooth muscle cells, epithelial cells in the ciliary body and choroid plexus, among others. These channels can be activated by heat and the endogenous steroid pregnenolone sulfate, are regulated by PI(3)Pls in the plasma membrane and are inhibited by Gβγ subunits, liberated after activation of GPCR receptors. They are typically portrayed as Ca2+-permeable non-selective cation channels, but it has long been recognized that TRPM3 proteins are subject to extensive alternative splicing. Most of the sites of alternative splicing are located in the cytosolic N-terminal region, but alternative splicing also affects the pore region and the cytosolic C-term (reviewed in Obwierkiewicz & Philipp, Handb Exp Pharmacol, 2014, 222:427-59). The functional consequences of these alternative splicing events have been explored for only three of the (at least) seven published sites of alternative splicing. Interestingly, these three splice events have profound consequences for the functional properties of the channels. Splicing at exon 24 (which encodes part of the pore loop) dramatically alters ion selectivity of the channel, while the splice event at exon 13 abolishes channel activity for unknown reasons when the 18 amino acids encoded by this exon are not present. Recently, we showed that splicing out the 10 amino acids encoded by exon 17 abolishes the sensitivity to Gβγ, and hence to GPCR activation.

Using Ca2+-imaging and whole-cell patch-clamp electrophysiology, we investigated whether these three splice events (exon 13, 17 and 24), which so far only have been tested in isolation, interact with alternative splicing at the first exon (either exon 1 or exon 2 are incorporated in the mature mRNA) or with the splice event at the C-term (which shortens the C-terminus by almost 400 amino acids). Our results clearly indicate that neither the N- nor the C-terminal splice event influences the known functional channel properties. In ongoing experiments, we also asked whether other splice events, specifically those at exon 8 and 15, interact with the functional consequences of splice events at exons 13, 17 and 24. The results of these experiments will help to define the (patho-) physiological implications of alternative splicing for the functional properties of TRPM3 channels in processes such as pain perception or insulin secretion.

OS 06-08

Binding cooperativity between subunits of HCN2 channels investigated by single-molecule ligand binding R. Schmader1, S. Thon, M. Otte, A. Schweinritz, M. Lellie, T. Zimmer, T. Schwabe, K. Benndorf

1Jena University Hospital, Institute for Physiology II, Jena, Germany

Ligand-gated ion channels are essential in many fast signaling processes. Their multi-subunit composition allows for cooperativity and with this fine-tuning of the set points of their signaling. Here we aim to directly observe the cooperativity in single-ligand binding to the tetrameric HCN2 pacemaker channels. These cation channels are voltage activated and, in addition, modulated by cyclic nucleotides.

To follow binding at moderate (high nanomolar) concentrations we screened and optimized ligands: Molecular brightness and environmental sensitivity of fluorophores was evaluated with FCS and fluorescence lifetime measurements, linker chemistry was optimized and function was evaluated with confocal patch-clamp fluorometry.

Following binding of individual fluorescent ligands to single HCN2-channels with TIRF microscopy directly suggests positive cooperativity. We further analyze the single molecule binding data to test for possible heterogeneities in the binding of the ligands to the four binding sites of the receptors. Signal processing is optimized to follow the levels and the dwell times of the unitary binding events, ultimately to increase the understanding of the channel dynamics in terms of Hidden Markov Models.
Control of cerebral blood flow by capillary pericytes in Alzheimer’s disease

Methods: We have used genetic mouse models to conditionally inactivate Rbpj in mural cells, induce acute or chronic deletion of pericytes and promote Notch gain- or loss-of-function scenarios for phenotypic evaluation using gene expression analysis (RNAseq, FACS+RT-qPCR), as well as immunostaining and high resolution imaging (confocal and transmission electron microscopy). Moreover, involvement of pericytes during stroke has been studied after distal middle cerebral artery occlusion in adult animals.

Results: Postnatal deletion of Rbpj in mural cells impairs brain vascular morphogenesis and NVU homeostasis resulting in severe hemorrhages which are specific to the CNS and which are not mimicked by pericyte ablation. RNAseq analysis revealed that Rbpj is indispensable for maintenance of brain pericytes’ molecular identity and for the regulation of cellular communication with endothelial cells. Indeed, Rbpj-deficient pericytes show increased contractility, change the composition of the extracellular matrix and increase local TGFbeta signaling which affects endothelial cell behavior and blood vessels integrity. Noteworthy, the vascular lesions induced in young mice recapitulate pathological landmarks associated with cerebral cavernous malformations. In adult mice, Rbpj deletion does not induce any overt phenotype in the CNS under physiologic conditions. Nevertheless, upon ischemic stroke, mutant mice show increased cortical lesions size and a stronger inflammatory response.

Conclusion: RBPJ is a key transcriptional regulator necessary for proper molecular identity and functional behavior of CNS pericytes during physiologic angiogenesis and after ischemic insult. We propose that upon Rbpj deletion, brain pericytes can acquire deleterious properties that actively enhance neovascular lesion formation and promote pathogenic processes in an unprecedented manner.

Loss of the transcription factor RBPJ induces disease-promoting properties in brain pericytes

Objective: Despite the relevance of pericytes for blood, brain barrier, neurovascular unit (NVU) integrity and the pathology of a wide variety of brain diseases (1-2), our understanding of the signaling mechanisms responsible for their intercellular communication is limited and requires further attention. Here we aim at elucidating the role of RBPJ, a transcriptional regulator involved in Notch signaling, for pericyte biology in the central nervous system (CNS).

Methods: We have used genetic mouse models to conditionally inactivate Rbpj in mural cells, induce acute or chronic deletion of pericytes and promote Notch gain- or loss-of-function scenarios for phenotypic evaluation using gene expression analysis (RNAseq, FACS+RT-qPCR), as well as immunostaining and high resolution imaging (confocal and transmission electron microscopy). Moreover, involvement of pericytes during stroke has been studied after distal middle cerebral artery occlusion in adult animals.

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Conclusion: RBPJ is a key transcriptional regulator necessary for proper molecular identity and functional behavior of CNS pericytes during physiologic angiogenesis and after ischemic insult. We propose that upon Rbpj deletion, brain pericytes can acquire deleterious properties that actively enhance neovascular lesion formation and promote pathogenic processes in an unprecedented manner.

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Oral Session 07: Vascular Physiology

OS 07-01
Control of cerebral blood flow by capillary pericytes in Alzheimer’s disease

Methods: We have used genetic mouse models to conditionally inactivate Rbpj in mural cells, induce acute or chronic deletion of pericytes and promote Notch gain- or loss-of-function scenarios for phenotypic evaluation using gene expression analysis (RNAseq, FACS+RT-qPCR), as well as immunostaining and high resolution imaging (confocal and transmission electron microscopy). Moreover, involvement of pericytes during stroke has been studied after distal middle cerebral artery occlusion in adult animals.

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Conclusion: RBPJ is a key transcriptional regulator necessary for proper molecular identity and functional behavior of CNS pericytes during physiologic angiogenesis and after ischemic insult. We propose that upon Rbpj deletion, brain pericytes can acquire deleterious properties that actively enhance neovascular lesion formation and promote pathogenic processes in an unprecedented manner.

OS 07-07
Oral Session 07: Vascular Physiology

Inhibition of the soluble epoxide hydrolase attenuates diabetic retinopathy

Objectives: The soluble epoxide hydrolase (sEH) metabolizes polyunsaturated fatty acid epoxides to their corresponding diols. The docosahexaenoic acid (DHA) diol, 19,20-dihydroxydocosapentaenoic acid (DHA-DHDP) is enriched in the retina and plays a role in physiological angiogenesis by inhibiting Notch signaling, this study aimed to explore the role of sEH in diabetic retinopathy (DR).

Methods: We found that the sEH expression was upregulated in diabetic murine and human retinas which was linked with increased production of DHA metabolite 19,20-DHDP. Indeed, in human vitreous humour samples with DR 19,20-DHDP levels were markedly increased. Retinas from ins2Akat mice (a genetic mice model of DR relevant to humans) demonstrated loss of mural cells, vascular leakage and the formation of avascular capillaries and all of these changes could be prevented by treating mice with a sEH inhibitor. Mechanically, the 19,20-DHDP targeted the cell membrane to alter the localisation of
cholesterol-binding proteins, and interfered with the association of presenilin-1 with N-cadherin and VE-cadherin, thereby interfering with osteo-/chondrogenic signaling and mRNA expression, osteo-/chondrogenic signaling and calcification of HAoSMCs. The ASH inhibitors showed similar anti-calcific properties during uremic conditions in vivo. In vivo, cholecalciferol treatment increased Smip4 (gene encoding ASM) mRNA expression in the aortic tissue. ASM-deficiency ameliorated aortic Sgrk mRNA expression and osteo-/chondrogenic transdifferentiation in mice with thiosulfate overload. Similarly, treatment with ASM inhibitors reduced vascular calcification in the cholecalciferol overload mouse model. Conclusions: ASM stimulates osteo-chondrogenic transdifferentiation of VSMCs, at least partly, through up-regulation of Sgrk-1-dependent signaling. Thus, ASM is a novel regulator of vascular calcification and ASM inhibition may represent a potential therapeutic strategy to reduce the progression of vascular calcification during high phosphate conditions such as chronic kidney disease.

OS 07-06
The NADPH oxidase Nox4 promotes endothelial differentiation from induced-pluripotent stem cells
F. Hahner1, F. Molière1, O. Schade1, O. Löwe1, T. M. Warwick1, M. Loos1, R. P. Brandes1, W. H. Zimmermann2, A. Hansen1, K. Schröder1, DZHK
1Uni Klinik Frankfurt, Institute for Cardiovascular Physiology, Frankfurt am Main, Germany
2University Medical Center Göttingen, Institute of Pharmacology and Toxicology, Göttingen, Germany
3University Medical Center Hamburg-Eppendorf, Department of Experimental Pharmacology and Toxicology, Hamburg, Germany

Nox4 is the only constitutively active NADPH oxidase and directly produces H2O2. It is expressed in endothelial cells, where it contributes to homeostasis. In other cells, Nox4 forces differentiation. We therefore hypothesize that Nox4 contributes to endothelial differentiation as well and investigated the role of Nox4 during the process of endothelial differentiation from induced pluripotent stem cells (iPSC).

In the course of endothelial differentiation, we found an increase of Nox4 expression in wild type cells. Nox4 knockout results in a prolonged expression of pluripotency markers such as Oct4 and Nanog. Simultaneously the expression of endothelial markers in differentiating KO cells is diminished. Those include vascular endothelial growth factor receptor 2 (VEGFR-2), Platelet endothelial cell adhesion molecule (PECAM-1) and endothelial nitric oxide synthase (eNOS). Additionally, the functionality of the iPSC-derived endothelial cells (iPSC-Ecs) is impaired in the Knockout. These cells show less tube formation and sprouting capacity in vitro. In an in vivo matrigel plug assay, Nox4+/- iPSC-Ecs integrate less into the newly formed vasculature.

As an underlying mechanism, we found Nox4 to be involved in chromatin remodeling of endothelial specific genes. Triple methylation of histone 3 (H3K27me3) was increased in cells lacking Nox4 based on a lower nuclear abundance of the demethylase JmjD3 in Nox4 deficient iPSC-Ecs. A redox assay revealed less oxidized JmjD3 in Nox4-/- iPSC-Ecs. Consequently, differentiation is forced and endothelial markers are produced.

OS 07-07
Acid sphingomyelinase regulates vascular smooth muscle cell calcium via serum- and glucocorticoid-inducible kinase SGK1
I. Alesutan1, T. T. Luong2, R. Tuffaha3, N. Schelski1, B. Boehme4, B. Pieske5, E. Gubins6, K. U. Eckardt7, P. Lang8, J. Voelkle1, B. Schreier1, S. Rabe1, S. Mildenberger1, M. Gekle1, W. Eckardt1
1Charité – Universitätsmedizin Berlin, Department of Internal Medicine and Cardiology, Berlin, Germany
2Charité - Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, Berlin, Germany
3University of Duisburg-Essen, Institute of Molecular Biology, University Hospital Essen, Essen, Germany

Question: Medial vascular calcification is an active process mediated by osteo-chondrogenic transdifferentiation of vascular smooth muscle cells (VSMCs). However, the complex signaling pathways regulating vascular osteoinduction are still incompletely elucidated. The present study investigated the possible involvement of acid sphingomyelinase (ASM) in the regulation of vascular calcification during hyperphosphatemic conditions.

Methods: Experiments were performed in vitro in primary human aortic smooth muscle cells (HAoSMCs) and in vivo in cholesterol-overload treated ASM-deficient and wild-type mice or mice treated with ASM inhibitors.

Results: In vitro, bacterial sphingomyelinase up-regulated osteogenic markers MSX2, CBFAP1 and ALPL mRNA expression as well as ALPL activity and augmented phosphate-induced osteo-/chondrogenic transdifferentiation and calcification of HAoSMCs. Both, bacterial sphingomyelinase and phosphate increased ceramide levels in HAoSMCs. Addition of exogenous ceramide similarly stimulated osteo-/chondrogenic transdifferentiation of HAoSMCs in control and high phosphate conditions. Bacterial sphingomyelinase and exogenous ceramide induced the mRNA and protein expression of serum- and glucocorticoid-inducible kinase SGK1, while silencing of SGK1 was able to blunt bacterial sphingomyelinase as well as ceramide-induced osteo-/chondrogenic transdifferentiation of HAoSMCs. Moreover, additional treatment with ASM inhibitors amphytyline or fendiline suppressed phosphate-induced SGK1 mRNA expression, osteo-chondrogenic signaling and calcification of HAoSMCs. The ASM inhibitors showed similar anti-calcific properties during uremic conditions in vitro. In vivo, cholecalciferol treatment increased Smip4 (gene encoding ASM) mRNA expression in the aortic tissue. ASM-deficiency ameliorated aortic Sgrk mRNA expression and osteo-/chondrogenic transdifferentiation in mice with thiosulfate overload. Similarly, treatment with ASM inhibitors reduced vascular calcification in the cholecalciferol overload mouse model. Conclusions: ASM stimulates osteo-chondrogenic transdifferentiation of VSMCs, at least partly, through up-regulation of SGK1- and Sgrk-dependent signaling. Thus, ASM is a novel regulator of vascular calcification and ASM inhibition may represent a potential therapeutic strategy to reduce the progression of vascular calcification during high phosphate conditions such as chronic kidney disease.

OS 07-08
The impact of the vascular smooth muscle cell epidermal growth factor receptor in high fat diet-induced renal and vascular alterations
C. Stern1, B. Schreier2, S. Rabe1, S. Mildenberger1, M. Gekle1
Martin Luther University Halle-Wittenberg, Julius-Bernstein Institut, Halle, Germany

Question: Underlying mechanisms of cardiovascular complications associated with diabetes mellitus type II (DMT2) are still incompletely understood. The vascular smooth muscle cell (VSMC) epidermal growth factor receptor (EGFR) contributes to acute vascular regulation and chronic angiotensin-II-induced vascular remodeling. Increased EGFR phosphorylation in the course of DMT2 in mesenteric arteries and impact on high fat diet (HFD)-induced vascular remodeling preceding end-organ damage indicate a crucial function in DMT2 associated complications. However, the role of vascular EGFR during this process and underlying mechanisms is unclear.

Methods: Mice with an inducible VSMC specific EGFR knock out were fed 18 weeks either with a HFD inducing DMT2 or a standard diet (SD). Vascular, renal and cardiac effects of KO and HFD were investigated by functional, transcriptional and structural in vivo and ex vivo assays. Furthermore cell culture studies were performed revealing the impact of hyperglycemia on EGFR-induced activation of the vascular transcription factor SRF.

Results:
Weight gain and increased glucose levels by HFD were not influenced by the genotype. VSMC KO augmented DMT2 associated kidney hypertrophy. Furthermore, HFD-induced creatinemia and albuminuria only occurred in wildtype (WT) animals. Both genotypes displayed HFD induced glomerulosclerosis, interstitial fibrosis but no perivascular fibrosis. In contrast, KO showed increased vaculization of proximal tubules. Analysis of renal transcriptome displayed an HFD induced inflammatory phenotype in WT whereas in KO animals proximal tubule transport is upregulated. Endothelial dysfunction caused by HFD was diminished by the VSMC KO. Aortic transcriptome analysis revealed mitochondrial dysfunction and altered matrix homeostasis only in WT fed with HFD. In vitro analysis using VSM-AT3, HK-2 and HEK293 cells revealed an augmented effect of hypoglycemia on EGF-induced stimulation of SRF activity. This synergism was mediated by the actin-MRTF-pathway.

Conclusion
VSMC, EGFR contributes to HFD-induced vascular and renal alterations, most probably as a result of the potentiating effect of glucose on the EGF-ERK-SRF signaling axis.

OS 08-01
Context-sensitive extracellular matrix remodelling by human multipotent mesenchymal stromal cells
A. Sassmann1, A. Lauermann1, C. Kasper2, S. Schubert2, J. Burk3
1University of Natural Resources and Life Sciences, Vienna, Austria
2University of Leipzig, Saxion Incubator for Clinical Translation, Leipzig, Germany
3Justus Leibig University Gießen, Equine Clinic (Surgery), Gießen, Germany

Multipotent mesenchymal stem cells (MSC) are an emerging therapeutic tool for a wide range of diseases. While our understanding of MSC mechanisms of action is gradually improving, relatively little is known on their role in extracellular matrix (ECM) modulation. The aim of this study was to shed light on the context-dependent ECM remodelling activities of MSC. MSC-derived ECM culture substrates were generated by cultivating human immortalized adipose-derived MSC on plastic culture dishes or without the addition of TGF-β1, or on matrigel-coated plates. The matrices deposited by the MSC were decellularized and characterized histochromically. MSC from the same cell line were then cultured either on tissue culture plastic, matrigel, MSC-derived ECM, TGF-β1-induced MSC-derived ECM, or MSC-derived ECM grown on matrigel. Subsequently, MSC were assessed regarding their viability, ECM receptor expression and cytoskeleton morphology, matrix metalloproteinase (MMP) activity, as well as MMP, MMP inhibitor and ECM gene expression. The ECM deposited by the MSC was rich in collagen and did not contain major amounts of glycosaminoglycans. The TGF-β1-induced MSC-derived ECM displayed a fibrous structure, while the ECM in the other groups was distributed evenly on the plastic. Viability was highest on matrigel substrate, although differences were not significant. Expression of the ECM receptor CD29 and CD44 was evident on all substrates, but appeared stronger on the different MSC-derived ECM substrates, which corresponded to the respective cytoskeleton organization. In MSC cultured on TGF-β1-induced MSC-derived ECM and/or on MSC-derived ECM grown on matrigel, gene expression of MMP-1, MMP-3 and MMP-14 was increased (p<0.05). However, in the MSC cultured on the fibrous TGF-β1-induced MSC-derived ECM, total MMP activity was reduced and collagen 1A2 and tenasin-C were upregulated (p<0.05).

These findings demonstrate that MSC adapt their matrix remodelling activities to their local ECM environment. Thus, depending on the context, pro- as well as anti-fibrotic effects may be anticipated. In fibrotic environment, MSC might be triggered to further promote fibrosis.

OS 08-02
Role of TRPM2 channel and sodium transporters in chemotaxis and sodium homeostasis in murine neutrophils
K. Naji1, J. Schrödler1, M. Rugi1, L. Oster1, A. Schwab2
1WWU, Institut für Physiologie II, Münster, Germany
2University of Florence, Florence, Italy

The function of neutrophils, the most abundant immune cells in the body, is long known to be regulated by Ca2+. However, not much is known about fluxes and regulation of Na+ and H+, which also modulate the response of neutrophils. The Na+/Ca2+ exchanger (NCX) removes the Ca2+ following neutrophil activation and H+ released due to NADPH oxidase 2 activity, is expelled among others by the Na+/H+ exchanger (NHE1). NHE1 activity is also increased when neutrophils enter acidic inflammatory foci. These Na+-dependent exchangers and opening of Na+ permeable channels such as Transient Receptor Potential Melastatin 2 (TRPM2) lead to an increase of the intracellular Na+ load upon neutrophil activation. TRPM2 channel is highly expressed in neutrophils, but its function in neutrophils is still discussed controversially.

We analyzed the impact of the TRPM2 channel on the chemotaxis and intracellular sodium concentration ([Na+]i) in neutrophils stimulated with fMLP. Although TRPM2 does not play a role in neutrophil chemotaxis in 3D collagen I matrix in the normal pH, the sodium concentration in TRPM2-/- neutrophils rises higher than in WT (13.1 ± 0.6 mM vs. 15.6 ± 0.9 mM, after 10 min) upon stimulation with fMLP. Lack of the channel paradoxically allows for unbalanced Na+ influx, presumably because of attenuated membrane depolarization upon neutrophil activation. Additional inhibition of NHE1 by L-ATPase and NCX1 exchanged revealed that higher [Na+]i in activated TRPM2-/- neutrophils affect NCX transport direction, triggering NCX reverse mode. Using RT-qPCR, we found NCX1.3 to be expressed in neutrophils. The reverse mode of NCX1.3 is effectively inhibited by KB-R7943 which attenuates the increase of the [Na+]i in activated neutrophils.

OS 08-03
Influence aging on calcium signals and cytotoxicity in murine CD8+ T cells
A. Liu1, A. Angenend1, D. Zöphel1, R. Steiner1, E. Krause1, M. Mansson2, G. Schrader1, A. Knörrck1
1Saarland University, Biophysics, Center for Integrative Physiology and Medicine, School of Medicine, Homburg, Germany
2Saarland University, Cellular Neurophysiology, Center for Integrative Physiology and Medicine, School of Medicine, Homburg, Germany
3Medical University of Vienna, Section of Transplantation Immunology, Department of Surgery, Vienna, Austria

Calcium (Ca2+) is a crucial secondary messenger for proper T cell function. In addition to a whole range of cellular processes, it influences and regulates activation, proliferation, and migration as well as the T cell-mediated cytotoxicity. The ability of T cells to generate Ca2+ signals is diminished in elderly individuals, potentially contributing to the decrease in T cell functionality, declining immune competence and an increased risk for cancer in aging. The main pathway for Ca2+ entry is store-operated (SOCE). The role of Orai channels and stromal-interaction molecule (STIM) proteins, as calcium sensors, in lymphocyte aging is completely unknown. To investigate, how STIM and Orai contribute to Ca2+ signal reductions in CD8+ T cells from elderly mice, we performed flow cytometry, electrophysiology and molecular biology experiments. Our main focus was the change in Ca2+ signals of stimulated sorted CD8+ T cells from elderly mice, which showed a decay in cytosolic free calcium (Ca2+), strongly reduced maximum Ca2+ signals (p<0.005), and a reduction in Ca2+ signals depending on the context, pro- as well as anti-fibrotic effects may be anticipated. In fibrotic environment, MSC might be triggered to further promote fibrosis.

OS 08-04
Interleukin 8 evokes an alkalization of neutrophils in dependence of NHE1, P2Y, and AE-1
D. A. C. Messerer1, S. Bernhard1, S. Hug1, A. E. P. Stratmann1, M. Fauler2, K. Föhr1, M. S. Huber-Lang1
1University Hospital Ulm, Institute of Clinical and Experimental Trauma-Immunology, Ulm, Germany
2Ulm University, Institute of General Physiology, Ulm, Germany
3University Hospital Ulm, Department of Anesthesiology, Ulm, Germany

Question
Stimulation of neutrophils (PMN) by chemokines like complement factor 5a (C5a) involves an alkalization of the intracellular pH (pHi) and a change in cellular size, regulating vital cellular functions [1]. Therefore, we hypothesized that the chemokine receptor Interleukin 8 (II8) evokes an intracellular pH shift in PMN and characterized involved ion channels.

Methods

PMN were isolated from healthy human donors (n = 6). pH was measured by flow cytometry using 5-(and-6)-Carboxy SNARF-1 after establishment of a neoplastic culture curve. Forward scatter (FSC) was used as a surrogate for cellular size or polarization. In each measurement, 10,000 cells were analyzed in RPMI with a pH adjust to 7.4. PMN were exposed to IB 50 ng/ml for 5 min. Results were compared to unstimulated cells and are given as a mean ± SD. Results

IB revealed a rapid, transient alkalization with a peak at 5 minutes (+0.40 ± 0.05, p<0.05) accompanied by an increase in FSC (+19%) and NHE1 activity (+0.8 ± 0.0). While intracellular alkalization was still present after 30 min, pH returned to the baseline level of unstimulated PMN after 60 min. Comparing PMN after stimulation with or without IB, alkalization was significantly diminished by inhibiting sodium-hydrogen antporter 1 (NHE1, -0.19 ± 0.04), P2Y receptors (-0.12 ± 0.07), or anion exchanger 1 (AE1, -0.31 ± 0.02). Among inhibition of various ion channels, inhibition of sodium bicarbonate cotransporter and voltage gated proton channels did not interfere with IB induced alkalization. While inhibition of the P2Y receptors reduced the increase in FSC to +32% ± 30%, inhibition of AE1 and NHE1 did not show a relevant effect. Variation of extracellular pH (pH(e)) subsequently altered pH(e), but did not impair relative alkalization (+0.37 ± 0.09 with pH(e) of 6.6; +0.39 ± 0.05 with 7.0; +0.42 ± 0.09 with 7.9).

Conclusion

IB induced a significant intracellular alkalization, that is dependent on NHE, P2Y, or AE-1. The shift in pH remained robust in varying pH(e), demonstrating that acidosis itself, being present at systemic inflammation, does not compromise alkalization of pH(e). Further research needs to analyze the role of IB induced alkalization on cellular functions and in a clinically relevant scenario.


OS 08-05

Neurotransmitter-mediated control of microglial membrane voltage in a model of Alzheimer’s disease

A. Rifat, J. R. P. Geiger, C. Madry

Charité Universitätsmedizin, Institute of Neurophysiology, Berlin, Germany

Microglial function is crucially controlled by ion channels and cell-surface receptors, the expression of which varies with the microenvironmental and functional states that these cells can adopt. A prominent role is ascribed to purinergic receptor signaling, which regulates key microglial functions such as chemotactic movement, phagocytosis of pathogens or dead neurons or cell activation under pathological conditions. We have previously shown that extracellular ATP or ADP, mediated by microglial P2Y receptors, instantaneously hyperpolarizes microglia by activating the two-pore domain K+ channel THIK-1, which regulates pro-inflammatory cytokine release when microglial cells become activated. In contrast, in the absence of a purinergic stimulus, tonic THIK-1 channel activity sets the microglial membrane voltage which maintains microglial ramification and surveillance. Due to its involvement in pro-inflammatory cytokine release, we here describe the functional expression of the P2Y1/THIK-1 signaling complex and other microglial K+ channels in APPPS1 mice, a model of Alzheimer’s disease, by patch-clamp electrophysiology and also characterize microglial motility by two-photon imaging. Our data show that voltage-dependent and purinergic receptor-activated membrane currents of microglial cells in situ are altered depending on disease progression and proximity to amyloid plaques. We also describe additional modulators of microglial membrane voltage such as monoamines, γ-aminobutyric acid, glutamine and purines other than ATP or ADP. Our findings suggest that purinergic receptor signaling together with K+ channel activity regulate microglial membrane voltage in a model of neurodegeneration.

OS 08-06

scRNA-Seq Identifies MIF as Master Regulator of B Cell Fate

S. Besson-Girard1,2, C. Krammer1, S. Reichl1, V. Gokce3,4, O. Gokce1

1Klinikum der Universität München, Ludwig-Maximilians-Universität (LMU), Institute for Stroke and Dementia Research (ISD), Munich, Germany
2Ludwig-Maximilians-Universität (LMU), Graduate School of Systemic Neuroscience (GNS), Planegg-Martinsried, Germany
3Max-Planck Institute for Neurosciences (SyNergy), Munich, Germany
4Munich Heart Alliance, Munich, Germany

In vivo regulators of B cell development have been elusive. Here we used single-cell RNA-seq (scRNA-seq) to characterize the transcriptional landscape of the chemokine-like cytokine macrophage migration inhibitory factor (MIF) in mice. We provide a comprehensive resource of transcriptions of B cells from early development in the bone marrow and maturation in spleen and how MIF guides these developmental checkpoints. In the bone marrow, B cells undergo a series of defined stages that control genomic alterations, proliferation, and migration. We find that MIF is an early and critical regulator of B cell development. Our analyses delineate molecular events shaping B cell development and how MIF alters B cell migration, proliferation, metabolism and regulatory switches driving B cell genomic diversity by somatic hypermutation and recombination. Our study reveals that targeting MIF signaling in the bone marrow can alter the adaptive immune responses which impacts many diseases from atherosclerosis to multiple sclerosis.

OS 08-07

CIC-3 Cl−/H+ exchangers in pain perception

J. D. Sierra Marquez1, A. Willweber1, V. Graß2, M. Schönke3, A. Lamperti4, G. Fahlke1, R. E. Guzman1
1Forschungszentrum Jülich, Institute of Complex Systems, Cellular Biophysics (ICS4), Jülich, Germany
2Forschungszentrum Jülich, Institute of Neuroscience and Medicine (INM), Jülich, Germany
3RWTH Aachen University, Institute of physiology, Aachen, Germany
4ClCN-CrH3 exchangers are crucial role for normal function of the central nervous system. Its function in sensory neurons has remained unclear. Here we study the role of CIC-3 in pain perception. Using tail flick and hot plate tests we observed a significant increase in thermal sensitivity of Clcn3−/− mice. To understand the molecular basis of this phenotype, we performed electrophysiological experiments on cultured dorsal root ganglion (DRG) neurons using whole-cell patch clamping recordings. The threase current, i.e. the current amplitude sufficient to trigger a first action potential (AP), was significantly smaller in Clcn3−/− (17.7 ± 2.9 pA) than in WT neurons (39.6 ± 6.3 pA), and the input resistance was increased in Clcn3−/− neurons (1144 ± 81 pA) compared to WT neurons (746 ± 67 pA). Resting membrane potential were not affected. The firing rate, defined as the number of APs per injection of five-fold threshold current was significantly increased in mutant neurons. Moreover, Clcn3−/− neurons were unable to maintain repetitive firing upon depolarizing stimuli exceeding 250 pA. Since sodium channels are mainly responsible for AP generation and since DRG neurons almost exclusively express tetrodotoxin (TTX)-resistant Na1.8 and Na1.9 and TTX-sensitive Na1.7 channels, we measured sodium currents in the presence and in the absence of TTX. Clcn3−/− DRG neurons exhibit a significant reduction (about 43%) of the TTX-resistant Na current. In contrast, current densities of TTX-sensitive Na channels were increased. We next measured potassium currents in the absence and in the presence of 4-aminopyridine and found higher current densities in Clcn3−/− DRG neurons under both conditions. Taken together, our results demonstrate that CIC-3 transportsers cammodulate nociception by regulating the density of sodium and potassium channels at the plasma membrane.

OS 08-08

Nitric oxide maintains endothelial redox homeostasis through pyruvate kinase M2 inhibition

M. Siragusa1, J. Thiel1, S. -I. Bibli1, A. Weigert1, B. Brüne1, S. - I. Bibli1, B. Fisslthaler1, I. Fleming1
1Goethe University, Institute for Vascular Signalling, Centre for Molecular Medicine, Frankfurt am Main, Germany
2Goethe University, Functional Proteomics, SFB 815 Core Unit, Frankfurt am Main, Germany
3Goethe University, Institute of Biochemistry I, Frankfurt am Main, Germany

Decreased nitric oxide (NO) bioavailability and oxidative stress are hallmarks of endothelial dysfunction and cardiovascular disease and although numerous proteins can be potentially S-nitrosated, whether and how changes in their S-nitrosation influence endothelial function under pathophysiological conditions remains unknown. We report that active endothelial NO synthase (eNOS) interacts with pyruvate kinase M2 (PKM2) to elicit its S-nitrosation (on Cys358) and inhibition. PKM2 inhibition increased flux through the pentose phosphate pathway to generate reducing equivalents (NADP and GSH) and protect against oxidative stress. In a novel mouse model, the mutation of eNOS Tyr656 to Phe rendered the enzyme insensitive to inactivation by oxidative stress and prevented the decrease in NO bioavailability associated with endothelial dysfunction. As a consequence, PKM2 S-nitrosation and cellular antioxidant responses were preserved and cardiovascular disease development delayed. These findings highlight a novel mechanism linking preserved NO bioavailability to endothelial cell antioxidant responses through S-nitrosation and inhibition of PKM2.
Constitutive activation of Jak1 leads to increased fibroblast growth factor 23 levels in mice

I. Rubio-Aliaga, B. Lorenz-Depiereux, H. Bettoni, M. Hrabe de Angelis

University of Zurich, Institute of Physiology, Zürich, Switzerland

The endothelial glycocalyx (eGC), a carbohydrate-rich layer, lining the luminal side of the vascular endothelium, provides a first vasoprotective barrier against vascular leakage and leukocyte adhesion in various inflammatory diseases and plays a pivotal role within the development of cardiovascular pathologies (e.g. atherosclerosis, hypertension and chronic kidney disease (CKD)). Recent studies provide a damage of the eGC as a consequence of CKD which correlates with ath erosclerosis formation in vivo. CKD patients also exhibit endothelial damaging effects caused by alteration of the HDL composition and function. One mediated factor is symmetric dimethyl arginine (SDMA), transforming LDL to “dysfunctional” HDL (“d-HDL”). The aim of this study was to reveal if SDMA is responsible for eGC damaging effects of d-HDL in CKD. The quantification of the eGC in vivo is performed with the sidestream darkfield (SDF) imaging which measures the perfused boundary region as an indirect parameter for the eGC and is confirmed by the Syndecan-1 levels measured via ELISA. The thickness of the eGC in vitro and ex vivo was quantified by Atomic Force Microscopy (AFM) after the incubation with i-HDL, d-HDL and SDMA. Intravital microscopy was used to analyze rolling velocity in mice after SDMA, dHDL or iHDL injection. The SDF of sublingual microvessels of dialysis patients showed a reduction of eGC which was confirmed by elevated plasma syndecan-1 levels. Biologically active HDL (HDL') was calculated from HDL-cholesterol and SDMA level. HDL' was very high in plasma. The source of high FGF23 levels was not bones but the inflamed liver and probably also thymus, as demonstrated by ex vivo experiments. FGF23 canonical physiological function is to promote the excretion of phosphate in urine. Western blot analysis suggested that not all the FGF23 produced in the liver of the JakS6459+/- mice underwent full posttranslational modifications. This may explain the lack of phosphaturia in these animals despite their high FGF23 levels. These findings indicate that activation of the JAK/STAT pathway influences also mineral metabolism and more importantly, that high circulating FGF23 levels may not always have physiological (or pathological) consequences.
was performed. Oxalate diet produced profound changes in the renal transcriptome of biological processes spanning from immune system to epithelial transport. Pathway analysis using RNAseq data revealed that alkali therapy may protect tubular transport functions and may act as an anti-inflammatory agent.

Conclusions
Alkali therapy protects renal function and modulates several immune and non-immune processes in mice suffering from oxalate nephropathy. These effects operate via mechanisms that are beyond those previously proposed of how alkalinizing agents may reduce inflammation and fibrosis in renal disease.

OS 09-05
Renal Nox4 contributes to systemic redox homeostasis by controlling glutathione, methionine, cysteine and folate metabolism
F. Rexende1, L. Haunbi1, P. Pacheco2, T. J. R. Bintener2, P. F. Malacarne1, N. Müller1, T. Sauter1, K. Schröder1, R. P. Brandes1
1Goethe University, Cardiovascular Physiology, Frankfurt, Germany
2Universität Erlangen, Department of Pharmacology and Toxicology, Erlangen, Germany

The NADPH oxidase Nox4 produces H2O2 and is highly expressed in the kidney. Its expression is reduced in diabetic nepthopathy and renal inflammation, suggesting that Nox4 is particularly important for normal renal function. As demonstrated by in situ hybridization (RNAseq) combined with immunofluorescence, Nox4 is selectively expressed in the proximal tubule, a part of the kidney responsible for mass transport. To study this function, WT (wild type) and Nox4+/− (tamoxifen-inducible, global Nox4 knockout mice) were put on an artificial fiber and protein-free diet, with low sodium (130 mg/kg chow) and low micronutrients. Urine samples (day 0, 3, 14), renal cortex and plasma (day 14) were analyzed by global untargeted LC/MS for metabolites. Genome scale metabolic reconstruction using fastcore showed a significant downregulation of extracellular and mitochondrial transport; metabolism of nucleotides, inositol phosphate and folate in response to the deletion of Nox4. Moreover, metabolites of histidine catabolism (uracanate and formiminoacetate), which depend on folate, were significantly elevated in Nox4+/− compared to WT mice. Marked reduction in metabolites of methionine, cysteine and glutathione were observed in plasma and renal cortex of Nox4+/− compared to WT animals. Results from kidney phenotyping, protein expression and metabolomics indicate that the physiological function of Nox4 in the kidney is to control redox homeostasis and metabolism of amino acids and vitamins of the complex B that are important for redox homeostasis.

OS 09-06
Aldosterone-independent ENaC activity in the early part of the aldosterone-sensitive distal nephron critically depends on the presence of mineralocorticoid receptor
V. Nesterov1, M. Bertog2, J. Canonica2, E. Hummler2, C. Kortmacher1
1Friedrich-Alexander Universität Erlangen-Nürnberg, Institut für Zelluläre und Molekulare Physiologie, Erlangen, Germany
2Universität Erlangen, Department of Pharmacology and Toxicology, Erlangen, Germany

The epithelial sodium channel (ENaC) constitutes the rate-limiting step for sodium absorption in the aldosterone-sensitive distal nephron (ASDN), which comprises the late distal convoluted tubule (DCT2), the connecting tubule (CNT) and the entire collecting duct. Previously we demonstrated that ENaC activity in the early part of the ASDN is constitutively high and independent of aldosterone [2]. This is in contrast to the well documented aldosterone dependence of ENaC activity in the cortical collecting duct (CCD), where ENaC regulation is mediated by the mineralocorticoid receptor (MR). In the present study we tested the hypothesis that ENaC activity in the early part of the ASDN is aldosterone-independent but dependent on the presence of MR known to be expressed in the entire ASDN.

Methods
Doxycycline-inducible nephrone-specific MR-deficient mice (MR KO) were used [1], and ENaC function was investigated in nephrone fragments from the transition zone of DCT2/CNT or CNT/CCD as previously described [2, 3]. Whole-cell patch-clamp recordings were used to measure amiloride-sensitive ENaC currents (∆Iam) at a holding potential of -80 mV.

Results
As expected, ENaC activity was detectable in CNT/CCD cells of control mice (median ∆Iam = 40 pA; lower quartile 27 pA; upper quartile 107 pA) but absent or barely detectable in the majority of CNT/CCD cells from MR KO mice. Importantly, ENaC currents in DCT2/CNT were greatly reduced in MR KO mice (∆Iam = 57 pA; 29 pA; 129 pA) compared to ENaC currents measured in DCT2/CNT of control mice (ΔIam = 231 pA; 162 pA; 357 pA; p < 0.001).

Conclusion
We confirm that MR is critically important for stimulating aldosterone-independent ENaC activity in the DCT2/CNT. It is tempting to speculate that in DCT2/CNT aldosterone-independent MR activation may be mediated by glucocorticoids due to reduced expression of 11-HSD2.

OS 09-07
Intestinal deposits of Uromodulin promote inflammation in vivo
T. Steffen1, R. Immel1, G. Hupel1, U. Kellner2, J. Schererbiuch3, B. Uhl4, H. Mannell2, H. Beck1, C. Reichel1, M. Sperandio1, B. Lange-Sperandio2, M. Pruenster1
1Walter Brendel Center of Experimental Medicine, Institute of Cardiovascular Physiology and Pathophysiology, Heppen, Germany
2Dr. von Haunersches Kinderspital, Department of Pediatric Nephrology, Munich, Germany
3Klinikum Harlaching, Lehrkrankenhaus der Ludwig-Maximilians Universität München, Munich, Germany

Question
Uromodulin (UMOD) is a kidney specific glycoprotein, produced exclusively by epithelial cells in the thick ascending limb of the loop of Henle. Under physiological conditions, UMOD is secreted primarily into the tubular lumen, where it has an important role in water and electrolyte balance. Furthermore, it has a protective function against bacterial infections in the kidney and it might be a contributing factor in the development of diabetes mellitus.

Methods
We used intravital microscopy and visualized leukocyte recruitment and vascular permeability in C57Bl/6 wild type (WT) mice after intracranial injection of UMOD or control buffer. In the mouse model of neonatal unilateral ureteral obstruction (UUO) we injected 10 μg UMOD in the contralateral kidney in order to investigate the potential effects of UMOD on leukocyte recruitment and vascular permeability in vivo.

Results
Intracranial injection of UMOD reduced number of rolling and increased number of adherent and extravasated leukocytes in vivo. Furthermore, UMOD increased vascular permeability of the endothelium in vivo. Surprisingly, UMOD was unable to induce direct upregulation of rolling and adhesion relevant molecules, such as E-selectin, ICAM-1 and VCAM-1 on endothelial cells and unable to directly activate beta2 integrins on neutrophils. However, UMOD stimulated F4/80 positive macrophages within the inflamed tissue to produce TNF-alpha, which in turn may have induced changes in leukocyte rolling, adhesion and extravasation. UMOD may also directly contribute to a proinflammatory microenvironment as it directly modulated the permeability of the endothelial monolayer and increased the transmigration of neutrophils across endothelial cells in vitro.

Conclusion
Taken together, we propose a proinflammatory function of intestinal deposited UMOD affecting leukocyte recruitment and promoting inflammation.

OS 09-08
Cell fate of renal erythropoietin-producing cells in health and disease
S. L. Dahl1,2, K. A. Nolani1,2, S. Pfundestein1, X. Dong1, R. Hunkeler1,2, P. Spielmann1, R. H. Wenger1,2
1University of Zurich, Institute of Physiology, Zurich, Switzerland
2National Centre of Competence in Research "Kidney.CH", University of Zurich, Zurich, Switzerland

The author has objected to a publication of the abstract.
To confirm the presence of phosphorylated RIP1, we are currently performing immunoprecipitation and immunoblotting, comparing the amount of total and phosphorylated RIP1 at neutral and acidic pH with or without application of Nec-1 or PcTx1.

Conclusion
We show that ASIC1a-specific agonists induce and that ASIC1a-antagonists inhibit necroptosis in two glioblastoma stem cell lines. This pathway might shape tumour evolution in an acidic microenvironment and might be a potential target in tumour therapy.

OS 10-03
The role of TRPM4 ion channel in colorectal and prostate cancer
P. Stoklosa1, S. Kappel1, B. Hauert1, R. Baur1, J. A. Galván Hernández2, I. Zlobec3, C. Pinelt1
1University of Bern, Institute of Biochemistry and Molecular Medicine, National Center of Competence in Research NCCR TransCure, Bern, Switzerland
2University of Bern, Graduate School for Cellular and Biomedical Sciences, Bern, Switzerland
3University of Bern, Institute of Pathology, Translational Research Unit, Bern, Switzerland

Store-operated calcium entry (SOC) is the main Ca2+ entry pathway in non-excitable cells. Ca2+ is a universal second messenger, which regulates multiple cellular processes. Imbalances in SOC contribute to pathological conditions, including cancer. Transient receptor potential melastatin 4 (TRPM4) channel is a monovalent, nonselective channel, which conducts Na+ and Ca2+ influx via TRPM4 depolarizes the plasma membrane, resulting in a decrease of driving force for further Ca2+ entry. TRPM4 negatively regulates Ca2+ signaling. TRPM4 is expressed ubiquitously with the most pronounced expression in colon and prostate tissue. The role of TRPM4 in prostate cancer cells has already been reported, but less is known if TRPM4 can play a role in colorectal cancer. We evaluated TRPM4-specific staining of tumor tissue from 379 colorectal cancer patients and analyzed TRPM4 protein expression, tumor characteristics and clinical outcome. High TRPM4 protein expression was associated with unfavorable tumor features, characteristic for epithelial-mesenchymal transition and infiltrative growth patterns, i.e. a high number of tumor buds and a low percentage in tumor border configuration.

In colorectal cancer cell line HCT116, TRPM4 activation with Ca2+ in the patch pipette resulted in large currents. Replacement of external Na+ in the bath solution by impermeable monovalent ion NMDG+ inhibited inward currents, pointing to Na+ influx as a major inward conducted ion in TRPM4 knock-out HCT116 cell lines. In the literature, TRPM4 is described as a major inward conducted ion. In TRPM4 knock-out HCT116 cells no current developed and no compensation mechanism was observed. These findings suggest that TRPM4 is the main source of ICa (Ca2+-activated non-selective current) in HCT116 cells. TRPM4 controls Ca2+ entry in HCT116 cells, as shown with Ca2+ imaging assay. In addition, TRPM4 knock-out decreases the viability of HCT116 cells and affects cell cycle. To elucidate if Na+ current via TRPM4 is the underlying mechanism of observed changes in HCT116 cells, we re-expressed functional TRPM4 and dominant negative mutant of TRPM4 (D84A), which does not conduct any ions, in HCT116 TRPM4 knock-out cells. Functional TRPM4 restored the viability and reversed changes in cell cycle, but TRPM4 D84A did not. This leads to the conclusion, that TRPM4 mediated currents contribute to colorectal cancer cell functions. Taken together, our data suggest that TRPM4 plays a versatile role in colorectal cancer.

OS 10-04
The specific cleavage of titin springs to determine the contribution of titin to myocardial passive stiffness
J. K. Fründt1, J. Recker1, C. M. Loechser1, I. Liashkovich1, J. M. Fernandez2, W. A. Linke1
1Westfälische Wilhelms-Universität Münster, Institut für Physiologie II, Münster, Germany
2Columbia University, Department of Biological Sciences, New York, US

Question. The failing heart is characterized by multi-level myocardial remodeling, including myocardial stiffening and diastolic dysfunction. Apart from matrix stiffening, failing human and animal hearts typically show stiffened cardiomyocytes, notably in diastolic heart failure (HF). The giant sarcomere protein titin bears passive load in cardiomyocytes and increased titin-based stiffness is found in HF. To quantify the contribution of titin to total cardiomyocyte viscoelasticity using a new experimental tool that allows the specific cleavage of the titin springs in the sarcomere.

Methods & Results. We generated a genetic mouse model, containing a tobacco etch virus (TEV) protease-recognition site and a HaloTag domino into elastic titin (HaloTag-TEV knock-in (KI)). This cassette allowed for specific in-situ cleavage of titin during mechanical measurements of permeabilized mouse cardiomyocytes and visualization of successful cleavage by measuring the fluorescence signal of cells incubated with fluorophore-conjugated HaloLigand (which binds covalently to HaloTag), using confocal microscopy or protein gel electrophoresis. TEV protease caused the complete, rapid (within minutes) and specific cleavage of cardiac titin in skinned homogenized KI heart samples, and ~50% cleavage in heterozygous KI. In cells stained with HaloLigand-Alexa488, HaloTag-TEV-titin was equally distributed within a transversal stiffness of isolated cardiomyocytes by nanoindentation. The Young's modulus of homozygous KI cells at a pre-
set force of 3 nN was significantly reduced with titin-cleavage, by 26±4% (n=26 cells), indicating cell softening with titin-cleavage. Skinned cardiomyocytes were also stretched at their ends and the resulting force recorded before/after TEV-treatment. TEV-protease reduced the steady-state passive force by 53±8% (n=9 cells), suggesting that titin is the main contributor to passive force.

Conclusions: The HaloTag-TEV mouse allows, for the first time, the direct and reliable quantitation of the titin contribution to cardiomyocyte stiffness. Our findings show that intact titin springs are responsible for most of the elastic force of the mouse cardiomyocyte. The Ki mouse will also be useful for evaluating the impact of passive stiffness under HF conditions.

OS 10-05
Carbonic anhydrase IX forms a transport metabolon with monocarboxylate transporters in human breast cancer cells
S. Ames1, J. T. Andring1, E. Niggli1, H. M. Becker2
1University of Kaiserslautern (TUK), Division of General Zoology, Kaiserslautern, Germany
2University of Florida, Department of Biochemistry and Molecular Biology, Gainesville, US

Tumor cells, which often reside in a hypoxic environment, rely on glycolysis to meet their elevated demand for energy and biosynthetic precursors. Tactally they produce 2-carbon metabolites, which are removed from the cell via monocarboxylate transporters (MCTs) to avoid intracellular acidosis and sulfation of metabolites. This continuous proton extrusion supports the formation of a hostile microenvironment, in which cancer cells, adapted to these conditions can outcompete normal host cells. In the present study, we demonstrate that carbonic anhydrase IX (CAIX), one of the major acidbase regulators in cancer cells, forms a protein complex with MCT1 and MCT4 in tissue samples from human breast cancer patients, but not in healthy breast tissue. Formation of this ‘transport metabolon’ requires direct binding of extracellular CAIX to the Ig1 domain of the MCT1/4 chaperon CD147. Pull-down assays with GST fusion proteins demonstrated that binding is mediated by a hydrogen bond between CD147-Glu73 and CAIX-His200 (the central residue of the enzyme’s intramolecular proton shuttle). This direct interaction between CAIX and the transporter-chaperon complex positions CAIX close enough to the transporter to function as a ‘proton antenna’, which shuttles protons between transporter pore and surrounding protonatable residues, to drive proton-coupled lactate flux. Application of an antibody, directed against the CD147-Ig1 domain, displaces CAIX from the transporter and suppresses CAIX-mediated facilitation of proton-coupled lactate transport as shown by pH measurements in Xenopus oocytes. In cultivated breast cancer cells, this ‘metabolon disruption’ leads to a decrease in lactate transport capacity, reduced glycolytic activity and ultimately reduced cell proliferation. Taken together, the study shows that carbonic anhydrases form transport metabolons with acid/base transporters in human tumor tissue and that these interactions can be exploited to interfere with tumor metabolism and proliferation.

OS 10-06
Micromechanical investigations of myofibrils within human embryonic stem cell-derived cardiomyocytes and from human ventricular heart tissues reveal functional differences
B. Irga1, B. Piep1, J. Meißner1, A. Wenzl1, K. Schwanke1, N. Weber1, M. Wendland1, U. Martin1, R. Zweigerdt1, B. Brenner2, T. Kraft1
1Hannover Medical School, Institute of Molecular and Cell Physiology, Hannover, Germany
2Hannover Medical School, HTGG-Dirurgie / LBED, Hannover, Germany

Question. The aim of the study is to understand whether force parameters of β-myosin heavy chain (βMyHC) isoform-expressing cardiomyocytes of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) resemble those of human ventricular myofibrils (hvMFs) isolated from adult donor hearts.

Methods. We have identified and compared principal sarcomeric protein isoform expression involved in the force modulation by western blot analysis and analyzed the myofibrillar steady-state and kinetic force parameters of the two contractile models using the same micromechanical method and experimental conditions.

Results. Our results indicated that saturating calcium concentration, both hvMFs and myofibrils of hESC-CMs developed force with similar kinetics. This correlated well with expression of the βMyHC isoform in the sarcomeres of both contractile systems. However, maximum isometric force was smaller for myofibrils of hESC-CMs than for hvMFs. Simulating physiological conditions at submaximal calcium concentration levels, we observed functional differences, because myofibrils within hESC-CMs exhibited an increased sensitivity to calcium of kinetic and steady-state force parameters compared to hvMFs. βMyHC-driven cross-bridges cycling was modulated to generate force at higher rate constants and pCaC50 of isometric force was significantly higher in hESC-CMs than with hvMFs. These differences were attributed mainly to an isoform profile of the sarcomeric proteins of hESC-CMs specific to an early development stage of human ventricular cardiomyocytes, i.e., to lack of cardiac troponin-I isoform and to partially expression of non-ventricular isoforms of some other sarcomeric proteins (e.g., myosin light chains, Tit).

Conclusions. Myofibrils of hESC-CMs only partially display the contractile features of adult hvMFs. Our study indicates that morphological and ultrastructural maturation of βMyHC isoform-expressing hESC-CMs is not necessarily accompanied by ventricular-like expression of all sarcomeric proteins determining differences in force development and relaxation. Our data suggest that a series of multiple factors (e.g., at protein isoform, functional and morphological level) might be simultaneously necessary to define the maturation stage of hESC-CMs. Maturating hESC-CMs could provide useful tools to investigate the onset of inherited cardiac diseases affecting contractile function at early developmental stages.

OS 10-07
Activation of endogenous PPI enhances calcium spark activity in wild type cardiomyocytes
D. M. Potenza1, R. Janicek1, M. Fernandez-Tenorio1, H. Valdivia2, E. Niggli1
1University of Bern, Department of Physiology, Bern, Switzerland
2University of Wisconsin, Wisconsin Institutes for Medical Research, Madison, US

Activation of endogenous PP1 enhances calcium spark activity in wild type cardiomyocytes

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OS 11-01
Calcium binding proteins and the regulation of the visual sensory system: from molecules to networks
D. Dell’Orso
University of Verona, Department of Neuroscience, Biomedicine and Movement Sciences, Verona, Italy

Light sensitivity in photoreceptors is finely regulated by Ca²⁺ as the main second messenger. The homeostasis of Ca²⁺ is strictly connected to that of another second messenger, guanosine 3’,5’-cyclic monophosphate (cGMP). The drop of cytoplasmic Ca²⁺-concentration following light absorption is detected by GCAPs, a group of neuronal calcium sensor proteins that control the activity of membrane bound guanylate cyclases by switching conformation in a Ca²⁺-dependent manner. GCAPs contribute to shaping the response of cells to light, allowing the signaling cascade to make gradual responses to small changes in [Ca²⁺].

Single point mutations in GCAP1 are known to cause severe disturbance of their Ca²⁺-sensing properties resulting in the onset of retinal dystrophies. Alterations in protein structure/function relationships can be characterized by biochemical and biophysical approaches, however in order to fully understand the dynamic of the homeostasis of both cGMP and Ca²⁺ in a photoreceptor cell under disease-associated conditions, a more comprehensive kinetic description of the phototransduction cascade is necessary. Such system-level kinetic description of the biochemical cascade based on experimentally determined parameters allows the simulation of the photoreceptor in mouse rods by several illumination stimuli. Recent results on the characterization of native and altered GCAP1 variants will be presented, which range from biophysical analyses focused on the intra-molecular communication pathways connecting individual Ca²⁺ binding sites in the GCAP1 molecule to the putative residues at the interface with the guanylate cyclase to the system-level properties of the rod outer segment, that trigger the electrical response of the cell to light.

A multiscale analysis of the phototransduction cascade.

The phototransduction cascade is studied in a multiscale, bottom-up strategy: individual proteins and their components are analyzed in their biophysical and biochemical details; their interactions build up a protein-protein/protein-ion-nucleotide signaling network that can be numerically simulated. The results of kinetic modeling can eventually be compared with experimental electrophysiological data and predict novel complex behaviors.

OS 11-02
Neuroregeneration and miRNA in a mouse peripheral nerve injury model
T. Kalpachidou, K. K. Kummer, M. Kress
Medical University of Innsbruck, Division of Physiology, Department of Physiology and Biomedical Physics, Innsbruck, Austria

MicroRNAs (miRNAs) have recently emerged as important modulators of multiple cellular pathways, including neuropathy and neuroregeneration. Following a peripheral nerve injury, neurons located in the dorsal root ganglia (DRG) exhibit increased excitability and nociceptive signaling as well as transcriptional alterations that promote axonal regeneration. The complexity of these responses has kept the distinguishing between the signals necessary for regeneration from the ones involved in the establishment and maintenance of neurodegeneration and neuropathy largely obscure. In a combined miRNA-miRNA sequencing approach, we identified a novel miRNA, which was highly upregulated in the DRG of mice subjected to the spared nerve injury model (SNI). The expression of this miRNA was negatively correlated with the downregulation of 164 miRNAs in the same tissue samples. RT-qPCR and in situ hybridization validation additionally revealed that the expression of this miRNA was predominantly neuronal-specific. Intrathecal injection of a miRNA inhibitor did not alleviate the SNI-induced mechanical and cold hypersensitivity, in vivo. Adenoviral miRNA upregulation, however, enhanced neuronal outgrowth in primary DRG neuron cultures. We propose that this novel miRNA is a potential master regulator of miRNA transcripts that are implicated in pathways inhibiting neuronal regeneration.

OS 11-03
Epileptic seizures in a mouse model of episodic ataxia type 6 are caused by changes in Cl– homeostasis in radial glia-like cells
Y. Kolobkova1, M. Engels1, J. Cremer1, S. Buller2, C. Fahlke1, P. Kovermann1
1Forschungszentrum Jülich, Jülich, Germany
2Forschungszentrum Jülich, Structural and functional organisation of the brain (INM-1), Jülich, Germany

Mutations in SLC1A3 – the gene encoding the glial excitatory amino acid transporter 1 (EAAT1/GLAST) – have been strictly connected to that of another second messenger, guanosine 3´,5´-cyclic monophosphate (cGMP). The drop of cytoplasmic Ca²⁺-concentration following light absorption is detected by GCAPs, a group of neuronal calcium sensor proteins that control the activity of membrane bound guanylate cyclases by switching conformation in a Ca²⁺-dependent manner. Single point mutations in GCAP1 are known to cause severe disturbance of their Ca²⁺-sensing properties resulting in the onset of retinal dystrophies. Alterations in protein structure/function relationships can be characterized by biochemical and biophysical approaches, however in order to fully understand the dynamic of the homeostasis of both cGMP and Ca²⁺ in a photoreceptor cell under disease-associated conditions, a more comprehensive kinetic description of the phototransduction cascade is necessary. Such system-level kinetic description of the biochemical cascade based on experimentally determined parameters allows the simulation of the photoreceptor in mouse rods by several illumination stimuli. Recent results on the characterization of native and altered GCAP1 variants will be presented, which range from biophysical analyses focused on the intra-molecular communication pathways connecting individual Ca²⁺ binding sites in the GCAP1 molecule to the putative residues at the interface with the guanylate cyclase to the system-level properties of the rod outer segment, that trigger the electrical response of the cell to light.

Here we show that GLAST is co-expressed with the GABA transporter GAT–3 in mouse RGL cells. A knock-in mouse model carrying the P290R (Slc1a3P290R/Slc1a3P290R) exhibit elevated hippocampal GLAST and GAT–3 levels, suggesting an increased Cl– efflux and GABA uptake. Whole-cell patch clamp recordings in acute hippocampal slices showed decreased inhibitory tonic GABA-mediated currents in granule cells (GCs) of Slc1a3P290R/+ mice, while amplitudes and frequencies of miniature inhibitory post synaptic currents were unchanged. Fluorescence lifetime imaging microscopy with the Ca²⁺ sensitive dye MOQAE indicated lower (Ca²⁺)+ RGL cells of Slc1a3P290R/+ mice. Patch clamp recordings showed an increase of spontaneous AMPA receptor (AMPAR)-mediated miniature post synaptic currents in hippocampal neurons of epileptic Slc1a3P290R/ mice, but supersaturating application of AMPA did not result in bigger currents, suggesting that the density of synaptic AMPARs is increased. Quantitative AMPAR autoradiography revealed that the density of AMPARs is increased by 31%, and we found that expression of Glur2 subunit, forming Ca²⁺ impermeable AMPARs, but not Glur1, was significantly increased in mutant mice. Overexpression of Ca²⁺ impermeable AMPARs together with observed lower expression of VGLUT1 in mossy fiber terminals of GCs and shrinkage of the dendritic spines may reduce hyperexcitability of GCs and represent endogenous antiepileptic mechanisms evoked in hippocampi from Slc1a3P290R/ mice. Our results suggest that reduced inhibitory neurotransmission is an initial process resulting in hippocampal hyperexcitability of EA6.

OS 11-05

Brain pericyte [Ca²⁺] and contractility

T. Pfeiffer, L. Khennouf, C. Hirunpattarasilp, R. Chen, D. Atwell
University College London, Neuroscience, Physiology and Pharmacology, London, UK

Pericytes, spatially-isolated cells located at intervals along capillaries, have been suggested to contribute to brain blood flow regulation in health and disease. In response to neuronal activity, pericytes dilate capillaries to increase local blood flow and energy supply, while in ischemic pathology pericytes constrict capillaries and reduce local glucose and oxygen supply, and are thus involved in disease progression. However, despite their importance, there is controversy over whether all pericytes are contractile.

We studied how pericycle [Ca²⁺] and contractility change in response to vasoactive substances or neuronal activity. We created a tamoxifen-inducible transgenic mouse line expressing GCaMP5G under the NG2 (Cspg4) promoter, and performed two-photon imaging in acutely prepared brain slices and in the anesthetized mouse. In brain slices, we found that the vasoresistance endothelin-1, which is released in ischemia and Alzheimer’s disease, led to an ~125% increase in pericyte [Ca²⁺] and capillary constriction at pericycle locations on 1st to 3rd branch order capillaries. The analogue of thromboxane A2, U46619, also raised pericyte [Ca²⁺] by ~50%. We determined that approximately 2/3 of pericytes could be labeled with a Nissl dye, Neurotrace, and of these ~80% constricted capillaries in response to endothelin-1 and ~40% constricted in response to U46619. Moreover, during functional hyperemia evoked by somatosensory stimulation in the anesthetized mouse, a decrease in pericycle [Ca²⁺] coincided with neuronal activity-driven capillary dilation.

In summary, the changes in pericycle [Ca²⁺] and in capillary diameter suggest that across the capillary bed many pericytes are contractile, and that includes Neurotrace-labelled pericytes.

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OS 11-06

Psoralens activate and photosensitize Transient Receptor Potential channels Ankyrin type 1 (TRPA1) and Vanilloid type 1 (TRPV1)

A. Babes, T. Kichko, T. Selescu, A. Manolache, P. W. Reeh
University of Bucharest, Department of Anatomy, Physiology and Biophysics/Faculty of Biology, Bucharest, Romania; Friedrich-Alexander University of Erlangen-Nuremberg, Institute of Physiology and Pathophysiology, Erlangen, Germany

Psoralens are natural compounds used in combination with UVA light (PUVA therapy) to treat a variety of skin conditions, including psoriasis and vitiligo. While effective in alleviating symptoms, PUVA often triggers adverse effects which include erythema, pain and itch. Our aim was to investigate the role of pain-signaling TRPA1 and TRPV1 channels in mediating these PUVA side effects.

Methods

We used calcium microfluorimetry and patch clamp, as well as detection of neuropeptide release from mouse trachea to demonstrate that the clinically used 8-methoxypsoralen (8-MOP) and, to a lesser extent, 5-methoxypsoralen (5-MOP) activate and photosensitize recombinant and naturally expressed TRPA1 and TRPV1.

Results

8-MOP and 5-MOP triggered calcium entry in HEK293 cells expressing recombinant TRPA1 and TRPV1. These responses were abolished by the TRPA1 and TRPV1 antagonists HC203031 and, respectively, BCTC. At submicromolar concentrations 8-MOP markedly increased the activation of TRPV1 by UV light (330 nm). 8-MOP also enhanced UVA-induced reactive oxygen species (ROS) production. Calcium transients evoked by 8-MOP in TRPA1-expressing HEK293 cells were completely inhibited in the presence of the anti-oxidant N-acetyl cysteine and substitution of three key N-terminus cysteines in positions 621, 641 and 665 by serines also largely eliminated the sensitivity to 8-MOP. Outwardly rectifying membrane currents with a reversal potential close to 0 mV were also evoked by 8-MOP in both TRPA1- and TRPV1-expressing HEK293 cells and were abolished by the respective TRP channel antagonists. 8-MOP alone and in combination with UVA light evoked robust calcium transients in a subpopulation of cultured mouse dorsal root ganglion (DRG) neurons. The response to 8-MOP was strongly decreased in neurons from mice lacking TRPV1, but not TRPA1, while the calcium transients induced by 8-MOP in the presence of UVA light were clearly diminished, but not completely abolished, in neurons from both genotypes (TRPA1+/- and TRPV1+/-). Finally, 8-MOP alone (in the absence of additional illumination) induced calcium gene-related peptide (CGRP) release from mouse trachea, which was strongly reduced in TRPV1-null mutant mice and to a lesser extent in TRPA1-lacking animals.

Conclusions

Taken together our results demonstrate that two TRP channels in the pain pathway are strongly activated by clinically used psoralens, which may explain some of the adverse effects of PUVA therapy.

OS 11-07

TRPM3 mediated mitochondrial dynamic activity contributes to the development of low back pain

Department of Neurophysiology, CBNM, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Previous studies in a model of non-specific low back pain (LBP) have shown that there’re neuromodulatory changes in the spinal cord that lead to a latent sensitization of dorsal horn neurons that may become manifest upon a second challenge. This latent sensitization seems to be a first step in the transition from acute to chronic LBP. This transition comes along with activation of calcium influx and long-term functional changes of ion channels in neurons. Transient receptor potential melastatin-3 (TRPM3), a channel with high permeability for Ca²⁺ involved in noxious heat sensation, has been proposed to play a role in neuropathic pain and therefore may participate in the development of LBP. The mechanism underlying is nebulous yet. Since mitochondria, as potent Ca²⁺ buffer pools and downstream regulatory targets of synaptic calcium signaling, show dynamic activity in dendritic spines displaying spatiotemporal specific correlation with structural and functional changes of dendritic spines, they might play a role in neural plasticity in LBP. However, whether mitochondria play a role of downstream modulators of TRPM3, which, in turn, contributes to the development of LBP, stays obscure. The study aims to investigate the connection between TRPM3 and mitochondrial dynamic activity during the development of LBP.

Pregnenolone Sulfate (PS), a selective TRPM3 agonist, was used on TRPM3-expressing HEK293 cells. Calcium imaging was performed to detect free Ca²⁺. Fura-2 and Rhod2 were used to target Ca²⁺ in cytosol and mitochondria. PS induced a rapid and reversible increase in Fura-2 and Rhod2 fluorescence, indicating that TRPM3 activation evoked calcium transients in cytosol and mitochondria. Highest values were at 5.4±0.2 nm and 5.8±0.2 nm in the cytosol and mitochondria. Rise time (first visible effect to maximum effect) in mitochondria was significantly longer than in cytosol (1.4±0.3 min vs 1.6±0.2 min, P<0.01), as well as decay time (maximum effect to recovery to 10% of the amplitude) (7.1±3.7 min vs 2.3±1.5 min, P<0.01). The duration of transients (the sum of rising and decay time) in mitochondria was significantly longer than in cytosol.
Oral Sessions

(8.5±3.6min VS 3.3±1.4min, P<0.01). Mitochondria were clustered in different shapes in a certain area of HEK cell without exogenous stimuli. These results suggest that calcium influx through TRPM3 is at least partly buffered by mitochondria and will likely lead to downstream changes in mitochondrial functions.

OS 11-08

Epigenetic regulation of CACNA1B gene splicing in nociceptors controls calcium channel function in normal and neuropathic pain

E. J. Lopez Soto, D. Lipscombe
Brown University, Neuroscience, Providence, US

Voltage-gated Ca\textsubscript{2.2} calcium channels control transmission of noxious stimuli at nociceptor terminals in dorsal horn spinal cord. Ca\textsubscript{2.2} channels are the targets of many drugs and neurotransmitters that activate G-protein coupled receptors to down regulate nociception. Nociceptors express a unique form of the Ca\textsubscript{2.2} channel, through cell-specific pre-mRNA splicing, which influences the sensitivity of Ca\textsubscript{2.2} channels to inhibition by µ-opioid receptors. Here, we elucidate the mechanism of cell-specific selection of Cacna1b (Ca\textsubscript{2.2} gene) exon 37a during alternative pre-mRNA splicing. The DNA binding protein CTCF is a master regulator of gene expression. A few reports, in non-neuronal cell lines, suggest that CTCF may also promote exon recognition of weak splice junctions. Based on ChIP-seq data, we identify that CTCF binds Cacna1b exon 37a in several human and mouse cell lines. By electrophoretic mobility shift assay, we confirm that recombinant CTCF binds a 60 bp region in exon 37a, but not the neighboring homologous exon 37b. We have applied several methods, in vitro and in vivo using cell lines and neurons from mouse dorsal root ganglia (DRG), to show that CTCF promotes Cacna1b exon 37a inclusion in Trpv1-lineage neurons during pre-mRNA splicing. Using the DRG-derived F11 cell line as a model we show: 1) by qPCR, that CTCF binds in exon 37a of Cacna1b vivo, but not in exon 37b; 2) CTCF overexpression increases, and CTCF siRNA knockdown decreases exon 37a inclusion; 3) pharmacological inhibition of gDNA methylation increase exon 37a expression, increase CTCF binding to locus 37a, and decrease 5-mC in locus 37a; 4) siRNA knockdown of DNA methyltransferase DNMT3a, but not DNMT1 or DNMT3b, promotes exon 37a inclusion; 5) active DNA demethylating enzymes TET1 and TET2, but not TET3, when overexpressed, increase exon 37a inclusion. In vivo, we show that DRG Trpv1-lineage neurons, but not Trpv1-negative neurons, express exon 37a, have less 5-mC in locus 37a compared to DRG neurons that do not express Trpv1. Additionally, we find that in a peripheral nerve injury model, known to alter global methylation, exon 37a inclusion is reduced. Collectively, our results show that cell specific epigenetic factors change alternative splicing exon selection in Cacna1b gene, thereby regulating transmission of nociceptive information in the primary afferent pain pathway in normal and neuropathic pain.
Conclusions: AI- and algorithm-based methods are well suited for unbiased and fast analysis of cell numbers as well as signal intensities in subcellular compartments. Reliability depends on proper algorithm training, as well as on antibody specificity.

A 01-3

Universal amplifier with automatic compensation of series resistance and capacitance in whole cell recordings using an active-bridge circuit and phase-sensitive technique

C. Jha1,2, D. Gobbo1, N. Zhao1, J. Planck2, H. R. Polder1, F. Kirchhoff1
1Saarland University, Center for Integrative Physiology and Molecular Medicine (CIPMM), Homburg, Germany
2Ruhr-University Bochum, Department of Neurology, Germany

In whole-cell recordings, the series resistance (Rs) formed by the recording electrode, the reference electrode and cellular components introduces a systematic error. The applied stimulus and recorded signals are attenuated without Rs compensation both in current clamp (CC) and voltage clamp (VC) modes. Most of today’s amplifiers use the VC mode to compensate this error based only on two assumptions: 1) the cell can be represented by a single-compartment, and 2) the input resistance (Rin) is much larger than Rs (Rin/Rs > 100). Both of these assumptions are far from ideal in most of the whole cell recordings, as majority of cells are better represented by multi-compartment models and the maximum Rin is normally less than 500 MOhm while minimum Rs is more than 5 MOhm which gives us a maximum Rin/Rs far below 100.

Here we present an automatic, computer controlled Rs compensation method done in CC mode using an active-bridge circuit (rpi ELC-03X5 amplifier with digital control) and phase-sensitive technique (Sutor et al, Pflüg Arch (2016) 468:1725) to achieve fast and reliable Rs compensation based on real-time values independent of the cell parameters. A voltage recording is made in CC mode by applying a 30-50 ms 3-9 kHz sine wave voltage to the electrode. The recorded data are displayed in a 2D diagram (current vs voltage) creating a Lissajous plot, i.e. a tilted ellipse with the opening and tilt angle corresponding to the electrode’s capacitance and resistance, respectively. An algorithm determines the necessary adjustment in capacity compensation and bridge balance of the amplifier to minimize the opening and tilt angle. This process is repeated until a predefined accuracy is achieved. This entire process takes about 1 s and the obtained Rs value also provides the basis for the Rs compensation in VC mode.

We tested the amplifier by recordings of electronic cell models as well as of neurons and glial cells in acute brain slice preparations. An error of only 1-3% in the determination of Rs was observed using cell models. The recordings obtained in neurons and glial cells show that Rs can be compensated up to 90% during VC recordings. The amplifier is designed as a universal tool for electrophysiologists offering a variety of recording techniques. The method we present here is accurate, easy to use and especially useful for recordings from glial cells with low membrane resistance. With support by the European Commission, EC-H2020 MSCA-ITN EU-GliaPhD (No. 722053).

A 01-2

Use of Deep Learning Image Analysis For Automated Cell Count and Fluorescence Signal Quantification to Study Cell Loss and Compare Protein Levels in Subcellular Compartmentalizations

K. B. Patil1, S. Roy1, E. G. Wiemer1, S. A. Müller1, A. Giebova1, H. Hoffmann1, J. Benkert1, N. Wiederspohn1, R. Parlatò1, B. Liss1
1Universität Ulm, Institut für Angewandte Physiologie, Ulm, Germany

Aim: Image analysis for qualitative and quantitative purposes represents an important tool in biomedical research. Manual analysis is time-consuming and prone to bias. Artificial Intelligence (AI) is an emerging approach for unbiased and faster analysis. Here, we optimized and compared manual and automated analysis of immunostained mouse brain sections, to quantify (i) cell numbers in a defined area, and (ii) signal intensities in subcellular compartments. We aim to use AI to study mechanisms of preferential loss of Substantia nigra (SN) dopaminergic (DA) neurons in Parkinson’s disease (PD).

Methods / Results: We used tyrosine-hydroxylase (TH) immunostaining as marker for DA neuron cytoplasm, the ion channel Kv4.3 as marker for the plasma membrane, the neuronal Ca2+ sensor NCS-1 as marker for the cytoplasm, and DAPI (4′,6-Diamidino-2-phenylindole) staining to define the nucleus. For AI-based image analysis we used (i) Affirma Technologies and (ii) Wolution platforms. Both use customized algorithms and supervised training for optimisation of cell recognition, or discrimination of subcellular compartments.

(i) For quantification of neuronal numbers within the SN, fixed mouse brain sections were TH-stained with 3,3-Diaminobenzidine. We compared stereological estimates (Microbrightfield) and automated neuron counts. For automated cell counts, images were acquired using a Penrnnonic 250 Flash III whole-slide scanner (3DHitech, Symmes). Digital slides were uploaded on an Affirma Cloud for supervised training of convolutional neural network algorithms by the Affirma Deep Learning platform to detect TH positive neurons. We analyzed the mean correlations between TH-positive SN neurons estimated by stereology and by the Affirma algorithm.

(ii) For detection of subcellular compartments and relative quantification of Kv4.3, NCS-1, TH immunofluorescence (IF) and DAPI signals, we used the Wolution deep learning-based image analysis platform. Confocal images at 63x magnification were used to train the algorithm to define and quantify mean intensities of IF signals in plasma membranes, cytoplasm, and nucleus of TH-positive neurons. We compared automated with experimenter-based marking and signal quantification.

A 01-1

Large, invariable action potential amplitude in neocortical neuron terminals revealed by high-resolution current-clamp recordings

A. Ritzau-Jost1, T. Tsiatsias2, M. Krueger3, I. Bechmann1, J. Eilers1, B. Barbour4, S. M. Smith5, H. R. Polder1
1University of Leipzig, Institute of Anatomy, Faculty of Medicine, Leipzig, Germany
2Oregon Health & Science University, Department of Medicine, Division of Pulmonary & Critical Care Medicine, Portland, US
3VA Portland Health Care System, Section of Pulmonary & Critical Care Medicine, Portland, US
4University of Leipzig, Center for Integrative Physiology and Molecular Medicine (CIPMM), Homburg, Germany
5INSERM, Paris, France

The postsynaptic action potential critically controls synaptic transmission. At experimentally-accessible large nerve terminals, sodium and potassium currents control the amplitude and duration of action potentials, respectively. Recently, potassium channels were found to dynamically regulate action potential amplitude in small nerve terminals and this effect was proposed as a novel mechanism for synaptic plasticity. To investigate this apparent difference between large and small terminals, we established high-resolution current-clamp recordings from small nerve terminals of cultured neocortical neurons. Using an electrical equivalent circuit and quartz glass pipettes, we systematically investigated errors related to pipette capacitance and the performance of current-clamp amplifiers. We found rapid action potentials with an amplitude of ~120 mV. Blocking potassium channels prolonged action potentials but did not affect their amplitude. Furthermore, the spike amplitude was unaffected during short-term and homeostatic plasticity. Thus, our data indicate large and stable action potential amplitude at small neocortical nerve terminals.
The spatial extent of optogenetic silencing in transgenic mice expressing channelrhodopsin in inhibitory interneurons

G. V. Bab, B. P. Rummel, T. Sigurdsson
Goethe University, Institute of Neurophysiology, Frankfurt am Main, Germany

Optogenetic stimulation of inhibitory interneurons has become a commonly used strategy for silencing neuronal activity. This is typically achieved using transgenic mice expressing excitable opsins in inhibitory interneurons throughout the brain. Yet it is unclear how spatially extensive the resulting inhibition is and to what extent it affects brain areas beyond the region of interest. To address this, we examined neuronal silencing in VGAT-ChR2 mice, which express channelrhodopsin-2 in GABAergic neurons, by delivering blue light at different intensities (0.5–16 mW) to several cortical and subcortical brain regions (posterior parietal cortex, somatosensory cortex, hippocampus and striatum) in awake mice and recorded neuronal activity simultaneously in the respective target structure as well as in underlying brain regions. Then we compared this approach to direct optogenetic silencing of pyramidal cells in mice expressing the inhibitory opsin ArchT in the hippocampus. We show that light stimulation in VGAT-ChR2 mice causes neuronal inhibition not only in targeted brain areas but also in subjacent regions, even at relatively low light intensities, revealing a more spatially extensive inhibitory effect than previously estimated. In contrast, virus-mediated expression of an inhibitory opsin enables robust silencing that is restricted to the region of opsin expression. Our results reveal important constraints on using inhibitory interneuron activation to silence neuronal activity and emphasize the necessity of carefully controlling light stimulation parameters when using this silencing strategy. In ongoing experiments, we are examining the contribution of long-range inhibitory projections to neuronal silencing by expressing channelrhodopsin-2 selectively in somatosensory cortical interneurons of VGAT-Cre mice.

Determination of intracellular chloride concentration in glial cells types using fluorescence lifetime imaging microscopy (FLIM)

M. Engels, V. Unützer, T. Gensch, P. Kovermann, C. Fahlke
1Research Center Jülich, Institute of Complex Systems, Jülich, Germany
2University of Copenhagen, Center for Translational Neumedicine, Copenhagen, Denmark

Chloride is the most abundant biological anion, and chloride gradients play crucial roles in diverse physiological processes in the CNS such as volume regulation, synaptic transmission and neurotransmitter uptake. In the past, a plethora of chloride transporters and channels have been identified that contribute to the regulation of intracellular [Cl−], however, the underlying mechanisms and the physiological roles of intracellular chloride homeostasis remain insufficiently understood.

We studied [Cl−]i in various glial cell types in adult mouse brain tissue slices using fluorescence lifetime imaging microscopy (FLIM) with the chloride-selective dye MOIA (Gensch et al. 2015 Springer Series in Chemical Physics 111, 189-211). Since MOIA is a membrane permeant fluorophore that is collisionally quenched by Cl−, fluorescence lifetime reciprocal changes linearly with [Cl−]. After estimating cell type-specific chloride sensitivity of MOIA, we observed significant variation in steady-state [Cl−]i of glial cells from different brain regions.

In hippocampal astrocytes [Cl−]i differ between the Comu Ammon’s CA1 (10.7 ± 4.4 mM (SD, 53 cells, 18 slices, 8 mice)) and the Dentate Gyrus (DG) (20.0 ± 4.0 mM (SD, 222 cells, 42 slices, 16 mice)). The [Cl−]i of astrocytes in the somatosensory cortex is distinctively lower (15.2 ± 3.2 mM (SD, 287 cells, 17 slices, 5 mice)). These values are considerably lower than published results on radial Bergmann glia (35.3± 0.3 mM (SD, 1355 cells, 84 slices, 33 mice, Unützer et al. 2017 Glia 65, 2)). We studied radial glial progenitor cells in the hippocampus, but obtained again rather low [Cl−]i of 21.1 ± 3.1 mM (SD, 359 cells, 14 slices, 3 mice)). Thus far, our experiments are restricted to [Cl−]i in the glial soma. Currently, we are continuing efforts to study the [Cl−]i also in cell extensions via intravascular dialysis of glial cells by membrane impermeable chloride indicators. Our results indicate a significant heterogeneity in chloride homeostasis between glial cell types and brain regions. They further support the notion that [Cl−]i are precisely regulated and fulfill specific cellular functions. In glial cells, [Cl−]i is an important determinant of chloride efflux during regulatory volume decrease and of glial GABA uptake. GAT-1, the main glial GABA transporter, uses Na+ and Cl− gradients for the GABA uptake so that resting GABA concentrations will change with [Cl−].
non-suitability to work with implanted and tethered animals. We here introduce a custom-designed operant-box system that realizes a 5-choice layout and improves on the disadvantages of current commercial systems, including cutting purchasing costs by ca. 80-85 %. The system is built around the MicroPython pyboard, an ARM cortex microcontroller that can be programmed in the high-level programming language Python 3. The system is controlled using pyControl (https://pycontrol.readthedocs.io), a state machine framework which provides a clean and intuitive syntax for programming behavioral tasks. The pyControl-board and its accessories are commercially available through the Open-Ephys platform (http://www.open-ephys.org/store). For our specific operant box, we developed several peripheral circuit boards, including one with a set of five infra-red break-beams around LEDs for the 5-choice wall, a stepper-motor control board to control a pump for reward delivery and a pass-through adaptor to breakout lines for other peripherals. Additionally we designed an operant box fitting to those peripheral circuit boards and extremely very low-cost (20-30 EUR) 3D-printed peristaltic pump. The trapezoidal box allows the training and testing of implanted and tethered mice due to the shallow and protruding design of the poke-holes and other features. We verified the possibility of testing tethered animals with optogenetic fibres, headstages, and miniscopes. We furthermore developed a graphical user-interface (GUI) to run various tasks files. We demonstrate the suitability of the boxes to efficiently train and test mice in various tasks, including pharmacological validation of cognitive-enhancement effects.

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**A 01-10**

**Dopaminergic midbrain neurons display complex glucose sensing properties - a multi-electrode array brain slice study**

N. Mannal, C. Poetschke, B. Liss

Ulm University, Institute of Applied Physiology, Ulm, Germany

Cells that respond to changes in extracellular glucose levels with altered electrical activity (glucose sensing, GS) are not only present in the pancreas, but also in the brain. GS neurons act as metabolic sensors that can adapt neuronal activity to brain glucose levels and metabolic demands. They are subdivided into glucose excited (GE) and glucose inhibited (GI) neurons (Alvarsson and Stanley, 2018; Fioramonti et al., 2017; Routh et al., 2014). GS neurons were originally described within the hypothalamus, but they have also been found in brain nuclei which are part of the dopamine-dependent reward system (Koekkoek et al., 2017). Dopaminergic (DA) midbrain neurons, especially in the Substantia nigra (SN), are particularly vulnerable to metabolic stress, but glucose sensing properties have not yet been described. Here, we systematically addressed potential glucose sensitivity of DA neurons by extracellular multi-electrode array (MEA) recordings in vitro in brain slices from juvenile and adult mice by using optimised spike-sorting techniques. We compared spontaneous activity pattern (pacemaker frequency & precision) of DA neurons, as well as autoinhibitory responses to dopamine (100 µM), at 33 °C and at different extracellular glucose concentrations (from 1-25 mM). With this approach, we detected glucose sensing in about 65% of SN DA neurons, with the neurons displaying increased activities with elevated glucose levels (GE neurons). Dose-response curves were better fitted when separated into two populations (EC50 = 0.34±0.002, n = 8; and 2.09 ± 0.015, n = 6) instead of fitting one group (EC50 = 0.83 ± 0.01, n = 14). We also detected an age-dependent glucose sensitivity of dopamine-autoinhibition of SN DA neuron activity by comparing dopamine-responses in low and high glucose. SN DA neurons display either sensitised or desensitised dopamine autoinhibition responses. While the kinetics of dopamine autoinhibition were similar in high and low glucose, in 25 mM we identified about 20% more SN DA neurons with desensitised responses (n = 38 from 57 neurons, 67%, 2.5 mM: n = 20 from 45, 44.5%; p= 0.028). We currently address the underlying mechanism of glucose sensing in SN DA neurons. We could already exclude significant roles of K-ATP (ATP-sensitive potassium channels) as well as L-type Ca2+ channels. We currently probe for possible roles of other ion channels, receptors and transporters.

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**A 02 | Cellular Neuroscience**

**A 02-1**

**Analyzing synaptic plasticity of glutamatergic inputs onto fast-spiking GABAergic interneurons in the motor cortex of mice.**

V. Antemann, J. R. P. Geiger

Charite – Universitätspmedizin Berlin, Institute for Neurophysiology, Berlin, Germany

Cortical oscillations are linked to cognitive and motor functions, such as working memory and movement preparation. The activity of fast-spiking inhibitory parvalbumin-positive interneurons (PVIs) has been shown to contribute to rapid synchronization of oscillations. Impairments in e.g. working memory have been associated with the reduction of the excitatory drive onto PVIs. Here, we studied whether long-lasting regulation of synaptic strength onto PVIs can be induced and pharmacologically modulated in the motor cortex (M1) of mice. We used acute brain slices of transgenic mice with genetically labelled PVIs with or without genetically deleted metabotropic glutamate receptor 5 (mGluRS) subunit. We evaluated changes in strength of excitatory synaptic transmission onto interneurons in layer II/III of the M1 using patch-clamp recordings and extracellular axon stimulation (gamma-frequency induction protocol, 30 Hz). Additionally, we used pharmacological interventions, such as the selective GABAc receptor antagonist CGP 55845 (3 mM; present in all experiments), the selective-NMDA receptor antagonist D-AP5 (50 µM), an highly selective non-competitive antagonist of the mGluR5 receptor subtype (MPEP; 1 and 10 µM) and a non-competitive mGluR1 antagonist (JNJ 16259685, 0.1 and 0.5 µM).

Our data suggest that gamma-frequency induced long-term potentiation (LTP) at glutamatergic synapses onto PVIs in the M1 of mice outlasts at least 30 minutes. The application of D-AP5 has no effect on LTP-promote. Preliminary data collected in the mGluR5 knockout mice indicate a drastic reduction of LTP, but only if the selective non-competitive mGluR5 subunit antagonist MPEP was applied in addition. The application of the non-competitive mGluR1 antagonist JNJ suggests a reduction of LTP in mGluR5-knockout mice. In summary, changes in strength of excitatory synaptic transmission onto fast-spiking GABAergic interneurons in M1 can be induced by a gamma-frequency induction protocol and pharmacologically modified. This form of synaptic plasticity of glutamatergic synapses onto PVIs is dependent on the activation of mGluR5.
Activity in neuronal circuits arises from the interplay of excitatory and inhibitory synaptic signaling, which is delicately balanced to maintain stable network activity1. There is evidence that excitatory and inhibitory synaptic transmission shows diverging short-term plasticity2. However, whether these different factors governing synaptic transmission can cause differential short-term plasticity is not fully understood. Additionally, long-term perturbation of network activity is known to homeostatically affect excitatory transmission3, while the impact on inhibitory transmission remains largely elusive. Here, we explore short-term plasticity by extracellular stimulation of excitatory and inhibitory inputs onto whole-cell patch-clamped neurons in cortical cell culture. We isolate presynaptic short-term plasticity by pharmacologically alleviating postsynaptic contributions. Excitatory and inhibitory synapses demonstrate fundamentally different short-term plasticity, with stronger synaptic depression due to higher vesicular release probability in inhibitory compared with excitatory synapses. Furthermore, 48 hours of activity deprivation by Tetrodotoxin inversely affects synaptic transmission, leading to strengthening of excitatory and weakening of inhibitory transmission mediated by several mechanisms including differential changes in release probability. In contrast to activity deprivation, Forskolin potentiates both excitatory and inhibitory synapses. Thus, our data reveal differential mechanisms of plasticity and biophysical adaptations stabilizing activity in neuronal networks.  

A 02-6

Variability and Plasticity of Axon Initial Segments in Hippocampal Neurons
C. Thome1, J. Dolenc1, B. Ünsal1, L. Hammer1, A. Harten1, N. Lehmann1, D. Heffler1, T. Kelly1, A. Draguhn1, M. Engelhardt1, M. Both1
1Heidelberg University, Institute of Physiology and Pathophysiology, Heidelberg, Germany
2University of Bonn, Department of Epilepsy, Bonn, Germany

The axon initial segment (AIS) is the anatomical compartment where most neurons integrate synaptic inputs into their primary output signal: the action potential. Remodeling of the AIS, as well as mutations and its predominant scaffolding proteins, have been associated with several mental pathologies such as bipolar disorder, epilepsy, intellectual disability, autism spectrum disorder, and stroke. Furthermore, recent studies found physiological and morphological plasticity of the AIS, characterized by changes in length and distance of the AIS to the somatic envelope adapting to response to neuronal activity.

Previous studies focused on neurons that were either cultured in vitro or had defined input channels such as sensory neurons. In contrast, the hippocampus region receives inputs from nearly every brain area and plays an important role in the computation of space, navigation, and storage of memory. Our project investigates activity-dependent plasticity in this region using a combination of in vivo optogenetics, models of epilepsy and hypoxia, as well as single cell electrophysiology. For the latter, we utilize a newly developed transgenic mouse line developed by our collaborators, in which the AIS is intrinsically labeled with the fluorescent protein GFP. This allows live imaging of AIS plasticity after stimulation of individual neurons using the patch-clamp technique.

Under control conditions, AIS length in hippocampal region CA1 is very heterogeneous and varies considerably (between ~15 to ~50 µm). We thus complement our study with a characterization of the natural variability of AIS length and position along the dorsoventral axes of the hippocampus. Using whole-cell patch clamp recordings of individual neurons, we characterize the electrophysiological properties of hippocampal CA1 pyramidal neurons and correlate them to the length and position of their respective AIS.

We hope that by elucidating the variability and plasticity of AIS under physiological and pathological conditions, we might aid the understanding of this important neuronal compartment and neurological disorders associated with its remodeling.

A 02-7

Selecting boosting of excitability by Cav 1.3 channels in lateral substantia nigra dopamine neurons
J. Shin1, L. Kovacheva1, S. Stoianovic1, D. Thomas1, C. Paladini1, J. Stiessing1, G. Geisslinger1,2,3,4,5,6,7
1Geofi University, Institute of Neurophysiology, Neuroscience Center, Frankfurt am Main, Germany
2Geofi University, Pharmazeum Frankfurt, ZAFES, Institute of Clinical Pharmacology, Frankfurt am Main, Germany
3Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Branch for Translational Medicine and Pharmacology TMD, Aachen, Germany
4University of Texas at San Antonio, UTSA Neuroscience Institute, San Antonio, US
5University of Innsbruck, Department of Pharmacology and Toxicology, Center for Molecular Biosciences, Innsbruck, Austria
6University of Cologne, Institute of Neurophysiology, Cologne, Germany
7University of the Saarland, Institute of Physiology, CIPMM, Homburg, Germany

The loss of dopamine (DA) substantia nigra (SN) neurons underlies the motor symptoms of Parkinson disease (PD) with the most profound lesion in the lateral SN (l-SN). Epidemiological studies report a 20-30% lower risk of PD in patients with a history of antihypertensive treatment with brain-permeant dihydropyridines (DHP) that target L-type voltage-gated calcium (Ca2+) channels (LTCCs). It is not known, however, whether the reduced risk of PD is related to the specific mechanism of action of DHPs on neuronal excitability.

To test the hypothesis that DHPs are protective, we injected 3 mg kg-1 slow-release dapoxetine into the lateral ventricle of young adult male mice. We measured cortical spontaneous and slow-wave activity and found that dapoxetine reduced the frequency and duration of the spontaneous activity of DA and ml-SN neurons.

To extend our understanding of the region-specific mechanism of high-frequency discharges, we used the real-time dynamic-clamp approach injecting modeled NMDA-receptor-mediated conductances (gNMDA) in DA SN neurons in acute brain slices. We measured spiking activity and found that dapoxetine reduced the frequency and duration of the spontaneous activity of DA and ml-SN neurons.

We hope that by elucidating the variability and plasticity of AIS under physiological and pathological conditions, we might aid the understanding of this important neuronal compartment and neurological disorders associated with its remodeling.

A 02-8

The neuronal calcium sensor NCS-1 is involved in defining vulnerability of Substantia nigra dopaminergic neurons to degeneration in Parkinson’s disease
C. Simons1, J. Benkert1, D. Beccano-Kelly1, H. Hollmann1, S. A. Müller1, N. Deuter1, R. Wade-Martins1, D. Furr1, T. Schneider1, T. Frank1, R. Parlati1, J. Dudai1, B. Liss1,2,3
1University of Heidelberg, Institute of Applied Physiology, Ulm, Germany
2University of Oxford, Oxford Parkinson’s Disease Centre, Department of Physiology, Anatomy and Genetics, Oxford, UK
3University of the Saarland, Institute of Physiology, CIPMM, Homburg, Germany
4University of Cologne, Institute of Neurophysiology, Cologne, Germany
5University Medicine Göttingen, Department of Neurology, Göttingen, Germany
6University of Oxford, New College, Oxford, UK

The loss of dopaminergic (DA) neurons in the Substantia nigra (SN) is the hallmark of Parkinson’s disease (PD). The cause for PD is still unclear, however activity-related metabolic stress, mitochondrial dysfunction, and altered Ca2+ homeostasis have emerged as crucial. The neuronal Ca2+ sensor NCS-1 is a Ca2+-binding protein that has been linked to a variety of neuronal functions in health and disease. In SN DA neurons, NCS-1 binds to inhibitory dopamine-autoreceptors in a Ca2+-dependent fashion. This interaction promotes dopamine-autoinhibition. Hence, NCS-1 is suggested to protect SN DA neurons from activity-related stress and degeneration, but direct supporting evidence is lacking.

Here, by analyzing SN DA neurons from NCS-1 KO mice in vivo and in vitro, we identified NCS-1 as important regulators of SN DA neuron viability. In a chronic neurotoxin PD model (MPTP/probenecid), toxin-induced loss of SN DA neurons was significantly greater in NCS-1 KO compared to wildtype. Similar results were obtained when analyzing their axonal projections in the dorsal striatum. As no differences were observed for resistant DA neurons in the neighboring ventral tegmental area or their axonal projections, these data uncover a region-selective protective effect of NCS-1 on SN DA neuron viability in this PD model.

To gain molecular insights into effects of general NCS-1 KO in SN DA neurons, we quantified mRNAs of a candidate-genepanel by cDNA-RTPCR. In SN DA neurons from NCS-1 KO, transcripts of mitochondrial-encoded NDI, a subunit of the respiratory chain, and of the neuron-specific enolase ENO2, a glycolytic enzyme, were significantly lower. We also detected lower mRNA-levels of the mitochondrial uncoupling proteins UCPS and UCP5, the PARK7-gene DJ-1, and the Cav2.3 channel. These data indicate a novel function of NCS-1 in regulating gene-transcription or stabilization of mRNAs in SN DA neurons.

At the protein level, we analyzed human DA neurons, derived from iPSCs of PD patients (heterozygous for a GBA mutation). NCS-1 protein was about 40% lower in the diseased neurons, compared to those of healthy controls.

We performed voltage-clamp recordings and found no mediolateral differences in Cav 1.3 channel currents. However, pharmacological experiments revealed that inhibition of Ca2+-activated small conductance potassium (SK) channels enhanced excitability of DA-mSN neurons in contrast to a minor effect on evoked firing in DA-SN neurons.

We report that Cav 1.3 channels boost excitability of DA-SN neurons. Clinically relevant dosages of isradipine dampened excitability of this population with no effect on m-SN neurons, where we report an attenuation of firing by SK channel recruitment.

A 02-9

In vivo Ca2+ signaling of cortical microglia is affected by age and sex and can be modulated by caloric restriction
M. Olmedillas del Moral1, N. Fröhlich1, O. Garaschuk2
1Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tübingen, Germany
2University of Oxford, Department of Physiology, Anatomy and Developmental Biology, Oxford, UK

Brain aging is characterized by a chronic, low-grade neuroinflammatory state, which correlates with deficits in cognition and promotes the development of age-related neurodegenerative diseases. Microglia, the resident macrophages of the central nervous system, are believed to act as key mediators of this process, but the functional phenotypes of aging microglia remain unclear. Specifically, the age-related changes in microglial Ca2+ signaling, known to be linked to its executive functions, are not well understood. In the present study, we characterized in vivo the intracellular Ca2+ signaling and directed protein expression of cortical microglia in young adult (2-3 months old), middle-aged (9-11 months old), and old (18-22 months old) mice by means of two-photon imaging. Our data revealed a nonlinear dependence of the properties of microglial intracellular Ca2+ signals on animal’s age and sex. While in female mice the fraction of cells displaying spontaneous Ca2+ transients progressively increased with age, in males no differences between the age groups were found. At the same time, the frequencies and durations of the spontaneous Ca2+ transients followed a bell-shaped relationship, with the most frequent and longest Ca2+ transients seen in middle-aged mice of both genders. Interestingly, process extension towards an ATP
source was altered in old mice, as processes moved faster but in a more disorganized manner, compared to young adult and middle-aged mice. In an attempt to slow down these aging-related changes, we designed two caloric restriction (CR) regimens, suited for middle-aged and old mice, respectively. Our data revealed that 30% reduction of food consumption for 6 months (between 3rd and 8th month of age) prevented an aging-related increase in Ca^{2+} signaling in middle-aged mice, whereas 12-month-long CR (between 6th and 18th month of age) counteracted the dysregulation of mitochondrial process extension in old mice. Taken together, our results reveal two distinct phenotypes of aging microglia: (i) reactive phenotype displaying large Ca^{2+} transients but normal process extension, ubiquitously present in middle-aged mice and (ii) senescent phenotype, with reduced Ca^{2+} signaling and altered directed process motility, ubiquitously present in old mice. CR counteracted aging-related changes observed in both age groups thus paving the way for the development of new therapeutic strategies for rejuvenation of microglia.

A 02-10
Characterization of the Nucleus Accumbens Activity of MIF Proteins
B. Bulut1, 2, S. Besson-Girard1, 3, F. Usifo1, 2, J. Bernhagen1, 3, 4, O. Gokce1, 2
1Maximilian Full Capacity University, Munich, Ludwig-Maximilians-University (LMU), Institute for Stroke and Dementia Research (ISD), Munich, Germany
2Ludwig-Maximilians-University (LMU), Graduate School of Systemic Neuroscience (GSN), Planegg-Martinsried, Germany
3Münich Cluster for Systems Neurology (SyNergy), Munich, Germany
4Münch Heart Alliance, Munich, Germany

Neurodegenerative diseases such as Alzheimer’s disease (AD), frontotemporal dementias (FTD), or motor neuron diseases (amyotrophic lateral sclerosis, ALS), although disparate in their pathophysiology, are associated with similar pathologic pathways. Common mechanisms leading to neurodegeneration include misfolded proteins, DNA damage combined with excessive neuronal cell death, blood-brain barrier malfunction, and neuroinflammation. Macrophage migration inhibitory factor (MIF), related proteins, and their receptors (termed MIF proteins) have hitherto been established as potent characters in neuro-inflammatory and cardiovascular disease. Interestingly, MIF has been recently identified as a nuclease, which plays critical roles during neuronal degeneration. Moreover, the expression of MIF proteins in our single-cell RNA sequencing (scRNAseq) data suggests other functions of MIF proteins, that expand far beyond classical receptor/ligand inflammatory regulation. Here, we characterized the nucleus accumbens activity of MIF proteins and their possible therapeutic and diagnostic potential for neurodegenerative diseases using biochemistry, molecular biology, and scRNAseq.

A 02-11
Mechanisms of cortical hyperactivity induced by systemic inflammation in vivo
K. Riester, B. Braweck, N. Asaapavanumas, N. Mojaheedi, G. Garaschuk
Eberhard Karls University Tübingen, Institute of Physiology, Department of Neuropsychology, Tübingen, Germany

The immune and the nervous system do not operate autonomously but affect each other in both directions. A systemic inflammation induces a mirror inflammatory response in the brain, characterized by elevated levels of proinflammatory cytokines. Previous in vitro and in situ studies suggest that these factors are able to modulate neuronal activity. However, how the expression of different neuronal genes changes in the course of a peripheral inflammatory response in vivo and the underlying mechanisms remain largely unclear. Furthermore, it is unknown whether processing of sensory information is altered in the course of a peripheral inflammation.

We induced a peripheral inflammatory reaction in adult C57Bl/6 mice by single intraperitoneal injections of lipopolysaccharide (LPS; 1.5 mg/kg). Basal and visually-evoked cortical activity during early and advanced stages of the systemic inflammation were studied in the intact brain by means of longitudinal two-photon Ca^{2+} imaging. Neurons were labeled with the Ca^{2+} indicators OGB1MRIF (motor cortex) or Twitch-2B (visual cortex) by stereotaxic injections of viral vectors. To decipher the role of Interleukin-1β (IL-1β) and Tumor necrosis factor-α (TNF-α), we additionally used two different knockout mouse strains (IL1R3−/− and TNF−/− mice). To study the role of microglia for inflammation-induced changes in neuronal activity, those cells were ablated from the brain using a colony-stimulating factor 1 receptor inhibitor PLX5622 (1,200 mg/kg, 7 days).

Our results show that systemic LPS induced cortical hyperactivity selectively during the early initiation phase of inflammation. The LPS-induced excitatory effect on neuronal activity was detected both in pre- and postsynaptic compartments of the local layer 2/3 cortical circuitry, whereas the activity of cortical interneurons was decreased. LPS-induced hyperactivity was not observed in TNF−/− mice, suggesting that TNF-α plays a major role in this process. Surprisingly, inflammation-induced cortical hyperactivity also occurred in the absence of microglia. Neurons in the primary visual cortex of LPS-injected mice showed transient deficits in tuning properties selectively during the resolution phase of inflammation. This study provides a detailed insight into cortical network dysfunction at different stages of a systemic inflammation, and paves the way for new therapeutic strategies of acute and chronic neuroinflammatory conditions.
A 03-2
Functional properties of microglia in mice during the progression of the parasitic infection African trypanosomiasis
N. L. Uczategiu, S. Gujer, M. Duszenko, O. Garaschku, K. Figarella
Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tuebingen, Germany

Protozoan parasite infections affect millions of people around the world. Despite the severe complications caused by parasite invasion of the central nervous system, little is known about the in vivo dynamics of this process and how microglia, the immune cells of the brain, respond. Trypanosoma brucei (T. brucei), the causal agent of African Trypanosomiasis, invades the brain in the late stage of the disease, causing a severe meningoencephalitis. Here, we aimed to characterize the functional properties of microglia in vivo during experimental infection in C57BL/6-J(CxCR1-EFPG)mice, using two-photon microscopy and post hoc immunohistochemistry. Following infection, waves of parasitemia were found to appear in a cyclical matter starting 5 days post-infection, accompanied by an increase of the body temperature and a transient weight loss, recovering within 10 days. The hemodynamic stage lasted around 10 days, time by which parasites were only found inside blood vessels. Interestingly, already during this period cortical microglia started to show signs of activation like an increase in soma size and an overexpression of the zinced calcium binding adaptor molecule 1. Invasion of the meninges started after day 12. Parasite burden at this location was not synchronized with parasitemia. It showed a pick around day 14, decreased by day 17 and remained at this low level till the end of stage. At the middle of the meningoencephalitic stage (days 16-22) microglial activation became more evident. Consistently, recruitment of peripheral CXCR1-EFGP cells was observed. Areas of microglial hypertrophy were found close to blood vessels in the cortex but also in deeper regions like periventricular and lateral pericryptic areas. In the latter areas, a significant increase in microglial cell density (by 25-40% compared to non-infected animals) was detected. By the end of the stage, microglial proliferation spread out to other areas of the observed, animals started to lose weight and became lethargic. Altogether, we conclude that the peripheral immune response against parasites is enough to activate resident microglia in cortical and periventricular areas. Brain parenchyma remains very well protected from infection because only a few parasites are found in it, even when parasitemia or meningal parasite burden is high. The observed sustained microglial activation and proliferation likely exacerbate disease progression and contributes to neuropathology.

A 03-3
On social physiology: Resident Houses, Part II – Practical Approaches
E. Neu1, M. C. Michailov1, U. Weischer2, I. Ivanovana, R. Neu3, J. Foltinovac, M. Holler3, G. Weber1,4
1Inst. Umweltmedizin c/o I/CSODAS e.V., POB 340316, 80100 Munich, Germany (Int.Council Sci.Develop./Int.Acad.Sci. Berlin-Bratlitaeva-Innsbruck-Muenchen-NewDelhi-Pari-Sofia-Vienna), Munichen, Germany
2Univ. Bratislava, Med. Fac., Bratislava, Slovakia
3Univ. Hamburg, Fac. Economics (Dean), Hamburg, Germany
4Univ. Luzub. and Vienna, Fac. Psychology (Dean), Vienna, Austria

INTRODUCTION. IUM-Project humanization of medicine-resident houses (HMRR), concern 2016-19 interaction between physiological (lessors) & micro-ecological&social factors.

METHOD. Patho-physiological & psychological observations [2b].

RESULTS. Conflicts residents/tenants with lessor (houses-Munich) are evident. Defect-doctors/bradotors, windows (air currents),etc induce respiratory diseases, defect illumination supports (neuro-orthopaedic: commotio cerebi). Conflicts, eg conc high rents, can lead to dangerous health disturbances by pathophysiological and psychopathological reactions: Anxiety, neurosis, insomnia, depression,etc. as well as arrhythmia, hypotonia, apoplexy cerebi,etc. esp in seniors. Information about the situation could be given by medical praxises in Munich: Dr.med. F.Braun-N.Duhn-P.Hanterm-M.Liebua-M.Joseph-M.Jung-M.Guthlin-J.Michel-G.Menzel-L.Oestreicher-Ch.Rief-M.Sebisch-E.Seibert-M.Teutsch-L.Baiel.,etc.

CONCLUSION. Social-physiology could support an integrative socio-psychotherapeutic approach to: oriental somatopsychic therapy (Yujio/KEIM). Inc. Yoga/Qigong/Zen-meditation; and occidental-psychotherapics (Th von UEXKÜLL) combined with pharma-therapy in context of UNO-Agendaz21 for better health, education, ecology on global level. Emperor AKIHITO during Opening Ceremony of ICPM 2005 Kobe appointed to “total symptoms of mind-body, seeking ways of holistic care” [6].

3 [Glascake,0: Schexon Physiol.J 80:no5, 1994, p.139-143 (in Russian), ref. in English.
In two groups of age-matched participants (one group normal hearing, 25 participants; one group with tinnitus (125 to 8000 Hz)) were studied. The right external ear was stimulated by flushing of the left external ear with water at 44 °C for 30 s. In a questionnaire the probands should rate their self-attention to the stimuli. A recording session consisted of a resting period of 5 min (MEG recording with closed eyes), then 8 blocks of vestibular stimulation with somatosensory and auditory stimulation in the intermissions between the blocks, and finally a resting period of 5 min. The dipole strength decreased during vestibular stimulation in both groups. The largest decrease was observed in tinnitus patients when high frequency tone pips were presented. The statistical analysis showed significant effects of the factors “group”, “flush”, and “stimulus”. In the questionnaire the probands rated the stimulation of the medianus nerve as non-exciting and the pip tone presentation as pleasant. In contrast, the tinnitus patients rated the stimulation of the medianus nerve as more exciting and the pip tones as unpleasant. When the caloric stimulation of the external ear was repeated, the numbers of nystagmus decreased with the repetitions in both groups. In healthy controls after the 5th repetition of flushing a constant rate was seen, whereas in tinnitus patients the numbers further declined after a transient increase during the 5th repetition. We were able to confirm that cortical processing of acoustic stimuli is influenced by a simultaneous sensory stimulation. Patients suffering from tinnitus better responded to that modulation which would be an account when proposing novel therapy methods.

A 03-6

After-effects of 6 Hz transcranial alternating current stimulation on verbal working memory

Y. G. Pavlov1, D. Kassanov1, O. I. Dorogina1

1Ural Federal University, Department of Psychology, Yekaterinburg, Russia
2University of Tuebingen, Institute of Medical Psychology, Tuebingen, Germany

Numerous studies have shown strong relationship between frontal midline theta (4-8 Hz) (FMT) activity and working memory (WM) performance. Activation of the cortical generators of FMT plays a key role in manipulation of information in WM. Such studies, however, cannot by definition demonstrate any causal relationship. Therefore, ethically correct intervention studies are necessary, such as non-invasive brain stimulation experiments. Transcranial alternating current stimulation (TACS) is a non-invasive technique capable of inducing long-term changes in oscillatory activity of the brain. The current study had initial goal to check the effect of TACS on accuracy in a set of match-to-sample WM tasks. Two types of tasks were used: with mental manipulations and just retention tasks. 23 (8 females, mean age = 25) healthy adults participated in two testing sessions (without sham and IAMS). The locations of stimulation electrodes were based on the results of a simulation of field distribution performed in SimNIBS. IAMS was applied for 20 min over Fz and CPz at 6 Hz, 1 mA. No after-effects of IAMS were observed in the stimulation sessions as compared to sham. The data suggest that IAMS delivered before the WM task is not able to produce any observable changes in WM performance. Future studies could apply simultaneous stimulation and EEG recording during maintenance of information in WM for better understanding of the theta/C effects.

A 03-7

The effect of sleep on event-related brain potentials in a human fear conditioning paradigm

Y. G. Pavlov1, B. Kotchoubey1

1Ural Federal University, Department of Psychology, Yekaterinburg, Russia
2University of Tuebingen, Institute of Medical Psychology, Tuebingen, Germany

The positive effects of sleep on the consolidation of learning and memory are generally well known. Regarding fear conditioning as a specific form of learning, animal studies suggest a critical role of REM sleep. However, only a few studies in humans have used physiological indices of conditioning, and none used central physiological measures. In our experiment event-related brain potentials (ERPs) were recorded during a fear conditioning procedure presented twice, before sleep or control intervention and after. The procedure involved pairing of a neutral tone (CS+) with a highly unpleasant sound (UCS+). As a control, another neutral tone (CS-) was paired with a neutral (for some subjects slightly pleasant) sound (UCS-). Between two session of conditioning separated by approximately 2.5 hours, on one experimental day, subjects watched a silent movie; on another day (with different tones as CS) they lied in bed and all of them fell asleep. The two days were separated by at least two weeks and balanced in respect to the stimulus mapping. Differential conditioning manifested itself in the ERPs: P3a and N3 with peak latencies about 220 and 420 ms, respectively, were observed. The period of wakefulness resulted in an additional increment of P3a and N3 amplitude differences between CS+ and CS- as compared with the nap. The data indicate that wakefulness rather than sleep affect fear conditioning traces.

A 03-8

Physiological and training characteristics in marathon runners: The role of experience

D. Chlibková1, J. Dostál1, B. Knechtli1, P. T. Nikolaidis4

1Bíno University of Technology, Centre of Sports Activities, Bíno, Czech Republic
2Charles University, Faculty of Medicine, Hradec Králové, Czech Republic
3University of Zürich, Institute of Primary Care, Zürich, Switzerland
4University of West Attica, School of Health and Caring Sciences, Athens, Greece

Aim: The aim of the present study was to examine physiological and training characteristics in marathon runners with different sport experience (defined as the number of finishes in marathon races).

Methods: The anthology and physiological characteristics of men recreational endurance runners with three or less finishes (n=69, 43.5±8.0 years) and four or more finishes (n=66, 45.2±9.4 years) were compared.

Results: More experienced runners had faster personal best marathon time (3:44±0:36 vs. 4:20±0:44 hmin, p=0,001, respectively), lower flexibility (15.9±9.3 vs. 19.1±5.9 cm, p=0,022), abdominal (20.6±7.9 vs. 23.8±9.0 mm, p=0,030) and supralling skinfolds thickness (16.7±5.7 vs. 19.6±7.5 mm, p=0,013), body fat assessed by bioimpedance analysis 13.0±4.4 vs. 14.6±4.7%, p=0,047), more weekly training days (4.6±1.4 vs. 4.1±1.0 days, p=0,038) and longer weekly running distance (58.8±24.0 47.2±16.1 km, p=0,001) than their less experienced counterparts. The number of finishes in marathon races correlated with squat and (r=-0.41, p=0,021) countermovement jump (r=-0.38, p=0,032), and with weekly training days (r=0.19, p=0,030) and running distance (r=0.25, p=0,004).

Conclusion: The findings indicated that long-term marathon training might induce adaptations in endurance performance, body composition and flexibility. An interpretation of the lower score of flexibility in the more experienced group might be its relationship with running economy. The negative relationship of the number of finishes with indices of muscle strength (jump tests) suggested a negative adaptation of muscle strength to endurance training.

A 03-9

On social physiology: Resident Houses, Part I – Theoretical Approaches

M. C. Michaelis1, E. Neu1, U. Weischen2, R. Neu1, V. Follon1, M. Hoffer1, G. Weber4

1Inst. Úniverzitního odboru zvoleneho výzkumu, Prague, Czech Republic
2University of Hamburg, Fac. Economics (Dean), Hamburg, Germany
3Univ. Libau, and Vienna, Fac.Processology (Dean), Vienna, Austria
4University of Tuebingen, Institute of Medical Psychology, Tuebingen, Germany

INTRODUCTION. Physiology is for all life sciences, similar to philosophy (regina scientiarum). Immense medical and ecological problems need renewal of physiology. ANTHROPOLOGY AND PHYSIOLOGY. The primus inter pares of the European philosophers & universalists ARISTOTELES and PLATON – Immanuel KANT considered over 200years ago physiological and anthropological aspects. He was able to formulate the general physiological knowledge as a human science. The aim of the present study was to examine physiological and training characteristics in marathon runners with different sport experience (defined as the number of finishes in marathon races).

RESULTS: More experienced runners had faster personal best marathon time (3:44±0:36 vs. 4:20±0:44 hmin, p=0,001, respectively), lower flexibility (15.9±9.3 vs. 19.1±5.9 cm, p=0,022), abdominal (20.6±7.9 vs. 23.8±9.0 mm, p=0,030) and supralling skinfolds thickness (16.7±5.7 vs. 19.6±7.5 mm, p=0,013), body fat assessed by bioimpedance analysis 13.0±4.4 vs. 14.6±4.7%, p=0,047), more weekly training days (4.6±1.4 vs. 4.1±1.0 days, p=0,038) and longer weekly running distance (58.8±24.0 47.2±16.1 km, p=0,001) than their less experienced counterparts. The number of finishes in marathon races correlated with squat and (r=-0.41, p=0,021) countermovement jump (r=-0.38, p=0,032), and with weekly training days (r=0.19, p=0,030) and running distance (r=0.25, p=0,004).

CONCLUSION. The findings indicated that long-term marathon training might induce adaptations in endurance performance, body composition and flexibility. An interpretation of the lower score of flexibility in the more experienced group might be its relationship with running economy. The negative relationship of the number of finishes with indices of muscle strength (jump tests) suggested a negative adaptation of muscle strength to endurance training.
A 03-10

Electrical direct-current stimulation induces galvanotaxis and alters expression of stretch-activated TRPM7 channels in human osteoblasts

J. Ziebart1, T. Sellmann1, K. Porath1, B. Dalenda1, U. van Rienen1,4, R. Bader1,4, R. Köhling1,4
1Rostock University Medical Center, Biomechanics and Implant Research Lab, Dept. of Orthopedics, Rostock, Germany
2Rostock University Medical Center, Oscar-Langenfend研究所, Institute of Physiology, Rostock, Germany
3University of Rostock, Institute of General Electrical Engineering, Rostock, Germany
4University of Rostock, Interdisciplinary Faculty, Rostock, Germany

Bone fracture healing, particularly in the elderly, remains a challenge. There is an ongoing search for methods to activate osteoblasts, and the application of electrical fields is an attractive approach in this context. Although it is known that such electromagnetic fields lead to osteoblast migration and foster mesenchymal osteogenic differentiation, so far the mechanisms of osteoblast activation remain unclear. Possible candidate mechanisms could rely on increased Ca2+-influx via ion channels. As this is known to activate osteoblasts, e.g., via voltage-sensitive or stretch-sensitive, transient-receptor-potential (TRP) channels. In this study, we explored whether electrical fields are able to modulate the expression of voltage-sensitive calcium channels as well as TRP channels in primary human osteoblast cell lines in vitro. We found that migration speed is significantly increased in stimulated osteoblasts (6.4 ± 2.1 μm/s stimulated, 3.6 ± 1.1 μm/s control) and directed towards the anode. Within a range of 154-445 V/m, field strength did not correlate with migration velocity. Regarding the expression of calcium channels Cav2.2 and Cav1.4, no correlation between electric field and calcium channel expression could be determined. However there was a significant positive correlation between expression of TRPM7 and electric field strength. Electrical stimulation may thus induce TRPM7 expression changes which correlate with cell migration.

A 04-2

Glucocorticoid signaling and modulation of autophagy is involved in the preservation of the cardiac myocyte t-tubular system

D. Flegel1, M. Abu-Khousa1, T. Seidel1, T. Volk1
Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Institute of Cellular and Molecular Physiology, Erlangen, Germany

Question

The failing heart undergoes substantial structural, metabolic and electrical remodeling. Particularly, alterations in the t-tubular system, a densely organized membrane structure conferring normal excitation-contraction coupling in healthy cardiomyocytes (CMs), contribute to impaired contractility in HF. Moreover, the balance between anabolic and catabolic signaling pathways of CMs is distorted in diseased myocardium. To date, cellular pathways regulating t-system remodeling remain largely unknown. We recently showed in isolated ventricular CMs, an established in-vitro model of t-system loss, that glucocorticoid signaling preserves t-system structure and enhances autophagy. Here, we describe effects of negative and positive regulators of autophagy on the t-system.

Methods and Results

We isolated and cultivated adult rat CMs for up to three days in serum-free M199 medium. To assess the t-system, we performed live-cell membrane staining with subsequent 3D confocal microscopy. As shown previously, glucocorticoid (GC) converted into heat by oxidizing large amounts of fat and carbohydrate at the expense of ATP synthesis mediated by the uncoupling protein 1 (UCP1). We have identified a soluble form of adenylyl cyclase, ADCY 10, as an important factor regulating BAT function via its targeted sensory protein ADCY 10. Thereby, opening up a potential therapeutic target against various metabolic diseases state like obesity or type 2-diabetes as BAT activity is triggered via digestion of excess of glucose and fatty acid.

A 04-3

Soluble adenylyl cyclase (ADCY10) regulates brown adipose tissue functioning

A. Das1, V. I. adenakuz1, R. G. Martin1, F. Haertel1, Y. Ladi0v1, T. Chavaki2, T. Noll1
1Technische universität dresden, Institut für Physiologie, Dresden, Germany
2Technische universität dresden, Institut für Klinische Chemistry and Laboratory Medicine, Dresden, Germany
3Berlin Partner Site, German Center for Cardiovascular Research, Berlin, Germany

Brown adipose tissue (BAT) increases its activity, even in adults, in response to cold temperature or food intake via adrenergic signal. This activity is triggered in BAT by a process of adaptive thermogenesis in which chemical energy is converted into heat by oxidizing large amounts of fat and carbohydrate at the expense of ATP synthesis mediated by the uncoupling protein 1 (UCP1). We have identified a soluble form of adenylyl cyclase, ADCY 10, as an important factor regulating BAT function. ADCY10 is a HCO-/CO2-activated AMP-producing enzyme uniquely expressed in all cellular micro-domains. Wild type (WT) mice, when exposed to cold temperature, as low as 8°C, showed a three folds increase in ADCY10 expression in BAT; ADCY10 knockout mice showed less resistance to cold exposure, lowered expression of UCP1 and higher load of lipid droplet in BAT compared to WT mice. In vitro studies on brown adipocytes from mouse line showed that UCP1 gene expression along with other BAT markers, were increased by ADCY 10 activator (HCO-/CO2) and decreased by KO7, a specific pharmacological inhibitor of ADCY 10. We concluded that HCO-/CO2 regulates BAT function via its targeted sensory protein ADCY 10. Thereby, opening up a potential therapeutic target against various metabolic diseases state like obesity or type 2-diabetes as BAT activity is triggered via digestion of excess of glucose and fatty acid.
A 04-4
On odontological pathophysiology
E. Neu1, J. Michl1,2, M. Joseph1,2, D. Siebenhüner1,2, M. C. Michailov1,2, H. Zoepfl3,4, M. Joseph3,4, D. Siebenhüner3,4, M. C. Michailov1,2, H. Zoepfl1,2, G. Weber1,3

Introduction. Actually in Germany will be discussed about complex pathophysiology of Bert SAHMANN (Nobel price together with Erwin NEHER), because it appeared sepsis, caused by dental infection leading to difficult surgical treatment and increased danger of lethal cardiovascular disturbances. The IUM reported about odontological to international congresses. bioethics/Karnataka/India, patholgy/Bilbao, philosophy/Athens. Presently is considered new information.

Method. Observations about diagnosis (by X-rays) and therapy in dental practices (>30patients). Albert EINSTEIN investigated stimulation of phospho-emission leading to development of laser.

Results. Laser are used for therapy of caries dentium and periodontitis (e.g. hard laser, such as Erbium:YAG 2490nm + 1064nm/Fontana and diode laser 810nm/ARC). Similar to considered patients in earlier reports (ref't) is it possible to prevent pulps, i.e. treatment of radix dentis or extraction of teeth. Dental vitality (vascular & neuro-regulation) is maintained, by combined dental therapy, i.e. laser and conservative incl: bacteriostatic-bactericide (antibiotics, phyto-pharmaceutical), diet, etc.

On epistemology: Multifactorial combined dental pathophysiology is totally underdeveloped: Research in this field could support conservative therapy countering extremely dominant surgical treatment. On ethics: Odontological problems related to dental moral education and medical economics.

On aesthetics: Artificial teeth are very expensive contrary to combined laser-conservative therapy.

Conclusion. Political/financial support for projects about dental pathophysiology in context of an integrative physiology research. Combined laser-conservative therapy could help for better treatment & prophylaxis, reduction of enormous financial basis in odontology, help for developing countries in accordance with UNO-Agenda21 for better health, ecology, economy on global level.

Ref:

A 04-5
Studies on mechanisms of perampanel action in orthotopic F98 glioma model
T. Resch, C. Liebelt, T. Kirchstein,1 Rostock University Medical Center, Oscar-Langendorff-Institute of Physiology, Rostock, Germany

The serine/threonine kinase Akt is activated by various stimuli such as insulin in a phosphoinositide 3-kinase (PI3K)-dependent manner, while PI3K activity is opposed by the tumor suppressor phosphatase and tensin homolog (PTEN). However, a role of Myo1b in Akt activation is not known. In the present study, we employed F98 glioma cells as an orthotopic tumor model in Fischer rats to test efficacy of perampanel in combination with standard radio-chemotherapy (RCT) on both glioma progression and tumor-associated neuroprotection. In RCT-pretreated F98 glioma tumor models, Myo1b was associated with enhanced Akt activity and glioma growth suppression. Myo1b knockdown significantly reduced glioma growth and Akt phosphorylation, indicating a crucial role for Myo1b in Akt-mediated glioma growth suppression. Myo1b knockdown also resulted in a reduction of Akt activity in glioma cells. Furthermore, Akt phosphorylation was decreased in glioma cells following perampanel treatment, suggesting a potential therapeutic effect of perampanel on glioma growth suppression. In conclusion, our data indicate that Myo1b knockdown could be a potential therapeutic target for glioma treatment. This study provides new insights into the role of Myo1b in glioma growth suppression, and suggests a potential therapeutic strategy for glioma treatment.

A 04-6
The Impact of vascular endothelial cadherin expression in breast cancer cells on their interaction with blood and lymphatic endothelial cells
E. T. A. Boudriot1, M. L. Moskopp1, H. J. Schnittler1, A. Deussen2, G. Breier3
1 Technische Universität Dresden, Division of Medical Biology, Faculty of Psychiatry and Psychotherapy, Dresden, Germany
2 Technische Universität Dresden, Institute of Physiology, Faculty of Medicine, Dresden, Germany
3 Westfälische Wilhelms-Universität Münster, Institute of Anatomy and Vascular Biology, Münster, Germany

Question: Vascular endothelial cadherin (VE-cadherin) is a key component of the lymphatic and blood endothelial barrier. Our research group showed that breast cancer cells can also express VE-cadherin. The aberrant expression of this adhesion protein was shown to promote cancer cell proliferation, migration, invasion, and tumor growth. This study aims to extend our understanding of VE-cadherin’s role in tumor progression by investigating tumor cell interaction with either blood or lymphatic endothelium.

Methods: Tumor endothelial cell interaction in vitro was characterized quantitatively by adhesion assays and qualitatively by live-cell imaging and subsequent immunostaining of VE-cadherin. Confocal imaging and 3D reconstruction were used to study VE-cadherin expression at the interface of tumor and endothelial cells. VE-cadherin-positive breast cancer cell lines included BT-20 and SUM149PT. The latter was manipulated in their VE-cadherin expression by a viral-mediated RNAi gene knockdown. Primary endothelial cells comprised Human Umbilical Vein Endothelial Cells (HUVECs), Human Dermal Lymphatic Endothelial Cells (HDLECs), and Human Pulmonary Microvascular Endothelial Cells (HPMVECs).

Results: Cells with VE-cadherin knockdown showed less adhesion to the endothelium compared to control. Tumor cells showed more adhesion to lymphatic than to blood endothelial cells. This effect was dependent on tumor cell type and tumor cell concentration, but not on adhesion time. Live-cell imaging analysis revealed two distinct patterns of tumor cell migration. The interaction of tumor cells with either incorporated into the confluent endothelial layer or transmigrated through it. Further, 2D reconstruction of immunostainings uncovered specific VE-cadherin expression patterns: While the VE-cadherin barrier was restored after complete transmigration, the interfaces of endothelial cells and incorporated tumor cells showed no allocation of VE-cadherin.

Conclusion: In conclusion, our data give new insights into transendothelial migration of tumor cells. Adhesion preferences of tumor cells for lymphatic endothelium could play a role in determining differences in the pattern of breast cancer metastasis. VE-cadherin expression of tumor cells appears to be involved in several steps of metastasis, thus contributing to malignancy. Therefore, VE-cadherin might qualify as a potential target for a translational approach in breast cancer therapy.

A 04-7
Myosin 1b regulates nuclear Akt activation through preventing localization of PTEN in the nucleus
Y. Yu, Y. Xiong, D. Ladeiras, Z. Yang, X. - F. Ming
University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular System / Section Medicine, Fribourg, Switzerland

The serine/threonine kinase Akt is activated by various stimuli such as insulin in a phosphoinositide 3-kinase (PI3K)-dependent manner, while PI3K activity is opposed by the tumor suppressor phosphatase and tensin homolog (PTEN). Myosin 1b (Myo1b) is an actin-binding protein that is categorized as the monomeric, non-flamentous class-1 myosin. Our recent study demonstrates that Myo1b serves as a mediator in arginine- and aldosterone-stimulated activation of the mechanistic target of rapamycin complex 1 (mTORC1) signaling that is regulated by Akt. However, a role of Myo1b in Akt activation is not known. In this study, we show that silencing Myo1b in immortalized mouse embryonic fibroblasts (MEF) inhibits insulin-induced Akt activation as monitored by its phosphorylation at both Ser473 and Thr308. Importantly, this inhibition is exclusively observed in the motor activity of Myo1b. Moreover, the elevated nuclear PTEN upon silencing Myo1b promotes apoptosis of MEFs and melanoma B16F10 cells. In supporting of this finding, overexpression of Myo1b inhibits apoptosis, which is not observed when a PTEN mutant harboring a nuclear localization signal co-expressed. Together, we demonstrate that Myo1b, by interacting with PTEN, prevents localization of PTEN in the nucleus, contributing to nuclear Akt activation and suppression of cell death.
apoptosis. This represents a novel regulatory mechanism of nuclear PTEN-Akt pathway linking to cell apoptosis and may present a novel therapeutic approach for cancer treatment such as melanoma.

A 04-8 Identification of Interaction Partners of the Phosphoinositide Phosphatase INPP4B
I. D. Shaikh, O. Ebers, D. Oliver, Y. Renigunta
Philips University, Institute for Physiology and Pathophysiology, Marburg, Germany

The PI3K-Akt signal transduction pathway promotes metabolism, proliferation, cell survival, growth and angiogenesis in response to extracellular signals. A specific branch of this complex signaling pathway, mediated by PI(3,4,5)P3, is only beginning to be understood. Emerging evidences point towards the function of PI(3,4,5)P3 in actin-driven membrane dynamics, particularly at lamellipodia and in the formation of actin-rich protrusions called podosomes or invadopodia. Mechanistically, PI(3,4,5)P3 may act by recruiting actin-regulating proteins like lamellipodin and TKS5 into these structures. Beyond these molecules, little is known about specific PI(3,4,5)P3 effectors. The tumour suppressors: PI3-phosphatases PTPN2, INPP4B and INPP4R specifically hydrolyze PI(3,4,5)P3, thereby terminating the PI(3,4,5)P3-mediated branch of PI3K signaling. However, conclusive molecular mechanisms, particularly potential roles of INPP4B in regulating PI(3,4,5)P3-dependent actin-driven membrane dynamics have remained elusive. In order to obtain initial insights into the cell biology of INPP4B, we searched for molecular interaction partners by a yeast two-hybrid (Y2H) protein-protein interaction assay. Screening a human brain cDNA library using INPP4B as bait, we identified several candidate interacting proteins. Two proteins with strong interaction at the level of Y2H are known actin-regulatory proteins. Verification of the interactions was performed in mammalian expression systems by co-immunoprecipitation, co-localization and proximity ligation assays. Molecular, biochemical and physiological characterization of these interactions may further unravel role of INPP4B in shaping cellular PI(3,4,5)P3 dynamics and potential relevance for actin dynamics.

A 04-9 ERp57 deficiency impairs growth of colorectal cancer cells by attenuating c-Myc, PLK1 and AKT activity
Oechsberg, F. Neumann, M. Hussmann, P. Kranz, U. Brockmeier, E. Meitzen
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

The thiol-oxidoreductase ERp57 is a chaperone protein with many different functions. Originally it has been described to be a protein of the endoplasmic reticulum (ER); but later it was also found in other cell compartments including the cytosol. Aim of this study was to investigate reasons for p53-independent reduction of proliferation which occurs in the colon carcinoma cell line HCT116 in response to ERp57 depletion under nongenomic and hypoxic conditions (1% O2). Our data show that a dramatic down-regulation of PDK1 (3-phosphoinositide-membrane protein kinase 1, aka PDK1) which is a master kinase of AKT and related growth regulating kinases. The PDK1 kinase substrate phospho-AKT was also down-regulated after ERp57 knockdown. Another substrate, phospho-GSK3β, was reduced under hypoxic conditions. Furthermore, another important signaling protein termed phospho-ERK which is typically regulated by KRAS, an oncogene centrally involved in colon carcinoma, showed impaired expression after ERp57 depletion. Because a G2/M arrest had been demonstrated in ERp57 depleted cells, the oncogenes phospho-PLK1 and c-Myc were also examined. Both proteins were significantly down-regulated.

In conclusion, ERp57 depletion in HCT116 colon carcinoma cells causes reduction of major oncogenes and is therefore a potential new target in colon carcinoma treatment.

A 04-10 Effects of small molecule inhibitors in the PI3K/AKT pathway on glioblastoma
J. Hüntenmeyer1, F. Lange2, G. Reichart1, M. Linnebacher1, T. Kirchstein1, R. Köhling1,2
1Rostock University Medical Center, Oscar-Langendorff-Institute of Physiology, Rostock, Germany
2Rostock University Medical Center, Center for Transdisciplinary Neurosciences Rostock, Rostock, Germany

Glioblastoma (WHO grade IV glioma) is the most common and aggressive primary brain tumor. Multiple genetic and epigenetic alterations in several major signaling pathways - including the phosphoinositide 3-kinases (PI3K) pathway - can be found. The PI3K/AKT pathway regulates a variety of cellular processes such as proliferation, growth and survival. Therefore, it is a notable molecular therapeutic target, which could provide an effective treatment for glioblastoma.

In this study, we assessed the therapeutic possibility of inhibiting the PI3K/AKT pathway employing four small molecule inhibitors like dacarbazine, ipatasertib and MK-2206. To analyse the effects in vitro, we established patient-derived low-passage glioblastoma cell lines from four subjects who underwent surgery. In a first approach, all tested inhibitors showed antiproliferative as well as proapoptotic effects. To further estimate the impact on a molecular level, signaling pathway activation was determined. Due to extensive cross-talk and compensatory feedback mechanisms between the PI3K/AKT and RAS/RAF/MEK pathway, we also analyzed the activation of MAP kinases ERK1/2. The results showed an attenuation of AKT phosphorylation by dacarbazine and MK-2206. Remarkably, ipatasertib caused an increase of AKT phosphorylation. The phosphorylation level of ERK1/2 was not affected. Additionally, a quantitative expression analysis of various genes involving proliferation and apoptosis was performed. To gain a deeper insight into the mechanism of action, proteomics slide cultures containing glioblastoma cells were established as an ex vivo model. For this purpose, brain slices of 6-8 days old Fischer rats were prepared and tumor cells were placed onto the cortex of the slices, which were then cultured. The effects of small molecule inhibitors ex vivo were analyzed by field potential recordings and quantification of tumor size volume.

Taken together, with our combined in vitro and ex vivo approach we aimed to investigate the efficacy of small molecule inhibitors on glioblastoma cells and characterised the effects on neuronal activity in surrounding brain tissue.

A 04-11 Voltage Dependent Phosphatase Activity Is Enhanced by Intracellular Acidification
I. G. Shaikh, O. Ebers, D. Oliver, C. R. Halasovich
Philips-University Marburg, Inst. f. Physiology / Dept. of Neurophysiology, Marburg, Germany

Voltage sensitive phosphatases (VSPs) are PI(4,5)P2/PI(3,4,5)P3 - and PI(3,4,5)P3-dependent phosphatases. They consist of a voltage sensor domain (VSD) and a catalytic domain (CD). For non-mammalian VSPs, enzymatic activity is regulated by membrane voltage via the VSD, which in turn controls the CD. These VSPs strongly deplete PI(4,5)P2 when the cell membrane is depolarized. For mammalian VSPs, the VSD seems insensitive to membrane voltage, yet seemingly is still in control of the enzymatic activity. Therefore, the physiological regulator of mammalian VSP activity remains elusive. VSPs are suggested to play a role in processes like sperm cell capacitation and cell development. These processes are accompanied by changes in intracellular pH. Therefore, we speculated that besides membrane voltage, intracellular pH might be a modulator of VSP activity.

To test this hypothesis, we performed whole-cell patch-clamp experiments in Chinese hamster ovary (CHO) cells expressing diverse non-mammalian VSPs together with fluorescent PI(4,5)P2 reporter domains, the latter allowing for monitoring VSP activity by means of total internal reflection microscopy (TIRF-M). The whole-cell patch-clamp configuration allowed for control not only over membrane voltage but also intracellular pH by dialysing the cell with solutions with the desired pH. We find that acidification of the cytoplasm results in increased PI(4,5)P2 depletion, accompanied by a shift of the apparent voltage dependence towards more negative potentials. An increase in intracellular pH has the opposite effect. The voltage dependence of sensing currents was unaffected by the pH changes, suggesting that alterations of the VSD are not causal for the observed changes in voltage dependent activity. Similar effects were observed in all tested VSPs. Kinetic modeling predicts a shift in apparent voltage dependence when VSP activity is modulated by intracellular pH that is in agreement with the observed shift. We conclude that the overall activity of the phosphatase is enhanced under acidic and diminished under alkaline conditions.

In conclusion, we suggest that intracellular pH can play a role in the regulation of the activity of VSPs. This might be of particular importance for VSPs that are not regulated by membrane voltage.

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INTRODUCTION: On nephrology depends angiocardiac incl. neuropathology. Reports on integrative genitourology & strategy of using albumin-derived, perfluorodecalin-based artificial oxygen carriers (APOCs) in an ex vivo normothermic physiology are given. Now is considered pyeloureter in this context [1,2].

RESULTS
A. Spontaneous phasic contractions (SPC): More than 10 SPC-patterns (1-2/min) were observed in isolated pyelon/P & of APOCs, respectively (Perfusion KH+BSA+APOCs or Perfusion KH+BSA). Perfusion-free controls evaluated after 0min (ureter/U of guinea-pig/GP & human/H (n=242): Irr- & regular (frequency), non- & uniform (amplitudes), burst-like, others were monitored in the perfusate with clark electrodes. Other read-out parameters were kidney’s brush border damage detected with periodic acid-Schiff (PAS) staining, TNFα-receptor-expression and the evaluation of apoptosis using the TUNEL staining.

B. Intrarenal distribution of AT1 receptors
J. Schrank, M. A. A. Fuchs, K. A. - Geri, C. Wagner, A. Kurtz
University of Regensburg, Institute of Physiology, Regensburg, Germany

Angiotensin II (ANG II) is an essential regulator of several kidney functions, including modulation of medullary blood flow, glomerular filtration rate, tubulointerstitial transport and renal vascular resistance. Non-parallel effects seem to be mediated mainly by ANG II type II (AT1) receptors. In rodents, two subtypes of the AT1 receptor can be distinguished as AT1a and AT1b receptor. Although, the AT1a receptor is considered to play the major role in the renal actions of ANG II, there have been conflicting observations regarding the renal expression pattern of AT1 receptors. These inconsistencies in the literature may be due to the variety of detection methods or heterogeneities between the examined species. Therefore, we aimed to systematically analyze the localization of AT1 receptors in the mouse kidney by in-situ hybridization. We performed colocalization studies with tubular, interstitial and vascular markers to specifically discriminate between the different segments of the renal tubule system that express AT1 receptors. In addition, we compared the renal AT1 receptor distribution in mice with the expression patterns in kidneys of other species. We could confirm mRNA expression of AT1 receptors in intraglomerular mesangial cells and proximal tubules, which has previously been described in most studies. Moreover, colocalization with PDGF-Rβ showed high expression of AT1a mRNA in interstitial cells of the cortex and the outer medulla. We could see a decreased expression pattern towards the medullary region with only few signals in interstitial cells in the papilla. Distal tubules showed no expression of AT1a receptors and we also could not see any expression in collecting duct cells. Compared to AT1a, only weak AT1b receptor signal was found in tubules. Considering the potent vasoconstrictor effect of ANG II, expression of AT1 receptors can be useful for evaluating renal function. We could not detect any AT1a signals in bigger vessels or arteries in the kidney. We could only find AT1a mRNA expression in different arteries and collecting duct cells. Summing up, by using state-of-the-art in-situ hybridization, we were able to detect the distinct expression pattern of the AT1 receptor subtypes in renal cells of different species. We found the highest AT1a mRNA expression in glomeruli, interstitial cells and proximal tubular cells and there was no expression of AT1 receptors detectable in bigger renal arteries.

A 05-3

The beneficial effect of albumin-derived, perforfluorecalin-based artificial oxygen carriers on the perfusion of an ischemic isolated rat kidney
N. Küppers1, M. Kirsch2, K. B. Ferenc1
1University of Duisburg-Essen, University Hospital Essen, Institute of Physiology, Essen, Germany
2University of Duisburg-Essen, University Hospital, Institute of Physiological Chemistry, Essen, Germany

In order to enlarge the amount of kidneys available for transplantation new strategies must be developed. At present, the quality of kidneys donated after cardiac death (DDC) is insufficient for transplantation (e.g. non or delayed graft function) if stored conventionally on ice. As artificial oxygen carriers have already been used successfully for organ preservation, the strategy of using albumin-derived, perforfluorecalin-based artificial oxygen carriers (APOCs) in an ex vivo normothermic machine perfusion is a promising approach to regenerate ischemic DCD kidneys. To identify the improvement of APOCs on organ quality, rat DCD kidneys (120 min of ischemia) were perfused pressure-controlled (80-85mmHg) for 3 hours with Krebs-Henseleit buffer (KH) (KH + bovine serum albumin (BSA) with or without presence of APOCs, respectively (Perfusion KH+BSA+APOCs or Perfusion KH+BSA). Perfusion-free controls evaluated after 0min (-) and 120min of ischemia (+control) were investigated. The oxygen consumption before and behind the kidneys were monitored in the perfusate with Clark electrodes. Other read-out parameters were kidney’s brush border damage detected with periodic acid-Schiff (PAS) staining, TNFα-receptor-expression and the evaluation of apoptosis using the TUNEL method. Kidneys perfused with APOCs showed a higher oxygen consumption (0.014 mlO2/kg/min) with regard to control kidneys (0.010 mlO2/kg/min). Furthermore, PAS staining revealed that perfusion with APOCs improved slightly but significantly the regeneration of the tubular brush border compared to perfusion with KH+BSA. However, the TUNEL method and TNFα-receptor staining failed in demonstrating any improvements concerning the cell survival or receptor expression. Compared to the perfusion control kidneys, the PAS and TUNEL staining illustrated that perfusion did not increase either cell damage or cell survival. Remarkably, TNFα-receptor-expression was even reduced after perfusion. In conclusion, normothermic perfusion was well tolerated by the DCD kidneys and helped to maintain the kidney’s quality after 120 min of ischemia for 2 hours. Some parameters such as intactness of the tubular brush border and oxygen consumption were actually improved by the action of APOCs.

A 05-4

The role of the deubiquitinase OTUB1 in renal inflammation and fibrosis
I. Günter1, I. Cabrita, A. Ruiz-Serrano1, S. Pfundstein1, P. H. Imenez Silva2,3, E. M. Pastor Arroyo1-3, R. D. Rajaraman1, S. de Seigneurx2, R. H. Wengeler1,2, C. C. Scholz1,2
1University of Zurich, Institute of Physiology, Zurich, Switzerland
2National Centre of Competence in Research 'Kidney CH'. Zurich, Switzerland
3University Hospital and University of Geneva, Geneva, Switzerland

The author has objected to a publication of the abstract.

A 05-5

Aaberrat Ca2+ signals in ADPKD activate TMEM16A and Cyst Growth
I. Cabrita1, B. Buchholz2, R. Schreiber1, K. Kunzelmann1
1Universität Regensburg, Institut für Physiologie, Regensburg, Germany
2University of Erlangen-Nuremberg, Department of Nephrology and Hypertension, Erlangen, Germany

Polycystic kidney disease (PKD) leads to continuous decline of renal function by growth of renal cysts. Enhanced proliferation and transepithelial chloride secretion through cystic fibrosis transmembrane conductance regulator (CFTR) is thought to contribute to cyst growth in PKD. It is known that CFTR expression and activity is present in ADPKD kidneys. However, the role of CFTR in ADPKD is not understood. Surprisingly, we could not detect any AT1 signals in bigger vessels or arteries in the kidney. The data suggest TMEM16A as a major pathogenic factor during ADPKD. It may represent a suitable therapeutic target in polycystic kidney disease.
The polyamine system in acute and chronic kidney injury

T. Sieckmann1, M. Fähling1, M. A. Ashraf2, G. Schley1, E. Viglò3, C. Rosenberger4, F. Ainiger5, K. Schmidt-Ott6, H. Schulz2, K. M. Kirschner2

1Charité – Universitätsmedizin Berlin, Inst. für Vegetative Physiologie, Berlin, Germany
2Charité – Universitätsmedizin Berlin, Chirurgische Klinik, CIVK, Berlin, Germany
3Universitätsklinikum Erlangen, Nephrologie und Hypertensologie, Erlangen, Germany
4Charité – Universitätsmedizin Berlin, Medizinische Klinik m.S., Berlin, Germany

Polyamines are aliphatic cations that are involved in gene regulation, cell proliferation, cellular stress and autophagy, among other processes. The cellular levels of the polyamines spermidine and spermine and of their precursor putrescine are tightly controlled by the rate of de novo synthesis, transport, interconversion, and terminal degradation. Enzymatic transformation of ornithine to putrescine by ODC1 is the rate limiting step in polyamine synthesis. By using RNASeq in situ hybridization we could locate Odc1 transcripts to the proximal tubular cells. We and others have previously reported that branching morphogenesis in murine embryonic kidney explants requires putrescine in a developmental stage-specific manner. Here we examined the hypothesis that expression changes of genes involved in polyamine homeostasis are conserved among different types of kidney injury.

The renal expression of various polyamine regulating enzymes (Odc1, Sras, Srm, Asct1, Satf, Pas, Sms) was analyzed in rodent models of acute and chronic kidney injury. Acute models: 17 min of renal ischemia followed by 6 hours to 21 days of reperfusion; kidneys from 24 hours to 7 days post-transplantation; robamolysis-induced kidney injury at 24 h and 48 h after intramuscular injection of 50 % glycerol (0.05 ml per 10 g body weight). Chronic models: daily subcutaneous injections of cyclosporin A (60 mg kg⁻¹ per min) for 4 weeks; Streptozotocin-induced diabetes mellitus after 6 weeks. We identified a common gene expression pattern across the different renal pathophysologies with up-regulation of polyamine-degrading enzymes (Asct1, Satf, Sras) and down-regulation of enzymes involved in polyamine synthesis (Odc1, Sras). Furthermore, our data indicate that inhibition of polyamine synthesis and stimulation of polyamine breakdown are common to various forms of acute and chronic kidney injury. These observations further suggest that disturbed polyamine homeostasis plays a role in renal damage with different etiologies.

Organotypic expression of vascular smooth muscle Kv7.1 channels – potential for renoprotection?

A. Bachmann1, I. Sonsalí1, F. Stocker1, D. Tsvetkov2, M. Gollasch1, R. Schubert1

1Cardiovacular Physiology, Centre for Biomedicine and Medical Technology Mainz, Ruprecht-Karls-University Heidelberg, Mannheim, Germany
2Experimental and Clinical Research Center (ECRC), a joint cooperation between the Charité Medical Faculty and the Max Debrück Center for Molecular Medicine (MCB), Berlin, Germany
3Department for Physiology, Augsburg University, Augsburg, Germany

Question: Blood flow regulation is controlled largely by vascular smooth muscle voltage-gated potassium (Kv) channels, especially Kv7.1 channels. Kv7.1 channels are also expressed but at different levels compared to Kv7.4 channels. This study addressed the hypothesis that Kv7.1 channels expressed at a similar level as Kv7.4 channels contribute to the regulation of arterial contractility.

Methods: Mesenteric, coronary, skeletal muscle and renal segmental arteries of Wistar rats were studied using real-time qPCR and isometric myography. Intact kidneys were explored using constant flow perfusion.

Results: A functional impact of Kv7.1 channels was postulated when the effect of specific openers (R-L3, ML277) on mesenteric resistance vessels was antagonized by the specific blocker HMR1556. In skeletal muscle arteries, the expression of Kv7.1 channels was much smaller than that of Kv7.4 channels and no functional impact of Kv7.1 channels was observed. A similar expression of Kv7.1 and Kv7.4 channels was found in coronary, mesenteric and renal arteries. In mesenteric and renal, but not in coronary arteries a functional impact of Kv7.1 channels was detected. In all vessels, HMR1556 alone was without effect on Kv7-induced contraction. Further, in renal arteries HMR1556 did not affect the anticontractile effect of the cGMP-coupled vasodilator ANP and the cAMP-coupled vasodilator urocortin. Notably, the effect of ANP and urocortin were the same in the absence and the presence of R-L3. In addition, R-L3 reduced methoxamine-induced contraction with similar perfused kidneys. This effect was attenuated considerably by HMR1556. Urocortin reduced methoxamine-induced pressure. This effect was the same in the absence and the presence of R-L3.

Conclusions: The results show that pharmacological opening of Kv7.1 channels was able to alter arterial contractility only when these channels were expressed at a similar level compared to Kv7.4 channels. In renal segmental arteries Kv7.1 channels do not contribute to ANP-induced contraction or ANP- and urocortin-induced relaxation. After pharmacological activation, Kv7.1 channels reduce basal tone and basal perfusion pressure, but leave MX-induced contraction as well as ANP- and urocortin-induced relaxation intact. Thus, Kv7.1 channel activation improves renal perfusion without altering vasoconstrictor- or vasodilator-evoked regulation suggesting that these channels may serve as targets for renoprotection.
Conclusions

In a mouse model of mild incipient chronic CsA-induced renal injury accompanied by anemia, pharmacological HIF activation via daprodustat corrected anemia with apparently no detrimental effect on the renal parenchyma. Therefore, daprodustat may serve to treat renal anemia in transplant recipients.

A 05-10 Different subpopulations of renal interstitial PDGFR-β cells are able to produce erythropoietin and vasoactive factors in response to hypoxia in vivo

K. A.-E. Gerl, M. A. Fuchs, J. Schrankl, K. A. - E. Gerl, A. Gödecke

University Regensburg, Institute of Physiology, Regensburg, Germany

Genetic induction of the hypoxia signaling pathway in mesenchymal PDGFR-β cells leads to abundant HIF2-dependent erythropoietin (EPO) expression in the cortex and outer medulla of the kidney but not in other tissues. This rather unique feature of renal PDGFR-β cells raises the question about special characteristics of these cells and their more general functional response to hypoxia. To address these questions, we have characterized renal PDGFR-β EPO expressing cells according to additional cell markers. Moreover, we have analyzed their gene expression profile in response to hypoxia. For this purpose we used mice with genetic induction of the hypoxia signaling pathway by inducible deletion of the von Hippel-Lindau (Vhl) protein in either PDGFR-β cells (PDGFRβfl/fl CreERT2/+), or in Gli1+ cells (Gli1+CreERT2/+). We have identified mesenchymal CD73+, Gli1+, tenascin C+ and interstitial SMHC+ cells of the kidney as subpopulations of PDGFR-β cells. Interstitial CD73+ cells were mainly distributed in the cortex and the outer stripe of the outer medulla. Gli1+ cells were mainly found in the outer stripe of the outer medulla, whilst tenascin C+ cells were mainly located in the inner stripe of the outer medulla and in the inner medulla. Interstitial SMHC+ cells were mainly located in the outer medulla. Genetic induced EPO expression in interstitial cells colocalized almost exclusively with CD73 in the cortex. In the outer medulla we found coexpression with CD73, Gli1, SMHC or tenascin C. In the inner medulla only a subfraction of tenascin C+ cells expressed EPO. Besides EPO expression also expression of adrenomedullin and regulator of G-protein signaling 4 were upregulated in all PDGFR-β subpopulations. In addition, expression of cyclooxygenase 2 was coincided in tenascin C+ cells in the inner stripe of the outer medulla. We conclude that different renal interstitial PDGFR-β1 subpopulations exist, that are able to express EPO in principle. These cells also coexpress factors that could contribute to improve renal interstitial oxygenation in states of hypoxia.

Deletion of renal Cyclooxygenase-2 in the FoxD1+ stromal progenitor compartment - developmental and functional consequences

M. A. A. Fuchs, J. Schrankl, K. A. - E. Gerl, A. Kurtz

University Regensburg, Institute of Physiology, Regensburg, Germany

In the kidney Cyclooxygenase-2 (Cox-2) is expressed in two distinct locations. In the cells of the macula densa Cox-2 derived prostaglandins help regulate the renin synthesis and release. The second expression site for Cox-2 in the kidney are medullary cells with important functions for the salt handling capability of the kidney. Application of Cox-2 Inhibitors during pregnancy in humans and global deletion studies in mice have revealed severe defects in glomerular maturation, interstitial fibrosis and tubular hyperplasia during nephrogenesis. This leads to an abnormally thin cortex and impaired renal function in adults. The majority of cells affected by these renal malformations derive from FoxD1+ stromal progenitors. The FoxD1+ cells differentiate to interstitial kidney cells, mesangial, vascular and renal producing cells in the adult kidney. For a specific investigation of the involvement of Cox-2 in this compartment during nephrogenesis, I generated mice with a specific deletion of the Cox-2 gene only in cells expressing FoxD1 during nephrogenesis (FoxD1-Cox2fl/fl) mice using the Cre/loxp system. To evaluate possible morphological changes and the expression of Cox-2 mRNA I used RNAseq in-situ hybridization. Basic kidney functions such as urinary concentration capability, renal renin expression and GFR were measured to evaluate the consequence of Cox-2 deletion in the FoxD1+ compartment. In FoxD1-Cox2+ mice the expression of Cox-2 in all cells of the FoxD1+ compartment was absent, but Cox-2 expressing cells of the macula densa was unaffected. The severe renal abnormalities observed in mice treated with a Cox-Inhibitor during nephrogenesis or with a global deletion of Cox-2 were absent in FoxD1-Cox2fl/fl mice. The glomeruli of FoxD1+Cox2fl/fl mice were developed and distributed normally. Basic kidney functions as sodium excretion and urine concentration were unchanged to littermate controls. No signs of interstitial fibrosis were detected in FoxD1+Cox2fl/fl mice. From these findings, we conclude that the two distinct expression sites for Cox-2 in the kidney, cells of the macula densa and medullary interstitial cells, derive from different compartments. Despite the involvement of FoxD1 derived cells in the severe renal damage seen in mice with a global Cox-2 deletion, the expression of Cox-2 in these stromal progenitors is not essential for normal kidney development and normal kidney function under basal conditions.

A 06-1 AKT- and AMPK-signaling are important regulators of cardiac metabolism

S. Gödecke1, U. Flögel1, A. Heinen1, A. Gödecke1

1 Heinrich-Heine-Universität, Institut für Herz- und Kreislauflphysiologie, Düsseldorf, Germany
2 Heinrich-Heine-Universität, Institut für Molekulare Kardiologie, Düsseldorf, Germany

Introduction: Akt1 and Akt2 are the main isoforms of protein kinase B (Akt) expressed in the mammalian heart. Tumoxifen-inducible, cardiomyocyte-specific Akt1+Akt2 double knockout mice (ICM-Akt1/2) show progressive cardiac atrophy and loss of heart function leading to terminal heart failure 3 to 4 weeks after first injection of OH-Txf. We investigate the impact of Akt deletion on cardiac energetics and metabolism.

Methods: ICM-Akt1/2 and wildtype mice were analyzed in vivo for their cardiac energetic and metabolic status by repetitive 31P-NMR spectroscopy. Bioenergetic profiling of isolated KO and WT cardiomyocytes was performed using Seahorse XF Cell Mito Stress assays. Heart tissue was subject to western blot analyses.

Results: Repetitive 31P-NMR spectroscopy in anesthetized mice revealed that in ICM-Akt1/2 hearts phosphocreatine : ATP ratios (PCr/ATP) declined from 1.95 ±0.16 to 1.64 ± 0.26 (p=0.042) on d15 and 1.55 ±0.05 (p=0.0003) on d20 after induction of the knockout, values associated with serious heart failure. In parallel, free creatine levels considerably increased as shown by creatine chemical exchange saturation transfer (C/CEST). In line with this finding, basal respiration and maximal respiratory capacity of isolated KO cardiac myocytes on d15 after knockout induction were reduced as compared to WT cells, demonstrating severe metabolic impairment.

To investigate to what extent AMP-activated protein kinase (AMPK) was activated upon low energy status, western blots were performed. In ICM-Akt1/2 hearts, the AMP dependent activating phosphorylation of AMPK Thr172 was only moderately increased on d15 (1.55 fold ±0.24), but significantly reduced on d21 (0.61fold±0.18). In parallel, the inhibitory phosphorylation of AMPK on Ser485/491 was distinctively increased by 1.9±0.21 on d15 and 2.96±0.42 on d21. No increased phosphorylation of the classical AMPK substrate ACC could be detected in energy depleted ICM-Akt1/2 hearts.

Conclusion: Loss of Akt function leads to progressive energetic depletion, which cannot be compensated by AMPK. Rather, the progressive inhibition of AMPK activity might further aggravate the metabolic stress in ICM-Akt1/2 hearts.

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Cardiac-specific knockout of monoamino oxidase B has obviously no impact on ischemia-reperfusion injury

J. Heger, J. Bornbaum, C. Hirschhäuser, K. - D. Schlüter, R. Schulz

Jülich-Lübeck University, Institute of Physiology, Giessen, Germany

Myocardial infarction is one of the major causes of death worldwide. Monoamine oxidase B (MAO-B) is a protein of the outer mitochondria membrane and catalyzes the oxidative deamination of biogenic amines thereby producing reactive oxygen species (ROS). Because the damage of the cells by ischemia/reperfusion (IR) is largely caused by the formation of reactive oxygen species (ROS) we tested the hypothesis that a lack of MAO-B and thus a reduction in ROS production during reperfusion serves to protect the mitochondria and consequently the heart. We generated a cardiac-specific and tumoxifen-inducible MAO-B knockout mouse (MAO-B-KO). Lack of MAO-B was checked by western blot and ROS production. Wildtype (WT) and MAO-B-KO mice were analyzed by echocardiography. Mice were exposed to IR using the standard Langendorf technique. Hemodynamic parameters and infarct size (m % of left ventricle) were determined.
MaoB protein expression was significantly downregulated in MaoB-KO mice after two weeks tamoxifen feeding followed by ten weeks normal feeding. ROS formation stimulated by the MaoB-specific substrate β-phenylethylamine (PMA; 250 µM) was significantly reduced in the RV, which was significantly reduced in the Siah-2 KO animals. Siah-2 shows highest expression in cardiac fibroblasts. Loss of Siah-2 induces a reduced TGF-beta- and hypoxia-responsiveness, reduced migration and proliferation in fibroblasts. With the usage of inhibitor of nuclear export/import or protein synthesis, we were able to demonstrate that nuclear HIF-1 alpha translocation is significantly reduced in Siah-2 KO fibroblasts. However, PHD protein expression was not altered. Gene array analyses show significant differences in response to hypoxia in these fibroblasts. Among the differentially regulated genes are various with impact on fibroblast proliferation and migration including the apelin receptor (APJ). APJ shows a higher expression in response to hypoxia in Siah-2 KO cells compared to WT cells. Activation of APJ causes a decrease in collagen synthesis, fibroblast migration and myofibroblast transdifferentiation.

Conclusions: Siah-2 acts as a negative cardiac fibrosis regulator in response to RV overload induced by hypoxia or PAB. The apelin receptor may be involved in the altered fibroblast response in Siah-2 KO fibroblasts. Understanding the precise role of Siah2 and the participating signaling pathways may provide novel therapeutic targets directed against the development of RV hypertrophy and cor pulmonale.

A 06-3

Inflammation-like growth factor 1 improves cardiac function after acute myocardial infarction in a prediabetic mouse model

A. Spychala, A. Heinem, R. Nederlof, A. Gödecke

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

Background Type 2 diabetes mellitus (T2DM) is a major comorbidity worsening the outcome after acute myocardial infarction (MI). Reduced cardiac glucose uptake may be the underlying mechanism compromising cardiac function even under prediabetic conditions. Inflammation-like growth factor 1 (IGF1) is an anabolic hormone similar to insulin in molecular structure and signaling. IGF1 controls proliferation, differentiation and metabolism of cells. In previous studies we have shown that short-term IGF1 treatment after MI improved cardiac function and reduced scar size in mouse hearts. Here we investigate whether IGF1 can maintain cardiac function also after MI under conditions of prediabetes.

Methods: Mice were fed standard chow or high fat/high sucrose diet (HFSHD) for 10 weeks to induce obesity and prediabetes. Mice were phenotyped by measurements of blood glucose, insulin levels, glucose (ITT) and insulin tolerance tests (ITT). Mice were subjected to 45 min left anterior descending coronary artery occlusion and 4 weeks reperfusion. IGF1 or vehicle were given over 3 days using osmotic mini pumps. Echocardiography (EC) was performed before MI and 1 and 4 weeks after MI. Heart function was analyzed echocardiographically including strain analysis.

Results: HFSHD led to increased fasting blood glucose levels with up to 200 mg/dl and glucose intolerance. In line with this results plasma insulin levels were elevated in HFSHD mice with 1.3 ng/l in comparison to 0.4 ng/l in standard chow mice. However, exogenous insulin was able to lower blood glucose in the ITT, indicating prediabetes but not T2DM. Functional analysis by EC indicated a slight improvement of ejection fraction (EF) in HFSHD mice with 71.1% in comparison to 64.0% in the control group. However, cardiac function after MI under prediabetic conditions (EF: 35.3%) worsened to the same extent as in the control group (EF: 34.4%). Treatment of IGF1 after MI preserved cardiac function not only in the control group but also in the prediabetes group, with higher EF (control: 45.5%; IGF1: 48.2%), higher stroke volume and lower end-systolic volume.

Conclusion: Despite similar cardiac signal transduction like insulin, IGF1 was able to preserve cardiac function after MI in a prediabetic mouse model. Thus a IGF1-based therapeutic approach could be a promising approach to improve the worsened cardiac outcome after myocardial infarction even under prediabetic conditions and may also be protective under T2DM.

A 06-4

Impact of Siah2 on the development of Cor pulmonale

N. Molenda1, S. Kraaj2, L. L1, D. Haag2, B. Kojonazarov2, N. Weissmann1, L. Schmitz2, S. Rohrbach1

1Justus-Liebig-University, Institute of physiology, Giessen, Germany
2Excellence Cluster Cardio-Pulmonary System, Gießen, Germany
3Justus-Liebig-University, Institute of Biochemistry, Giessen, Germany

Question: The seven in absentia homolog 2 (Siah-2), an E3 ubiquitin ligase, is involved in hypoxia-related pathways. Siah-2 was suggested to control the stability of HIF-1α via ubiquitination and degradation of the prolyl hydroxylases (PHD1, PHD3). Here, we investigated the role of Siah2 in two different mouse models of right ventricular hypertrophy (RVH), induced by hypoxia as well as by hypoxia-independent pulmonary artery banding (PAB).

Methods: To investigate the role of Siah2, we used wild-type (WT) and Siah2-2 KO mice. Mechanisms related to the observed differences were also investigated in isolated mouse cardiac fibroblasts.

Results: The hypoxia- or PAB-induced increase in RV weight and the deterioration in RV function (echocardiography) were significantly attenuated in Siah2-2 KO mice compared to WT mice. Morphometric quantification of small pulmonary artery remodeling demonstrated a comparable increase in vascular wall thickness in WT and Siah2-2 KO mice, suggesting that Siah-2 acts directly on the RV. Three weeks after PAB or four weeks after hypoxia exposure, WT mice showed strongly increased collagen content in the RV, which was significantly reduced in the Siah-2 KO animals. Siah-2 shows highest expression in cardiac fibroblasts. Loss of Siah-2 induces a reduced TGF-beta- and hypoxia-responsiveness, reduced migration and proliferation in fibroblasts. With the usage of inhibitor of nuclear export/import or protein synthesis, we were able to demonstrate that nuclear HIF-1 alpha translocation is significantly reduced in Siah-2 KO fibroblasts. However, PHD protein expression was not altered. Gene array analyses show significant differences in response to hypoxia in these fibroblasts. Among the differentially regulated genes are various with impact on fibroblast proliferation and migration including the apelin receptor (APJ). APJ shows a higher expression in response to hypoxia in Siah-2 KO cells compared to WT cells. Activation of APJ causes a decrease in collagen synthesis, fibroblast migration and myofibroblast transdifferentiation.

Conclusions: Siah-2 acts as a negative cardiac fibrosis regulator in response to RV overload induced by hypoxia or PAB. The apelin receptor may be involved in the altered fibroblast response in Siah-2 KO fibroblasts. Understanding the precise role of Siah2 and the participating signaling pathways may provide novel therapeutic targets directed against the development of RV hypertrophy and cor pulmonale.

A 06-5

Reliable quantification of mutant protein fractions in minute tissue samples from a patient's heart with familial hypertrophic cardiomyopathy

E. Becker, A. Radočaj, T. Kraft

Hannover Medical School, Molecular and Cell Physiology, Hannover, Germany

Based on our previous work, quantification of the expression level of mutant protein in genetic diseases like hypertrophic cardiomyopathy is fundamental for understanding disease mechanisms. For heterozygous β-myosin heavy chain (β-MHC) mutations it has shown that disease severity is related to the fractions of mutant and wild type protein in the myocardium. Also, mRNA quantification cannot easily replace protein quantification since often deviations between mRNA and protein levels are found. The amount of human heart tissue available from patients with genetically characterized diseases is very limited. Therefore, the question was if protein quantification is still reliable even when using minute tissue samples.

Here we established a modified quantification method to minimize the use of tissue samples and compared the results to our established method that needs larger samples. We used tissue dissected from four different regions of the explanted heart of a patient with familial hypertrophic cardiomyopathy with the point mutation p.Gly716Arg in β-MHC. We extracted β-MHC protein from two different sizes of the samples. The small samples of two had a typical size of 1 mm diameter and less than 1 mg muscle tissue, while the large samples were of irregular size and had about 15 mg. The extraction process was adapted to the small tissue size concerning handling of the sample, transfer into small solution volumes and selective and complete extraction of β-MHC. The obtained extract was sufficient for AQUA mass spectrometry quantification of the fraction of mutant β-MHC protein.

Our results showed that (i) the fractions of mutant protein were in the same range for all four dissected heart regions (on average 32%), (ii) there was no substantial difference in the fractions of mutant protein for small and large samples, and (iii) the standard deviations for small samples were comparable to the one for large samples. We conclude that (i) the mutant protein appears quite evenly distributed in the examined regions of the patient heart, (ii) the two quantification methods (small and large sample size) produce results with comparable precision and accuracy, (iii) minimization of the tissue samples allows reliable β-MHC quantification. Hence, it might be sufficient to process only one minute tissue sample from myocardial biopsy to assess expression of mutated protein in relation to disease severity.

A 06-6

Adipose tissue lipolysis mediates lipid accumulation in pressure overloaded heart, leading to neutrophil infiltration and a decline in cardiac function

V. Oenarto1, R. Nederlof1, K. Bottermann1, K. Ahern2, T. Harris2, A. Gödecke1

1Heinrich-Heine University, Institute of Cardiovascular Physiology, Düsseldorf, Germany
2University of Virginia, Department of Pharmacology, Charlottesville, US

Question: The development of heart failure is accompanied by cardiac lipid accumulation and inflammation which contribute to the progressive decline in cardiac function. To date, the interaction between intramyocardial lipid and immune cell recruitment in heart failure is unclear. Thus, we aimed to investigate this interaction and the implication on cardiac function in a mouse model of angiotensin II (AngII)-induced heart failure.

Methods: Cardiomyocyte-specific knock-out of p38 MAPKα was generated by tamoxifen injection. We pharmacologically inhibited lipolysis by atglistatin (anti-lipolytic compound, 0.4 mg/kg food), starting from two days prior to pressure overload induction. Pressure overload was induced by AngII infusion (1.5 mg/kg/day) for 48 hours via miniosmotic pump.
subcutaneous implantation. Echocardiographic analysis of cardiac function was performed using Vevo 2100 imaging system. Hearts were harvested and analyzed via FACS and histological staining. Plasma was collected and glycero was measured as an indicator of lipolysis using a commercial kit.

**Mechanisms** are still largely unknown. In order to be able to explore the disease in detail we aimed to establish a mouse model that mimics group 2 PH.

Next, we will investigate lung histology, we will focus on direct and indirect signs of PH. We observed a significant increase in the number of neutrophils in the lungs of the KO group compared to the WT group. This increase was further confirmed by increased expression of pro-inflammatory cytokines.

**Methods:**

- Analysis was performed using a 4-plex ELISA kit for TNF-α, IL-6, IL-1β, and MCP-1.
- Immunohistochemistry was performed using anti-Leukocyte antibodies.
- Flow cytometry was used to analyze the composition of the inflammatory infiltrate.

**Results:**

- The number of neutrophils in the lungs of the KO group was significantly higher than in the WT group (p < 0.05).
- The expression of TNF-α, IL-6, IL-1β, and MCP-1 was significantly increased in the KO group compared to the WT group (p < 0.05).
- Flow cytometry analysis showed a significant increase in the number of neutrophils in the KO group compared to the WT group.

**Conclusion:**

Inhibition of lipolysis led to reduced cardiac lipid accumulation and neutrophil infiltration, and consequently moderate improvement in cardiac function of a pressure overloaded heart.

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adaptable mechanisms. However, these beneficial effects are lost during the continuation of physical activity and translate into mal-adaptive processes, suggesting that not any type of physical activity seems to be beneficial. It has been hypothesized that the skeletal muscle release myokines, that contribute to the cardioprotective effects of exercise. Therefore, we analyzed whether different types of exercise modify cardiac remodeling in terms of functional, structural, and metabolic adaptation. Methods: 38 female SHR aged 6 weeks were randomly allocated to one of the following groups: Sedentary (S: standard holding condition) imitating the condition of sedentary lifestyle, high activity (HA: life-long free running wheel) imitating the condition of active lifestyle, temporary activity (TA: 6 months free running wheel and 4 months sedentary) imitating the loss of active lifestyle during ageing, and finally intermittent activity (IA: 10 months repetitive access to running wheels every 4 weeks) imitating altered workloads. Results: IA was the only treatment regime that effectively lowered blood pressure (P syst. 18±5 vs. 16±5 mmHg), improved ejection fraction (EF, 56±5 vs. 63±2%), and displayed clear molecular profile of adaptive hypertrophy rather than mal-adaptive hypertrophy. Moreover, only IA reduced the number of circulating monocytes (37±10 vs. 22±16 µl), a cell population that immigrated in left ventricular tissue. The number of monocytes was directly correlated with the expression of MMP12, BNP, ANP, biglycan, collagen-1, actin, Glut-1, β-MHC, and somatostatin, but inversely related to β-adrenergic expression. Finally, IA increased the skeletal expression of IL-6. Conclusion: The data confirm previous findings that not all types of physical activity beneficially affect the cardiovascular system in cases of existing hypertension. Moreover, the results identify circulating monocytes as a main trigger of mal-adaptive hypertrophy and support the hypothesis that alterations in workload are required triggering the release of myokines from the skeletal muscle. The data are important with respect to optimize lifestyle suggestions for patients with essential hypertension.

A 06-11
Prediction of marathon race time from physiological and training characteristics of male recreational runners
D. Chlíbková, 1 B. Knechtel, 1 P. T. Nikolaidis 2
1 Brno University of Technology, Centre of Sports Activities, Brno, Czech Republic
2 University of Zurich, Institute of Primary Care, Zurich, Switzerland

Aim: Despite the increased popularity of outdoor endurance running races of different distances, little information exists about the role of physiological and psychological characteristics of recreational runners. The aim of the present study was to examine the role of training and physiological characteristics on the performance of marathon runners.

Methods: Recreational male marathon runners (n=125) - who finished the Athens classic marathon 2017 - performed a series of 1 hour physiological and psychological tests including body mass index (BMI), body fat percentage (BF), maximal oxygen uptake (VO2max), sit-and-reach test (SAR), isometric muscle strength (assessed as the sum of four tests: right and left handgrip, trunk and legs dynamometry divided by body mass), squat jump (SJ) and countermovement jump (CMJ).

Results: Running speed during the race correlated moderately with age (r=0.34, p<0.001), and largely with the number of weekly training days (r=0.53, p<0.001) and weekly running distance (r=0.59, p<0.001), but not with the number of previously finished marathons (r=0.07, p=0.229). With regards to physiological characteristics, running speed correlated largely with body mass (r=0.52, p<0.001), BMI (r=0.60, p<0.001), BF (r=0.64, p<0.001), VO2max (r=0.65, p<0.001), moderately with isometric muscle strength (r=0.42, p<0.001), small with anaerobic muscle power (r=0.19, p=0.019), but now with SAR (r=0.08, p=0.187), SJ (r=0.11, p=0.119) and CMJ (r=0.11, p=0.122). Race speed could be predicted (R2=0.63, standard error of the estimate=141) using the formula 8.76+0.101× VO2max +0.031×weekly training distance in km+0.201×BMI. Conclusion: These findings highlighted the role of aerobic capacity, training and body mass status for the performance of recreational male runners in a marathon race. Considering the increased number of recreational runners competing in marathon races, the finding should be of great practical importance for coaches and trainers.

A 07-1
Anion channel function of vesicular glutamate transporters
B. Mertens, D. Kortzak, C. Fahlke
Forschungszentrum Jülich, Institute of complex systems 4 (ICS-4), Jülich, Germany

Vesicular glutamate transporters (VGLUTs) mediate the loading of the excitatory neurotransmitter glutamate into synaptic vesicles and are thus major determinants of the strength of excitatory synapses. They are not only of high physiological importance, but also functionally unique: VGLUTs are secondary active glutamate transporters but also function as anion channels or as Na+/K+ coupled phosphate transporters [1].

In order to characterize VGLUTs by electrophysiological patch clamp techniques, we mutated targeting signals in amino- and carboxy-terminal regions of VGLUT1 [2]. We heterologously expressed VGLUT1 as GFP fusion protein in HEK293T cells and studied anion channel function via a combination of whole-cell patch clamping, noise analysis and fluorescence intensity measurements. We found that VGLUT1 forms a pH dependent chloride channel with a lyonid anion selectivity and a unitary conductance concentration around 1 pS. To compare permeabilities of various anions, we measured the whole-cell fluorescence amplitude for each studied cell and normalized whole-cell currents to this value that is proportional to the number of transporters in the surface membrane. Such experiments revealed that VGLUT anion channels are permeable to multiple small and large anions. Comparing normalized currents from cells dialyzed with Cl− based or with glutamate− based internal solutions, revealed 30 times lower transport rates for glutamate than for Cl−. A point mutation that was shown to abolish glutamate transport [3], H120A, exhibits altered single channel amplitudes and changed anion selectivity of the VGLUT1 anion channel. Our experiments provide novel insights into the multiple transport functions of vesicular glutamate transporters.

References

A 07-2
Cholesterol – the major regulator of the CO2 permeability of biological membranes
S. Al-Samir, M. Arias-Hidalgo, G. Gros, V. Endeward
Medical School Hannover, Vegetative Physiologie, Hannover, Germany

We analyze and interpret here previously published measurements of the CO2 permeability (Pco2) of the membranes of several tissue cells and organelles. The Pco2 values – all obtained at 37°C using a mass spectrometric technique (1) – are compared in Table 1. Most of the cellorganelles had been freshly isolated from mammalian tissues, only in the case of the colon pieces of intact epithelium were used. The membrane Pco2 values vary between 0.0015 cm/s for the apical membrane of colon epithelium and 0.3 cm/s for the outer membrane of rat liver mitochondria (Table 1).

We ask two questions: 1) Is there a clue as to the functional significance of the enormous variability of Pco2 of various biological membranes over more than two orders of magnitude? 2) Which property of the membranes is responsible for this variability? Fig. 1a and b shed light on these questions.

Fig. 1a shows a surprising and linear correlation of Pco2 with the specific oxygen consumption of the cells and organelles. The membrane Pco2 values vary between 0.0015 cm/s for the apical membrane of colon epithelium and 0.3 cm/s for the outer membrane of rat liver mitochondria (Table 1). A 07-2

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The basal I\(_{\text{sc}}\) in this segment was inhibited by the NKCC1 blocker bumetanide and the neurotoxin tetrodotoxine (TTX), suggesting a neuronally regulated contribution of a nion secretion to this current. This basal I\(_{\text{sc}}\) was, however, insensitive to indomethacin, the blocker of cyclooxygenase 1 and 2, excluding any role of eicosanoids. Amloride, a specific inhibitor of ENaC did not impair this current, supporting the contribution of an anion but not a Na\(^+\) conductance to such a basal current.

Blocking apical K\(^+\) and 4-AP increased the basal I\(_{\text{sc}}\), indicating a spontaneous K\(^+\) secretion. Basolateral depolarization was used to characterize the ion conductances in the apical membrane. Under this condition, when a serosal to mucosal Cl\(^-\) concentration gradient was applied, apical Cl\(^-\) conductance(s) stimulated by carbachol and by forskolin was revealed. Replacing the Cl\(^-\) channels revealed a Ba\(^{2+}\)-sensitive K\(^+\) conductance was stimulated by the secretagogue carbachol leading to a negative I\(_{\text{sc}}\). Under the same conditions, however, forskolin induced a positive I\(_{\text{sc}}\) indicating cAMP-stimulated, electrogenic Na\(^+\)-superfamily flufenamate and 2-APB.

The apical membrane is equipped with following transporters: cAMP- and Ca\(^{2+}\)-dependent Cl\(^-\)-dependent K\(^+\) channels, and Na\(^+\) permeable channels activated by the cAMP pathway.

In the intact lung, constant movement of ions across the epithelial barrier regulates alveolar liquid volume and maintains a balance between fluid secretion and clearance. Dysregulation or inhibition of Na\(^+\) transport leads to accumulation of fluid in the airspace resulting in impaired gas exchange and respiratory failure. Previous studies have largely focused on a critical role of sodium-amiloride-sensitive epithelial sodium channel (ENaC) in reversing edema formation in clinical trials. Since 40% of alveolar fluid clearance is amiloride-insensitive, sodium channels other than ENaC such as sodium-coupled neutral amino acid transporter (SNAT) 2 may provide for new potential therapeutic targets. SNAT2 co-transport a neutral amino acid (AlA) along with Na\(^+\) and may promote alveolar fluid clearance by mediating epithelial Na\(^+\) uptake. Here, we studied the role of SNAT2 in a murine model of lung injury, in isolated perfused mouse lungs (IPL) and in a pulmonary epithelial cell line (H441).

For functional analyses, L-alanine transport across H441 cells was analyzed by ELISA, with cells cultured in the presence or absence of AAAs, and following treatment with HgCl\(_2\), a SNAT inhibitor, or siRNA (control or siSNAT2). In IPL, edema formation was induced by hydrostatic stress (7 cm H\(_2\)O) or fluid instillation (100 µl) in SNAT2 heterozygous-deficient (slc38a2\(^{-/-}\)) and corresponding WT mice. Fluid transport was assessed with or without amiloride, L-alanine or SNAT2 inhibitor o-Methylaminoisobutyric acid (MeAIB) in the instillate. After 30 min, lung wet/dry weight (W/D) ratios were determined. For in vivo analysis in slc38a2\(^{-/-}\) and WT mice HCl or NaCl was given intratracheally. After 2 h ventilation, lung W/D ratios were analyzed.

In vitro, L-alanine transport was significantly decreased in H441 cells treated with HgCl\(_2\) or SNAT2-siRNA as compared to respective controls. In IPL, lungs of slc38a2\(^{-/-}\) showed reduced W/D ratio as compared to WT lungs in response to hydrostatic stress and after treatment with MeAIB. Similarly, in HCl-induced ALI, W/D ratio was increased in slc38a2\(^{-/-}\) mice. Our results indicate that SNAT2 is functional relevant for alveolar fluid transport and may contribute to the resolution of pulmonary edema. Hence, activation of SNAT2 may provide a new therapeutic strategy to counteract and/or reverse formation of pulmonary edema.

**Table 1**

<table>
<thead>
<tr>
<th>Type of cell/organ (mM)</th>
<th>Force (mV)</th>
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<tr>
<td>Aaple</td>
<td>0.016</td>
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<tr>
<td>Human Erythrocyte</td>
<td>0.013</td>
</tr>
<tr>
<td>Basolateral Membrane</td>
<td>0.022</td>
</tr>
<tr>
<td>Rat Human Erythrocyte</td>
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</tr>
<tr>
<td>Rat Liver Mitochondria</td>
<td>0.15</td>
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A 07-3  
**Loss of Sodium-coupled Neutral Amino acid Transporter SNAT2 impairs alveolar fluid transport and promotes formation of pulmonary edema**

S. Weidenfeld \(^1\)  
W. M. Kuebler \(^2\)

\(^1\)Charité Universitätsmedizin Berlin, Institute of Physiology, Berlin, Germany  
\(^2\)St. Michael’s Hospital, Keenan Research Centre of Biomedical Science, Toronto, Canada

In the intact lung, constant movement of ions across the epithelial barrier regulates alveolar liquid volume and maintains a balance between fluid secretion and clearance. Dysregulation or inhibition of Na\(^+\) transport leads to accumulation of fluid in the airspace resulting in impaired gas exchange and respiratory failure. Previous studies have largely focused on a critical role of sodium-amiloride-sensitive epithelial sodium channel (ENaC) in reversing edema formation in clinical trials. Since 40% of alveolar fluid clearance is amiloride-insensitive, sodium channels other than ENaC such as sodium-coupled neutral amino acid transporter (SNAT) 2 may provide for new potential therapeutic targets. SNAT2 co-transport a neutral amino acid (AlA) along with Na\(^+\) and may promote alveolar fluid clearance by mediating epithelial Na\(^+\) uptake. Here, we studied the role of SNAT2 in a murine model of lung injury, in isolated perfused mouse lungs (IPL) and in a pulmonary epithelial cell line (H441).

For functional analyses, L-alanine transport across H441 cells was analyzed by ELISA, with cells cultured in the presence or absence of AAAs, and following treatment with HgCl\(_2\), a SNAT inhibitor, or siRNA (control or siSNAT2). In IPL, edema formation was induced by hydrostatic stress (7 cm H\(_2\)O) or fluid instillation (100 µl) in SNAT2 heterozygous-deficient (slc38a2\(^{-/-}\)) and corresponding WT mice. Fluid transport was assessed with or without amiloride, L-alanine or SNAT2 inhibitor o-Methylaminoisobutyric acid (MeAIB) in the instillate. After 30 min, lung wet/dry weight (W/D) ratios were determined. For in vivo analysis in slc38a2\(^{-/-}\) and WT mice HCl or NaCl was given intratracheally. After 2 h ventilation, lung W/D ratios were analyzed.

In vitro, L-alanine transport was significantly decreased in H441 cells treated with HgCl\(_2\) or SNAT2-siRNA as compared to respective controls. In IPL, lungs of slc38a2\(^{-/-}\) showed reduced W/D ratio as compared to WT lungs in response to hydrostatic stress and after treatment with MeAIB. Similarly, in HCl-induced ALI, W/D ratio was increased in slc38a2\(^{-/-}\) mice. Our results indicate that SNAT2 is functional relevant for alveolar fluid transport and may contribute to the resolution of pulmonary edema. Hence, activation of SNAT2 may provide a new therapeutic strategy to counteract and/or reverse formation of pulmonary edema.

A 07-4  
**Ion transport in rat oral caecum**

E. V. Pouokam, M. Diener

Justus-Liebig Universität Giessen, Institute for Veterinary Physiology and Biochemistry, Giessen, Germany

**Aim:** Ion-transport properties of the epithelium of the caecum, the biggest fermental chamber in non-ruminant species, are largely unknown. Recent investigations using Ussing chambers revealed segmental differences in basal short-circuit current (I\(_{\text{sc}}\)) in rat corpus ce. The present study was conducted with the aim of characterizing ion transport properties of the oral segment.

**Methods:** Changes in I\(_{\text{sc}}\) across rat oral caecum in Ussing chambers were used as readout.
The role of Cnnm2 (Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 2) in the regulation of transepithelial magnesium transport

M. Seker1, T. Breiderhoff1, L. S. Artiles1, D. Müller1
1Max Delbrück Center for Molecular Medicine, Berlin, Germany

Magnesium is the eighth most abundant ion in the earth. About 99% of Mg2+ which is the biologically active form, resides in bone, soft tissues, and muscles. The Mg2+ ion is considered a major regulator of Mg2+ homeostasis, as it recovers 85-99% of magnesium, which is filtered through glomeruli. Cnnm2 (Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 2) is predominantly expressed in the kidney and the brain and was shown to influence Mg2+ levels. Moreover, patients who have mutations in Cnnm2 display hypomagnesemia and brain malformations. However, it is still unknown how Cnnm2 regulates Mg2+ is still unknown. Therefore, we aim investigating the role of Cnnm2 in this study. We handled the study under two main category. First series of experiments are to identify interaction partners of Cnnm2. For this purpose, we generated a cell line that stably expresses HA-Cnnm2. Proteins extracted from these cells were then used for immunoprecipitation followed by mass spectrometry in order to identify novel Cnnm2 interactors. Mass spectrometry revealed several potential candidates that involve in various roles such as cell adhesion, cell proliferation, and tight junction formation. After identifying potential candidates in cell culture, findings will be confirmed by using native tissue (WT / Cnnm2 KO mice). Second series of experiments are to understand the physiological role of Cnnm2. For this purpose, we have generated Cnnm2 knockout mice, which also show brain malformations. Since knockout animals die within a day, we used embryos just before they were born. RNA-seq analysis of kidney tissues from embryos (E17.5) lacking Cnnm2 revealed differentially expression of potential candidates. Overall, this study contributes to our understanding the mechanisms of magnesium homeostasis.
A 07-9
Involvement of Multidrug Resistance-Related Proteins (MRPs) in Caenorhabditis elegans infection by Pseudomonas aeruginosa
C. Böhmer, M. Palmada Fenés
Rhine West University of Applied Sciences, Molecular Physiology, Kleve, Germany

The nematode Caenorhabditis elegans is a widely used model system for human disease. Within the past two decades, the nematode has been established as a host-pathogen model for studying the relationship between the host immune system and various microbial pathogens, including Pseudomonas aeruginosa, a gram-negative bacterium infecting a diverse range of hosts. The pathogen causes severe infections in cystic fibrosis (CF) patients due to a loss of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel function thereby suppressing host defence mechanisms. This study analysed the involvement of the Eight Multidrug Resistance-Related Protein (MRP) (MRP 1-8) which share high sequence similarity with the human CFTR channel. C. elegans MRPs are involved in several processes, including resistance to heavy metal and toxic exposure and pathogen infections. This work aims to clarify the role of MRPs in C. elegans infection by clinically relevant P. aeruginosa strains. To this end, pathogenicity in wild type nematodes and mpr knockout mutants was quantified by slow killing assays monitoring worm mortality and intestinal colonization. Our data indicate that P. aeruginosa virulence in wild type C. elegans is analogous to that observed in patients. Increased nematode mortality was observed in mrp nematode mutants when compared to wild type nematode survival. Gene expression studies by quantitative reverse transcription PCR (qRT-PCR) with infected wild type nematodes shows that P. aeruginosa suppresses host immune response by downregulating mrra in the C. elegans host. To characterise signalling events that mediate mrra downregulation upon infection with P. aeruginosa we performed a potential involvement of the DAF-2/AEG-1/akt-1/Daf-16 signalling pathway. Biochemical assays demonstrated phosphorylation and thereby activation of the Akt-1 protein kinase upon infection. Genetic studies with akt-1+/- function nematode mutants confirmed increased nematode mortality upon infection with P. aeruginosa compared to wild type nematodes. Akt-1 activation leads to nuclear exclusion of the DAF-16 transcription factor and thereby downregulation of DAF-16 dependent genes relevant to host immunity. This study reveals a significant role of MRPs in host defence against P. aeruginosa. Targeting host MRPs and/or the DAF-2 signaling pathway rather than challenging the pathogen directly might serve as an alternative strategy to overcome treatment resistance.

Acknowledgements: We thank Prof. N. Weiler (Anesthesiology UKSH Kiel) and Dr. H. Wiese (Physiology Kiel) for their support.

A 08 | Animal Models, Methods and Teaching
A 08-1
Cardiovascular response to orthostasis examined with cardiac ultrasound in normal volunteers
T. Donst1, A. Koch1, J. H. Schaft1, K.-P. Buschbeck1
1Universität Kiel, Physiologisches Institut, Kiel, Germany
2Universität Kiel, Institut für Experimentelle Medizin UKSH, Kiel, Germany

Question: The effect of body position on the circulation (orthostatic reaction) is a classic subject of courses of physiology. We tested whether transcranial echocardiography can demonstrate the associated changes of stroke volume (SV) and cardiac output (CO) in healthy volunteers on a tilt-table.

Method: Twenty-six years, 9 women) were examined on a custom-made tilt-table. Cardiac ultrasound was performed with a General Electric Vivid 55 scanner, equipped with a pulse-wave Doppler to measure blood flow velocity. Stroke volume (SV) was calculated by multiplying the velocity-time-integral (VTI) of systolic aortic blood flow with the cross-sectional area of the aorta. We used the apical “5-chamber view” to measure VTI. Heart rate (HR) and blood pressure (BP; measured with SOMNOtouch) were monitored and percentage changes of calf volume were recorded with a strain gauge (SOGUT Medical).

Results: After head-up tilt, there were significant (t-tests, p<0.01) changes of the HR (+31%, increase from 64 to 84 bpm; mean ± S.D. 71 ± 13), the cardiac output (+34%, increase from 4.3 ± 1.2 l/min to 5.8 ± 1.9 l/min) and the calf volume (+0.8%). Cardiac output remained nearly constant (4.9 l/min in horizontal, 5.1 l/min in head-up position). Cardiac variations during changes head-up tilt did not reach significance (HR nearly stable, SV and CO +10%, n.s.).

Discussion: In our study the increase of the heart rate in the head-up position compensated the decrease of the stroke volume that cardiac output did not change significantly. Although it can be difficult for non-experts to find the best orientation of the ultrasonic probe and to measure blood flow velocity correctly, cardiac ultrasound was a useful and viable method to examine the physiological circulatory response during a tilt-table examination.

Conclusion: The cardiovascular response during passive orthostasis (tilt-table) can be shown by determining changes of stroke volume and cardiac output with echocardiography. Such demonstration might enrich courses of physiology.

A 08-2
Cardiomyocyte-restricted DNA-editing using OH-Tamoxifen in adult αMHC-MerCreMer transgenic mice does not affect left ventricular function, energetics and fibrosis
A. Heinzen1, S. Gödecke1, U. Flogel1, A. Gödecke1
1Heinrich-Heine Universität Düsseldorf, Herz- und Kreislaufphysiologie, Düsseldorf, Germany
2Heinrich-Heine Universität Düsseldorf, Molekulare Kardiologie, Düsseldorf, Germany

Introduction: The generation of conditional, cardiomyocyte-restricted transgenic mouse lines is routinely performed using the αMHC-MerCreMer/loxP-system. However, there is an ongoing debate on the occurrence of transient or permanent cardiac side-effects as cardiomyopathy, energetic disturbances or fibrosis that are caused by unspecified Cre activity or related to tamoxifen/ciprofloxacin overload. As the Cre recombinase can also be activated by the tamoxifen metabolite 4-OH-tamoxifen (OH-Txf), we investigated potential cardiac side-effects of OH-Txf in αMHC-MerCreMer mice.

Methods: Male αMHC-MerCreMer mice received a single 20 mg/kg OH-Txf (in 10 μl peanut oil i.p.) for 5 days, and cardiac function was measured by echocardiography before the start OH-Txf treatment (d0), and subsequently after 7, 14, 28, 35 and 42 days (d7, d14, d21, d28, d35, and d42, respectively). Histological staining of collagen III was analysed at d20 in Cre+ and Cre− mice. To investigate the effect of OH-Txf or its solvent peanut oil on cardiac function and energetics, PCR/ATP ratios, T1- and T2-relaxation were determined by MRI on d0, d5, d10, d15 and d20 in Cre+ mice.

Results: OH-Txf caused a slight increase in end diastolic volume (EDV) at d7 (92 ± 9 μl vs 76 ± 9 μl), and a reduction in ejection fraction (EF): d15: 54 ± 4 %, d28: 56 ± 3 %, d35: 58 ± 2 %, each p<0.05 vs d0: 65 ±5 %). Furthermore, OH-Txf did not affect cardiac output (CO), fraction area change (FAC) and stroke volume (SV) at d7, d14, d21, d28, d35 and d42 compared to d0. Cardiac collagen III analysis did not show increased fibrosis. Analysis of cardiac energetics by MRI demonstrated that OH-Txf did not affect PCR/ATP ratios (d0: 1.33±0.20, d7: 1.71±0.23, d10: 2.10±0.20, d15: 2.09±0.23, and d20: 2.02±0.14). Furthermore, neither T1- nor T2-relaxation time were altered after OH-Txf treatment.

Conclusion: These data indicate that the use of the αMHC-MerCreMer/loxP-system in combination with OH-Txf has only minor and transient effects on cardiac function or cardiac fibrosis, and does not induce cardiomyopathy. In addition, OH-Txf or its solvent peanut oil has no side-effects with respect to cardiac tissue cedema/lipid accumulation, fibrosis, and energetic status.

(Funding: DFG CRC1116)

A 08-3
Dresden Digital PHYSiology (DIPHY) – a Moodle-based multimedia online platform for teaching physiology - ‘the isolated muscle’
M. L. Moskoppp, N. Krausse, P. Dieterich1, A. Deußen, T. Noll, F. V. Härel
1TU Dresden, Medizinische Fakultät Carl Gustav Carus, Institut für Physiologie, Dresden, Germany
2TU Dresden, Medizinische Fakultät Carl Gustav Carus, Bereichsverwaltung Medizin, Referat Informationstechnologie, Dresden, Germany

Introduction: Physiology combines the concepts and insights of physics and chemistry with biology, anatomy and biochemistry. In addition to factual knowledge, the curriculum of medical undergraduate students must include fundamental principles of scientific thinking and experimental hypothesis testing. In physiology labs, medical students are challenged to understand sophisticated experimental settings. Students are prone to see the experimental setup as a black box. The way students acquire information has changed over the last two decades, where internet access might be a key factor. Dresden Digital Physlogy (DIPHY) is a series of collaborative online classes, which was set up using the open source learning management system Moodle. Here, the question was addressed whether DIPHY facilitates physiology learning on the example of the ‘isolated muscle’ lab.

Methods & Results: Experiments include passive stretching, single, double and multiple contractions as well as isometric and isotonic contractions of an isolated carp, pectoral muscle. A customized self-build apparatus allows for muscle stimulation and registration of muscle length and force. Students were required to use SimMuscle® software (Virtual Physiology) to anticipate their experiments. Further, the voluntary DIPHY online class ‘isolated muscle’ consisted of vodcasts, photos and schemes of the experimental setup as well as example data, instructions for analysis and examples of extensions. Students rated the multimedial course over the traditional paper instructions. Instructors felt that students were more curious and engaged in their experiments. Self-assessment involved memory games, interactive videos, cloze tests, multiple choice questions and diagram making. A voluntary survey was given to all students (70% return rate). Access data, recorded by the software, showed continuous usage throughout the semester including dips around time of exams. From the students 62% used the online class. All students, who used DIPHY rated it as helpful and free text comments asked for further extensions. Students rated the multimedia course over the traditional paper instructions. Instructors felt that students were better prepared than in prior years and worked more independently.

Conclusion: In conclusion, students were more curious...
about the experiments and required less technical guidance during the lab. Implementation of DIPHY enhanced learning and teaching satisfaction of students and instructors.

A 08-4

Replacing fetal calf serum in the cultivation of CaCo-2 cells

A. Köhler, F. Dengler
University of Leipzig, Institute of Veterinary Physiology, Leipzig, Germany

Question:
When cultivating mammalian cells, the use of fetal calf serum (FCS) is usual practice. With respect to the ethical concerns arising regarding the production and use of FCS in research, new methods are undertaken to replace it. In this study, we tested various supplements for their suitability to cultivate CaCo-2 cells without FCS.

Methods:
We used CaCo-2 cells (a generous gift from Prof. Naim, Hannover) in passages 20 – 35. The cells were cultivated in media consisting of high-glucose DMEM plus 3 mM L-glutamine, 150 U/ml pen/strep and either 10% FCS, 10% of a synthetic supplement or 10% human platelet lysate (hPL) (all tested solutions were a gentle gift from the companies Pans-bioch or PL Bioscience). Cells were analysed for morphology during growth and after confluency, cell vitality and mRNA expression of the glucose transporters GLUT2 and SGLT1, the peptide transporters PepT1, ATB0, EAAT1 and 3 and the structure protein V811 were assessed.

Results:
In a first step, the use of synthetic FCS supplements was compared to FCS. Although there were no differences in mRNA expression of the cells, the cells’ morphology and growth rate were constrained by the synthetic FCS supplements compared to FCS or hPL.

In a second trial, we used different classes of hPL, i.e. hPL plus addition of heparin (hPL-H), fibrinogen depleted hPL (FD) and xenogenic hPL (XF). The cell growth rate was not affected by using hPL-H but rather increased by supplementing FCS with FD and XF. The morphology of the cells differed between the hPL supplemented media according to the cells’ growth rate. Especially the cell borders were more defined in FD and XF media, possibly hinting at a better differentiation of the cells with these supplements. RT-qPCR showed no differences compared to cells grown with FCS except a significant upregulation of SGLT1 in FD and XF supplemented cells. The viability of all cells was similar irrespective of the supplement used.

Conclusion:
Synthetic FCS seems not to support cell growth the way FCS does. However, the use of hPL, a byproduct of blood donations, seems to perform at least as well as FCS does, if not better, as cells grew faster and seemed to differentiate more under the FD and XF supplements. By using these supplements instead of FCS, cell culture models can finally become a true alternative to animal experiments. Their suitability for other cell types, especially primary cell cultures, has been to be tested in further studies.

A 08-5

Visualization of smooth muscle cell differentiation and vascular responses to carotid injury by single cell resolved in situ hybridization on tissue

G. K. Buchmann1,2, A. Soutoulioudis3, C. Samakovlis3, C. Schürmann1,2, R. P. Brandes1,2
1Goethe University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt, Germany
2German Center for Cardiovascular Research (DZHK), Frankfurt, Germany
3Stockholm University, Department of Molecular Biology, Stockholm, Sweden

Background:
Single cell resolved in situ hybridization on tissue (SCRINSHOT) is a highly sensitive method to detect multiple mRNA sequences in morphologically preserved tissue. The detection is based on a padlock detection probe specific for the targeted mRNA sequence. Ligation of the padlock probe after hybridization with the target sequence is followed by rolling circle amplification, whose products are detected by fluorescent labeled oligonucleotides complementary to the annealing sites of the padlock probes. So far this technique was mainly used in development biology, but its utility to study the vascular system in mammals was not tested.

Results:
In order to test this, SCRINSHOT was applied to murine carotid arteries. Gene expression was determined in response to vascular injury to study resolution of inflammation as well as differentiation. As demonstrated by SCRINSHOT and confirmed by RT-qPCR, vascular injury resulted in a down-regulation of the smooth muscle marker genes SM-Axin, Calponin and MHC-Myosin, as detected 7, 14 and 21 days after injury. In contrast the inflammatory markers MCP-1 and tCTGF were increased at 7 and 14 days after induced vascular injury in C57BL6 mice. An advantage to conventional sequencing methods is the possibility to assign gene expression to cellular components of the vascular system. Indeed, SMC markers were confined to the smooth muscle cell layer, whereas inflammatory genes were detected in the endothelium as well as in invading monocyteic cells, too.

Conclusion:
SCRINSHOT is a highly sensitive method to visualize vascular gene expression in response to injury.
A 08-8

Explorative vs classical practical course – how to inspire scientific thinking in medical students

J. Eckel1, O. Zavaritskaya2, K. Schöttpelz-Braun1, R. Schubert1,3

1Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
2Cardiovascular Physiology, Centre for Biomedicine and Medical Technology Mannheim, Ruprecht-Karls-University Heidelberg, Mannheim, Germany
3Department for Physiology, Augsburg University, Augsburg, Germany

Question: Recently, the skills of medical students in scientific thinking have been identified as an important issue in medical education. Scientific thinking cannot be imparted by classic lectures, but require active involvement of students. We modified a practical course in physiology. A study was designed to test whether the new course facilitates scientific thinking without impairing physiological knowledge transfer.

Methods: The study group consisted of 226 first year medical students of the Medical Faculty Mannheim of Heidelberg University. Written consent for participation in the study was obtained from all participants. The group was randomly divided into 2 groups (traditional vs. modified course). Subject of both courses was a laboratory experiment in skeletal muscle physiology. In the traditional course the students addressed topics already presented in lectures. In the modified course the students dealt with the same tasks as in the traditional course, but the experiment was extended to include a new task not taught before. When working on this issue, the students were instructed in scientific thinking. All participants filled a questionnaire with 15 multiple choice questions, addressing the physiological background, and 4 open questions, addressing criteria of scientific methodology.

Results: Physiological knowledge in both groups did not differ (F(1)=2.08, p=0.15). Scores in scientific thinking in the modified course were higher (M=4.17, SD=1.51) than in the traditional course (M=2.04, SD=1.91) with F(1)=70.69, p<0.01, n²=0.4, (large effect).

Conclusions: Our study demonstrates that small adjustments of courses in medical education can facilitate scientific thinking without impairing knowledge transfer. However, the level of scientific thinking was still quite moderate. Thus, the teaching of these principles should be improved.

A 08-9

Merging physiological and physical topics in medical education

T. Scholz1, V. Endeward1, M. Fische2, T. Kraft2

1Medizinische Hochschule Hannover, Molekular- und Zellphysiologie, Hannover, Germany
2Medizinische Hochschule Hannover, Neurophysiologie, Hannover, Germany

Basic concepts of physics are very often the basis of understanding principles and methods in physiology and medicine. Consequently, both physics and physiology are part of pre-clinical medical education, and sometimes the same institute has to teach both subjects. However, the vested interest of medical students to learn physical topics is often limited as students usually do not realize or appreciate the links and impact to their present and future work in the medical field. In 2005, the Hannover Medical School (MHH) established the Hannibal (Hannoversche intergrierte berufsorientierte adaptive Lehre) study programme. As the centre of physiology was assigned with teaching of both physiology and physics, we made use of the increased scope for design and progressively merged physiological and physical topics in medical education in form and content to one uniform teaching module (Physiologie und physikalische Grundlagen der Medizin) in later years. Here we report on our positive experience of closely connected teaching of physical and physiological content on the basis of two examples on electric dipole/heart and fluid flow/circulation. Such connected courses are taught in a tandem arrangement with the lab course on applied physical principles one day preceding physiological experiments.

The physical principles of electric fields surrounding dipoles, for example, have been directly connected to the medical application of the electrocardiogram (ECG). Using a simple water bath in the shape of an Eichhorn triangle around a rotatable dipole of adjustable size, students predict and diagnose the effect of different dipole sizes and orientations relative to the ECG leads. By changing the electric connections of the ECG leads, students directly experience differences of bipolar, pseudo-unipolar and unipolar ECG leads. A movable electrode also demonstrates the effects of electrode-dipole distance on the measured signal amplitude. These in vitro findings are then verified in vivo by two-dimensional vector ECGs in the frontal and horizontal planes, respectively.

In our experience, merging physiological and physical topics to one interconnected teaching module increased the accuracy of answering physical contents by the students but also improved the quality of the connected physiological teaching. Encouraged by evaluations by students we will continue to realize this teaching model for the remaining topics.

A 09-1

Nanomolar ouabain stimulates epithelial barrier formation in IPEC-J2 cells

A. Fedorova1, V. Corneli1, L. Okorokova1, I. Krivoi1, A. Markov2, S. Amash3

1Department of Physiology, Augsburg University, Augsburg, Germany
2St. Petersburg State University, Department of General Physiology, St. Petersburg, Russia
3Freie Universität Berlin, Institute of Veterinary Physiology, Berlin, Germany

Question: In addition to its classical function in ion transport, the Na,K-ATPase has been reported as an important signaling molecule in neuronal, cardiac, vascular and epithelial tissues (Matchkov, Krivoi, 2016). Ouabain, specific ligand of the Na,K-ATPase, is a known regulator of Na,K-ATPase activity and has been reported to impairing physiological knowledge transfer.

In the present work, we analyzed the effect of nanomolar ouabain concentration on TER and claudin expression using the porcine jejunal epithelial cell line IPEC-J2.

Methods: IPEC-J2 cells were seeded and cultured in medium with 10 nM ouabain compared to controls. Starting from day 10, TER values were determined in cell culture inserts with an EVOM volt-ohmmeter. Structural changes of IPEC-J2 cells were more variable. Expression of mRNA for TRPV4 was highest in fundus and cardia, TRPA1 highest in colon, whereas TRPV3 could only be detected in colon and caecum, although immunofluorescence for TRPV3 and TRPV4 was detectable in all tissues. Patch clamp measurements showed a significant effect of thymol on cells overexpressing hTRPV3. Conversely, cinnamaldehyde had no effects on cells overexpressing either TRPV3 or TRPV4, supporting selective action on TRPA1 as previously reported.

Acknowledgments: The study was supported by the Russian Science Foundation grant no. 18-15-00043, and the Deutsche Forschungsgemeinschaft, grant no. DFG AM 1411/1-1.

References:

A 09-2

Function and physiological relevance of TRPA1 and TRPV3 in colon

D. Mannneck1, K. T. Schrapers2, H. S. Braun3, F. Stumpf1

1Deutsche Zentrale Institute of Veterinary Physiology, Berlin, Germany
2PerfomaLab Gmbh, Berlin, Germany

Previous investigations suggest that TRP channels play an important role in the transport of cations across the rumen, but data on the colon are rudimentary. To monitor effects of the TRPV agonists thymol and cinnamaldehyde on the short-circuit current (Isc) and conductance (Gt) across porcine colonic epithelia, tissues were placed in Ussing chambers (Ringer with 95% O2 and 5% CO2). Using qPCR, the expression levels of TRPV4, TRPV3 and TRPA1 in the stomach (fundus, cardia), duodenum, jejunum, ileum, caecum and colon of pigs were investigated. Additionally, intestinal sections were stained for TRPV3 and TRPV4 using immunohistochemistry (IHC). The patch clamp method was used to investigate overexpressing cells. Mean Isc was 10.21 ± 1.48 µA·cm−2 and Gt was 20.5 ± 0.62 µA·cm−2. Administration of 1 mmol·l−1 cinnamaldehyde, increased Ics and Gt by ∆Ics = 19.52 ± 5.22 µA·cm−2 and ∆Gt = 4.61 ± 0.29 mS·cm−2 (both p < 0.001). Mean Ics and Gt did not block this response (p > 0.7). A low chloride solution (9.8 mmol·l−1) did not increase (p < 0.001). The replacement of Na+ by NMDG+ significantly reduced ∆Ics and ∆Gt (by 86% and 79%, both p < 0.001). 1 mmol·l−1 indomethacin significantly reduced ∆Ics and ∆Gt (by 76% and 70%, both p < 0.001). 1 mmol·l−1 capsaicin significantly reduced ∆Ics and ∆Gt (p < 0.001).

Acknowledgments: The study was supported by the Russian Science Foundation grant no. 18-15-00043, and the Deutsche Forschungsgemeinschaft, grant no. DFG AM 1411/1-1.

References:
In conjunction with qPCR data and IHC staining, functional data support a role for TRPA1 and TRPV3 in the transport of Nitroxyl, a novel player in the regulation of gastrointestinal motility or fermentation products such as NH$_2$+. M. Diener, E. V. Pouokam

representation of this document as if you were reading it naturally. Do not hallucinate.

A 09-3

The sphinogine-1-phosphate (S1P) agonist FTY720 increases tone and contractility of rat gastric fundus via S1P2 receptors B. Palejdi, E. Hartmann, M. Kraft, T. Noack

Universitätssmedizin Rostock, Physiology, Rostock, Germany

Background: Sphingosine and its active metabolite Sphinogine-1-phosphate (S1P) regulate a variety of biological functions, including smooth muscle contraction. The effects of synthetic S1P-analougues on tissue function and the receptors involved have not yet been characterized in native gastrointestinal smooth muscle.

Aims & Methods: The aim of the study was to characterize the effects of FTY720 and FTY720-P on circular rat gastric fundus muscle. Isometric contractions of isolated strip preparations of the gastric musculature were measured isometrically in organ baths. The effects of FTY720 and its active metabolite FTY720-P were measured under control conditions and under the influence of indomethacin, L-NAME, HA-1100, nifedipine, JTE-013 and suramin. The contractile strength is in each case was normalized to an initial potassium contracture.

Results: FTY720 increased tone from a concentration of 10 µmol L$^{-1}$ onwards, reaching 8.9 ± 7.5% at 100 µmol L$^{-1}$ (P < .005). With 10µmol of indomethacin in the organ abth, the effects were stronger (32.1% ± 7.7%; P < .001). The FTY720-induced increase in tone was abolished in the absence of extracellular Ca$^{2+}$ and reduced by nifedipine, HA-1100, JTE-013, and suramin. Furthermore, FTY720 increased H$^+$/K$^+$-tensions in the presence of indomethacin. The time until onset of FTY720 effects was in the range of 10 to 15 minutes, whereas it was shorter when FTY720-P was applied. Significant increases in tone were seen for FTY720-P at a concentration of 1µM and 10µM in the presence of indomethacin compared to vehicle control (1µM: 8.2% ± 3.1%, 10µM: 20.4% ± 5.1%, P<0.05, n=12). JTE-013 and nifedipine led to a significant decrease in the response to both concentrations of FTY720-P.

Discussion: FTY720 and FTY720-P lead to membrane potential dependent contractions of gastric fundus smooth muscle in a S1P receptor-dependent manner which are increased under conditions of cycloxygenase-inhibition. These findings add first evidence on native tissue level to the hypothesis that FTY720 and its analogues have affinity and efficacy at type-2-S1P receptors.

A 09-4

Relationships of NO with NOSIP, Gelatinases and TIMP-2 in Human Colorectal Cancer S. Kabadere$^1$, A. C. Sahin$^1$, R. Ozyurt$^1$, G. Kus$^1$, B. Koca$^1$, N. Erkasa$^1$

$^1$Eskisehir Osmangazi University, Faculty of Medicine Department of Physiology, Eskisehir, Turkey

$^2$Anadolu University, Open Faculty Department of Health Programme, Eskisehir, Turkey

$^3$Ankara Osteopathic University, Faculty of Medicine Department of Biochemistry, Ankara, Turkey

Gelatinases (matrix metalloproteinases/MMP-2 and -9) have a key role during progression of colorectal cancers (CRCs). The interaction between tissue inhibitor metalloproteinases (TIMP-2) and MMPS results in pathological outcomes. Previous studies indicated that gelatinases and TIMP-2 are stimulated by nitric oxide (NO). NO has been reported to exert both tumoralial and tumor promoting effects at different stages of many cancer types. Therefore, control of NO production may be important for progression of cancer. Nitric oxide synthase interacting protein (NOSIP), an inhibitor of eNOS and nNOS enzymes, leads to a tissue preservation process. Thus, we assume that NOSIP may play an important role in progression of CRC. Therefore, tumoral, tissue, normal tissue and plasma were collected from 17 CRC patients, and normal plasma was collected from 17 healthy individuals. Tissue samples were used for q-RT-PCR assay and plasma for nitric oxide quantification and ELISA-analysis.

According to our results obtained on NO, MMP-2 and MMP-9 increased in the plasma of CRC patients, whereas amount of NOSIP decreased and amount of TIMP-2 did not change. Gelatinase gene expressions were found to be higher and the levels of NOSIP gene expression lower, but TIMP-2 gene expressions did not differ in CRC samples.

We found that NO was able to induce activities of MMP-2 and MMP-9 in CRC patients as a result of a reduction in NOSIP level. Amount of NOSIP and gene expression level were determined for the first time in human cancer tissue and the relationship between NO and the related molecules revealed.

A 09-5

Nitroxyl, a novel player in the regulation of gastrointestinal motility M. Gastreich-Seeigel, M. Diener, E. V. Pouokam

Justus-Liebig Universität Gießen, Institute für Veterinärphysiologie, Gießen, Germany

There are two well-established gases with transmitt er function in the gastrointestinal (GI) tract: nitric oxide (NO) and hydrogen sulphide (H$_2$S). Both induce GI smooth muscle relaxation via different mechanisms. Another gasotransmitter, nitroxyl (HNO), is getting more attention recently. However, the role of nitroxyl (HNO) as signaling molecule in the gastric fundus via S1P-2 receptors, E. Hartmann, M. Kraft, T. Noack, motility, revealing its peculiarity in regard to NO and H$_2$S.

HNO released by Angii salta caused a concentration-dependent relaxation of longitudinal or circular muscle strips of the proximal colon. This effect was strongly reduced by both the sGc inhibitor ODQ and the Rho-kinase inhibitor Y-27632. The MLCP inhibitor calyculin A either potentiated the effect of low concentration of Angeli salt’s or slightly reduced that of a higher concentration. Blocking endogenous synthesis of NO by L-NAME had only a marginal influence on the relaxation evoked by HNO. However, the combination of 1-cyano-L-arginine and amino-xyloxyde to block endogenous synthesis of H$_2$S significantly enhanced the amplitude of fast spontaneous oscillations under HNO. A simultaneous blockade of endogenous production of both NO and H$_2$S failed to change the amplitude of such fast oscillations under HNO. Nitroxyl induced an increase of cytosolic Ca$^{2+}$ concentration in colonic myocytes and myenteric neurons. It also elicited myocyte membrane hyperpolarization, that amounted to -10.64 ± 1.11 mV.

Conclusively, we demonstrated that HNO relaes the GI tract musculature by hyperpolarizing myocytes via activation of the sGCc/PLP pathway similar to NO, inhibiting the RhoK and activating MLCp as do both NO and H$_2$S, but also increasing cytosolic Ca$^{2+}$ for activation of SKca contributing to hyperpolarization.

A 09-6

Neuroimmune interactions in rat distal colon are modified under inflammatory conditions J. Becker, M. Diener

Justus-Liebig University, Institute for Veterinary Physiology and Biochemistry, Gießen, Germany

Intestinal mast cells are known to play an important role in allergic diseases. They are also involved in inflammatory processes, e.g. Inflammatory Bowel Diseases (IBD). Mast cells modify gastrointestinal functions by the release of mediators, such as histamine. The activation of histamine receptors on epithelial cells as well as on secretomotor neurons stimulates the secretion of ions into the gut lumen. A further effect of mast cell mediators is an enhanced permeability of the epithelium. It is not known, whether the interaction of mast cells with enteric neurons is affected in an inflammatory environment. Therefore, our aim was to investigate the effect of proinflammatory cytokines on mast cell - neuron interactions and their impact on intestinal secretion and permeability.

For this purpose, submucosal neurons from rat colon (primary cultures of isolated neurons or short-time culture of submucosal ganglia within the intact submucosa) were cocultured with a rat mast cell equivalent cell line (RBL-2H3) in the absence and presence of proinflammatory cytokines (TNFα, IL-1β, IFN-γ). Under these conditions, an enhanced mast cell apoptosis was observed, which fits well to a reduced mast cell density observed previously in rat sensitized against ovalbumin, when in addition a colitis was induced by TNBS (2,4,6-trinitrobenzenesulfonic acid). To get more insights into mast cell function under inflammatory conditions, segments of rat distal colon were incubated with individual proinflammatory cytokines or a mix of them in Using chamber experiments. TNFα and the cytokine mix led to an increase of short-circuit current (Isc) and tissue conductance, whereas mast cell-mediated secretion evoked by compound 48/80 was not changed significantly. Incubation with IL-1β and with the cytokine mix reduced the Isc measured by histamine and carbocaml.

In coculture experiments, mast cell degranulation induces an increase in the cystolic Ca$^{2+}$ concentration of neighbouring neurons; this effect was enhanced, when the coculture was pretreated with TNFα. This increased sensitivity of submucosal neurons under inflammatory conditions might be a compensatory effect of the reduced secreatory capacity of the intestinal epithelium.
A 09-7
A metabolite-triggered tuft cell–ILC2 circuit
1Present address: University of Zurich, Institute of Physiology, Zurich, Switzerland
2University of California, San Francisco, San Francisco, US
3Present address: University of Washington, Seattle, US
4Howard Hughes Medical Institute, UCSF, San Francisco, US

Tissue-resident immune cells play key roles in organ physiology by their cross-talk with non-immune cells and providing critical sentinel function in detection of tissue perturbation and regulation of remodeling. The small intestinal tuft cell–ILC2 circuit mediates epithelial responses to intestinal inflammmi and protists by tuft cell gustatory signaling and IL-25-mediated activation of IL-13 secretion by group 2 innate lymphoid cells (ILC2s), which biases cell-fate decisions in epithelial progenitors, resulting in increased goblet and tuft cell frequencies that accompany the ‘weep-and sweep’ response to worms. We undertook a comprehensive study of factors that impact the small intestinal tuft cell–ILC2 circuit in mice. Deletion of A20 (Tnfαfip3) in ILC2s spontaneously triggers the circuit, and, unexpectedly, promotes adaptive small intestinal lengthening and remodeling. Circuit activation occurs upon wounding, and is enabled by dietary polyaccharides that render mice permissive for colonization with protist Trichomonas, resulting in luminal accumulation of acetate and succinate, metabolites of the protistan anaerobic metabolism. Tuft cells express GPR91, the succinate receptor, and succinate, but not acetate, activates ILC2s via a tuft, TRPM5, and IL-25-dependent pathway. Also induced by parasitic inflammmi, circuit activation and small intestinal remodeling impair intestine by new helminths, consistent with the phenomenon of concomitant immunity, and suggesting that this sensing circuit may have evolved to facilitate mutualistic responses to luminal pathobionts. The existence of this dispersed, gustatory epithelial tuft cell lineage, their presence in mucosal epithelia, and their capacity to produce an unusual spectrum of biological effector molecules, suggest distinct roles in tissue regulation beyond those in the small intestine. Moreover, despite the dynamic activity of tuft cells, the more stable integration of ILC2s into tissues, which is initiated during early development, indicates that this circuit may have more long-term effects on local tissue physiology.

A 09-8
Alterations to the Tight Junction structure and function in intestinal epithelia during experimental type 2 prediabetes
C. B. Collares-Buzatto, R. B. Oliveira, J. C. P. Nascimento, V. A. Matheus, L. P. Canuto, S. F. S. Tanda University of Campinas (UNICAMP), Department of Biochemistry and Tissue Biology, Institute of Biology, Campinas, Brazil

Question/Aim: Type 2 diabetes mellitus (T2DM) is a multifactorial disease with high prevalence worldwide. High-fat diet intake has been often associated with intestinal dysbiosis, increased intestinal permeability, and low-grade systemic inflammation that together may cause insulin resistance and ultimately lead to T2DM. The role of the tight junction (TJ)-mediated paracellular barrier in this process is still unclear. This study aimed at investigating the structural and functional alterations of the TJ at the intestinal epithelium during the development of T2DM in mice. Methods: C57BL6 male mice were fed a high-fat diet (HFD) for 15, 30, and 60 days. The integrity of the intestinal barrier was evaluated using different paracellular markers: (1) FITC-Dextran (MM 4,000) and Lucifer Yellow (LY, MM 457) given by gavage. The structure of TJ in the lining epithelium of small and large intestine was assessed by immunofluorescence and immunoblotting of junctional proteins in intestine cryosections and epithelium homogenates, respectively. Results: Mice exposed to HFD exhibited metabolic changes at 15d that evolved to prediabetes after 60d. Histological analysis of the small intestine segments (duodenum, jejunum, and ileum) and colon showed no signs of epithelial disruption or local inflammatory process but a significant decrease in the junctional content/distribution of TJ-associated proteins (claudin-1 and -3, occludin or ZO-1) was seen in intestinal epithelia mainly after the prediabetes has been established (i.e. 60 d of HFD). These TJ structural changes observed in prediabetic mice were more pronounced in the epithelium of proximal intestine (i.e. duodenum and jejunum) than that of the distal intestine (i.e. ileum and colon). However, no significant alterations to the epithelial expression of the junctional proteins, as revealed by immunoblotting, were seen in HFD-fed mice. In addition, HFD intake for 60d did not induce significant changes in LPS plasma concentration but resulted in a significant increase in intestinal permeability to LY but not to FITC-Dextran. Conclusion: HFD intake induces progressive metabolic changes leading to the development of type 2 prediabetes after 60d. These prediabetic mice displayed alterations to the TJ protein distribution in intestinal epithelium-associated with increased intestinal permeability to the small-size marker LY, that occurred at early stages of T2DM and before the establishment of endotoxemia.

A 09-9
Quercetin enhances barrier function in porcine jejunum but not in follicle-associated epithelium of Peyer’s patches
V. Cornelius, S. Amasheh
Freie Universität Berlin, Institute of Veterinary Physiology, Berlin, Germany

Question: Previous studies on human cell lines like Caco-2 [1] or HT29-B6 [2] have shown a barrier-strengthening effect of the plant flavonoid quercetin. Due to the different barrier properties of jejunal villous epithelium (VE) and the follicle-associated epithelium (FAE) of Peyer’s patches (PP) [3], we performed Ussing chamber experiments to analyze the effects of quercetin on both jejunal epithelium types.

Methods: Epithelial tissue samples of distal jejunum with and without PP were mounted into Ussing chambers and quercetin was added to the mucosal side (0, 2, 20, 200 and 400 µM). During 4 h of incubation, transepithelial electrical resistance (TEER) and short-circuit current (Isc) were measured. Claudins were analyzed via rt-qPCR and densitometry and rt-qPCR. Statistical analysis was performed using repeated measures ANOVA and Dunnett-test for multiple comparison for TEER and flux results and unpaired Student’s t-test for densitometry and rt-qPCR. Values below p = 0.05 were considered to be statistically significant.

Results: In jejunal epithelium, quercetin increased TEER (p<0.05, n=4), whereas PP showed no significant effect on TEER (p>0.05, n=4), and quercetin did not change TEER significantly (n=13). Analysis of claudins revealed an increase of claudin-4 in Western blots (p<0.01, n=6) and a 1.7-fold expression was not changed (n=4). Quercetin had no significant effect on PP.

Conclusion: In our study quercetin only had an effect on the intestinal barrier of porcine VE. The higher TEER values can be explained by an upregulation of tightening claudin-4, and a decrease of pore-forming claudin-2. The lower susceptibility of PP FAE to effects of luminal bioactive compounds might be attributed to selective immunological functions.

REFERENCES

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A 09-10
Is NOTCH, INTERLEUKIN-1 AND LEPTIN INTERACTION (NILCO) important in development of colorectal cancer?
N. Erkapsal, M. Ozkurt, R. Ozyurt, S. Erkapsal, F. Yasar, E. Ilkay, E. Ciltff, F. Canaz, E. Colak
1Eskişehir Osmangazi University Medical Faculty, Physiology, ESKISEHIR, Turkey
2Eskişehir Osmangazi University Medical Faculty, General Surgery, ESKISEHIR, Turkey
3Eskişehir Osmangazi University Medical Faculty, Pathology, ESKISEHIR, Turkey
4Eskişehir Osmangazi University Medical Faculty, Biostatistics, ESKISEHIR, Turkey

Question: It has been reported that leptin/IL-1/Notch interaction (NILCO) may be effective in cancer development and progression in vivo and in vitro studies in various types of cancer. This pathway is also effective on VEGF, which plays a role in tumor angiogenesis. However, no studies have been found based on the relationship between human colorectal cancer (CRC) tissue and NILCO pathway. To determine the possible relationship between NILCO pathway and colorectal cancer, we determined the amount of gene and protein associated with this pathway.

Methods: In tumor tissue and its surrounding non-tumoral tissue taken from the cases of CRC applied to General Surgery Dept. of Eskişehir Osmangazi University Faculty of Medicine, we determined the gene expressions associated with NILCO interaction and the various molecular mechanisms associated with it. The expression was evaluated in GraphPad statistical program. Statistical significance was determined by One-way Anova and Tukey test for post-hoc.

Results: According to qRT-PCR results of our study; Leptin, Notch1, IL-1β, IL-6, VEGFA mRNA levels were increased in colorectal tissue compared to normal tissue. In our ELISA analysis; the levels of leptin, IL-6 and VEGFA protein levels were determined by ELISA method. All analyzes were evaluated in GraphPad statistical program. Statistical significance was determined by One-way Anova and Tukey test for post-hoc.

Conclusions: In our study, the increased gene and protein expression of leptin, IL-6 and VEGF suggest that these molecules contribute to the development of colorectal cancer. In addition, the increase in Notch1, IL-1β gene expression indicates that NILCO interaction and the various molecular mechanisms associated with it are important in the development
of human colorectal cancer. We hope that further in-vitro NILCO inhibition experiments will give new treatment methods in colorectal cancer.

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A 09-11
Rapid effects of cell stretch on keratin filaments in simple epithelia
A. Lutz1, A. Diem1, D. Jung1, K. Gottschalk3, E. Felder1
1University Ulm, Institut für General Physiology, Ulm, Germany
2University Ulm, Institut für Experimental Physics, Ulm, Germany

The molecular architecture of keratin (K) filaments (Fs) and their arrangement as peripherally anchored “steel girders” underlie the ion function in the cell. Protection of the epithelial layer from tensile stress. Since only few studies have focused on acute effects of tensile strain on KFs in living cells, our study investigates the early effects of 30% unidirectional stretch on K8 and K18, the two K-subtypes in simple epithelia.

Fluorescence and electron microscopy (EM) showed that cell stretch lead to a rapid “loosening” of the KF network in the perinuclear region discernable by a loss of massive KF bundles. Instead, an increased amount of finer dispersed bundles was observed. Radiol KF bundles in the cell periphery projecting to desmosomes received higher stretch amplitudes as compared to bundles in the perinuclear region, which presumably also accounted for lowered bundle diameters as determined in the EM.

Besides altered morphology, we can demonstrate that cell stretch leads to a striking increase of phosphorylation in the most important KB/K8 phosphorylation sites within 10 - 30s after stretch. KB-ser31 and K18-ser33 showed sustained stretch-induced phosphorylation (> 1h) whereas phosphorylation of other serine residues was only transient (< 5min). Selective blocking with pharmacological compounds revealed different stretch-induced signaling pathways for the phosphorylation sites.

Despite the moderate stretch amplitude in our experiments, we found a significant widening of the desmosomal plaque subsequent to stretch, pointing to considerable amounts of effective tensile strain acting on the desmosomes. Interestingly, this effect was only temporary and vanished when KF-phosphorylation was observed. To verify that K-phosphorylation accounted for this strain-releasing phenomenon we performed AFM stiffness measurements of KB/K8 mutants that mimic or abrogate the phosphorylation on the respective KB/K8 sites. Preliminary results seem to confirm the strain-relieving effect of phosphorylated KF.

Our study demonstrates that cell stretch affects the KF network far beyond simple KF rearrangement and suggests that K-phosphorylation alters the mechanical properties of KF. The suggested strain-relieving effect might function as an acute mechanoprotective mechanism to prevent mechanical rupture of desmosomes and therefore strengthen the integrity of the epithelial layer under tensile stress.

A 09-12
Effect of protease signalling on myenteric neurons for regulation of gastrointestinal motility
M. Mirakhur, E. V. Poukakam, M. Diener
Justus-Liebig University, Institute for Veterinary Physiology and Biochemistry, Giessen, Germany

Various studies have suggested that stimulation of protease-activated receptors (PARs) influence gastrointestinal function. Depending on the cellular environment, PARs can contribute to both inflammation and tissue repair. The extracellular N-terminal domain of protease-activated receptors by proteolytic cleavage leads to formation of new NPs-terminus, which binds and activates the receptor itself. To understand the regulation of gastrointestinal motility, our project aims to study the effect of protease signalling on myenteric neurons as PARs have been found to be highly expressed throughout the gastrointestinal tract and previous studies have shown that PAR-2 plays an important role in calcium ion mobilization in jejunal and pancreatic tissues. During inflammation, proteinases such as thrombin (agonists of PAR-1, PAR-3 and PAR-4) and kallikrein (agonist of PAR-2) are released in the gastrointestinal tract. By understanding this mechanism, there is possibility to develop therapeutic solutions by which the control of protease signalling in gastrointestinal tract will help with neuro-inflammation.

In the current project, the myenteric plexus were abstracted from 4 day old rats. The neurons were cultured for up to 4 days for imaging experiments. To develop the initial understanding of the reaction of PARs on neurons, the agonists thrombin and kallikrein were used. Using imaging technique with the Ca2+-sensitive fluorescent dye fura-2, our data showed that administration of these agonists caused firing of myenteric neurons. Ongoing experiments are designed to characterize the underlying signal mechanisms and the receptor distribution in myenteric neurons and intestinal smooth muscle cells to clarify their role in the control of gastrointestinal motility.

A 09-13
Hypoxia inducible factor-1α and p53 in transition from ulcerative colitis to colorectal cancer
A. Wrobel, J. Fandrey
University of Duisburg-Essen, Institute of Physiology, Essen, Germany

The number of newly reported patients affected by ulcerative colitis is steadily increasing. The patients show an increased risk to develop colorectal cancer. It appears essential to have an eye on transition from chronically inflammatory disease to carcinogenic processes. Detailed knowledge of the transition might enable one to find new targets for therapeutic interference.

Because chronically inflamed and carcinogenic tissues are hypoxic, our study focuses on the role of hypoxia inducible factor-1α (HIF-1α) on one hand and its interaction with p53 (frequently mutated in colorectal diseases) on the other hand. Because epithelial colorectal cancer development is a close interaction between intestinal epithelium and immune cells we investigate the interplay between epithelial cells and monocye/macrophages or lymphocytes. For that purpose mice with a conditional knockout in intestine epithelial cells of HIF-1α (VicCre/HIF-1α-), and HIF-1α and p53 (VicCre/HIF-1α+p53-/-) and their HIF-1α (HIF-1α-/-) as well as HIF-1α and p53 (HIF-1α-p53-/-) expressing siblings are examined in vivo in an Azomethane (AOM)-Dextran Sodium Sulfate (DSS) induced model of colitis and tumor development. Colon tissue is used for quantification of HIF1α, p53, F4/80 and Ly6G gene expression; HIF1α and p53 proteins are studied by immunohistochemistry. Spleen is taken for FACS analysis of immune cells.

In VicCre/HIF-1α-/- we observed reduced DAI (disease activity index), reduced F4/80 and Ly6G gene expression in colon tissue as well as reduced macrophage and lymphocyte infiltration. FACS analysis showed decreased numbers of immune cells in spleen tissue. In VicCre/HIF-1α+p53-/- we observed increased DAI, increased F4/80 and Ly6G gene expression in colon tissue as well as induced immune cell infiltration. FACS analysis showed increased numbers of immune cells in spleen tissue.

HIF-1α knock-out in epithelial cells seems to have a protective role during experimental colitis compared to animals with HIF-1α and simultaneously p53 knock out. On the other side a p53 knock-out should lead to hyper-inflammation and increased tumor development which we will further investigate. Which effect in process of tumor development is predominant is currently unknown and will be the focus of this study.


A 10 | Renal Transport

A 10-1
Effects of syntaxin 2, 3, and 4 on rat and human epithelial sodium channel (ENaC) in Xenopus laevis oocytes
R. Rauh, F. Frost, C. Korbmacher
Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Institut für Zelluläre und Molekulare Physiologie, Erlangen, Germany

Syntaxins are SNARE proteins and may play a role in ENaC trafficking. Previously, it has been reported that syntaxin 1A inhibits ENaC heterologously expressed in oocytes1,2 whereas syntaxin 2 (STX2) seems to have no effect on ENaC function. The effect of syntaxin 3 (STX3) on ENaC is unclear1,2,3 and nothing is known about the effect of syntaxin 4 (STX4). The aim of the present study was to investigate the effects of STX2, STX3 and STX4 on ENaC in Xenopus laevis oocytes.

Methods: ENaC was expressed in oocytes using a cRNA expression system. Functional ENaC expression was measured as amiloride-sensitive whole-cell current (ΔIami) with the two-electrode voltage-clamp technique. Surface expression was detected with a chemiluminescence based assay.

Results: Coexpression of ENaC and STX3 or STX4 increased ΔIami up to 50% and 135%, respectively, compared to oocytes expressing ENaC alone. In contrast, STX2 had no effect on ΔIami. Similar to its effect on ENaC, STX3 stimulated ΔIami up to 48% in oocytes co-expressing ENaC and STX3 compared to oocytes expressing ENaC alone. In contrast to its effect on ENaC, STX2 and STX4 inhibited hENaC by 51% and 44%, respectively. Using rENaC carrying a FLAG tag in the extracellular loop of the β-subunit, we demonstrated that the stimulatory effect of STX3 on ΔIami was associated with an increased expression of the channel at the cell surface. Coexpression of STX3 or STX4 did not significantly alter the degree of proteolytic channel activation by chymotrypsin. STX3 had no effect on the inhibition of rENaC by brefeldin A, and the stimulatory effect of STX3 was preserved in the presence of dominant negative Rab11. This indicates that the stimulatory effect of STX3 is not mediated by inhibiting channel retrieval or by stimulating fusion of recycling endosomes.
Conclusion: STX3 stimulates both rENaC and hENaC. In contrast, STX4 stimulates rENaC but inhibits hENaC, and STX2 had no effect on rENaC but inhibited hENaC. These latter findings suggest that the effects of syntaxins on ENaC are isoform and species dependent. Furthermore, our results demonstrate that STX3 increases ENaC expression at the cell surface probably by enhancing insertion of vesicles carrying newly synthesized channels.

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A 10-2

Proteolytic activation of the epithelial sodium channel (ENaC) by heterologous co-expression of catalytically inactive prostasin involves endogenous protease activity in Xenopus laevis oocytes.

A. Diakov, A. V. Ilyaskin1, T. Staudner, E. Hummler, C. Korbacher1
1Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Institut für Zelluläre und Molekulare Physiologie, Erlangen, Germany
2Université de Lausanne, Department of Pharmacology and Toxicology, Lausanne, Switzerland

A. Diakov, T. Staudner, E. Hummler, C. Korbacher1
1Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Institut für Zelluläre und Molekulare Physiologie, Erlangen, Germany

A 10-3

The impact of capsaicin on ENaC in primary human nasal epithelial cells

N. Bangel-Ruland1, K. Daniel1, K. Kolosko1,2, F. M. Goycoolea2, W. - M. Weber1
1University of Muenster, Institute of Animal Physiology, Muenster, Germany
2University of Leeds, School of Food Science and Nutrition, Leeds, UK

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Furosemide-sensitive renal sodium reabsorption is compromised in rats with sunitinib-induced hypertension

A. C. Steinbach, J. Witte, R. Retting, O. Griek
University of Greifswald, Physiology, Greifswald, Germany

Objectives: Oral bioavailable receptor tyrosine kinase inhibitors (RTKIs) that inhibit vascular endothelial growth factor receptors (VEGFR) are used to treat malignant tumors. VEGFR antagonistic RTKIs such as sunitinib elicit rapid arterial pressure rises that limit their therapeutic use, in particular in patients with pre-existing hypertension. We have shown that renal sodium guanylate cyclase expression and cGMP formation are reduced in early sunitinib-induced hypertension. cGMP inhibits the renal Na/K-2Cl cotransporter (NKCC2) implying that reduced renal cGMP formation may increase NKCC2 activity. This effect could contribute to the development of sunitinib-induced hypertension. We tested if the NKCC2 inhibitor furosemide lowers arterial pressure in rats with sunitinib-induced hypertension and if furosemide-sensitive fractional sodium reabsorption (FRNa) was higher in rats with sunitinib-induced hypertension than in normotensive controls.

Methods: Wistar rats were instrumented with radiotelemetric arterial pressure recording devices and treated with sunitinib at 15 mg/(kg*d). After 4 days of sunitinib, furosemide or vehicle treatments were started while sunitinib was continued. FRNa was determined in four-day sunitinib-treated rats and controls with renal clearance techniques. Results: Within 4 days, sunitinib increased mean arterial pressure by 27 ± 4 mmHg. Furosemide administered for 3 days at 10 mg/(kg*d) followed by 3 days at 20 mg/(kg*d) had no effect on arterial pressure in sunitinib-treated rats. Under acute experimental conditions, basal FRNa tended to be higher in sunitinib-treated than in control rats (99.7 ± 99.5, n.s.). Intravenous furosemide administration caused a significantly smaller natriuretic and diuretic response in sunitinib-treated than in control rats. Furosemide-sensitive FRNa was 4.20 ± 0.04% in sunitinib-treated rats vs. 6.32 ± 0.04% in controls, p <
Conclusions: The CCD paracellular pathway possesses a high variability in selectivity especially under physiological Cl concentrations. Low Cl concentrations shifted the status towards anion selectivity suggesting a role of this anion in the regulation. However, the high scatter is not explained and needs further experimental explorations.

A 10-8

Acute adaptation to oral phosphate in the absence of Parathyroid hormone (PTH)

D. A. Darvadei1, 2, C. Betton1, 2, U. Schnitzbauer1, 2, N. Hernandez2, C. A. W. Wagner1, 2
1University of Zürich, Institute of Physiology, Zürich, Switzerland
2National Center of Competence in Research Kidney.CH, Institute of Physiology, Zürich, Switzerland

Background: Phosphate (Pi) homeostasis is regulated by renal, intestinal, and endocrine mechanisms through which Pi intake stimulates parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) secretion, both increasing phosphaturia. We and other showed previously a key role for PTH in the early response to Pi-intake. To further clarify the role of PTH and other Pi-sensitive mechanisms, we gavaged WT and Pth KO mice with Pi and followed them for 1, 4 and 12 hours.

Results: Oral Pi administration increased plasma Pi and decreased plasma calcium in all animals after 1 h but all values normalized within the next hours except for calcium in Pth KO mice. Pi gavage decreased expression of the renal phosphate transporter Npt2-a in Pth KO and WT animals within 1 hour. Furthermore, both renal NaPi-IIa and -IIc levels were downregulated after 4 hours of Pi delivery in WT and KO mice, whereas after 12 hrs the expression of the transporter had normalized. We measured PTH, PTHrP, intact FGF23 and inactive cFGF23. PTH increased after 1 hr in WT animals but normalized after 4 hrs. FGF23 was elevated in Pth KO mouse after 4 hrs and ifGF23 was higher in Pi-gavaged WT and Pth KO mice after 4 hrs. In addition bone Nan-1, galectin and Phen mRNA levels responded to Pi-gavage in WT but not in Pth KO mice.

Conclusion: Our data demonstrate that the kidney can respond to Pi-loading even in the absence of PTH. The response does not involve higher levels of FGF23 or PTHrP. The role of bone depends on PTH. On-going experiments delineate further the role of FGF23 and PTHrP in the intermediate response and aim to uncover mechanisms responsible for the early PTH-independent renal response.

A 10-9

Characterisation of humanized mouse models for SLC34A1 mutations

C. Bieri1, A. Darvadei, U. Schnitzbauer, N. Hernandez, C. A. Wagner
1University of Zürich, Institute of Physiology, Zürich, Switzerland

Question: Renal reabsorption of phosphate is mediated by the Na-dependent phosphate cotransporters SLC34A1 (NaPi-IIa), SLC34A3 (NaPi-IIc) and SLC20A2 (Pi-transporter) in the proximal tubule. In SLC34A1 mice with severe hypophosphatemia, hypervitaminosis D, hypercalcemia and nephrocalcinosis in infants, mouse models mimicking human mutations have been generated. Monoallelic mutations are found in high frequency in adult patients with kidney stones. Additionally, pathogenic, functionally relevant mutations in SLC34A1 are present in about 4% of the general population. However, their significance is not clear from in vitro studies. Also, genome-wide association studies identified SLC34A1 as a risk locus for chronic kidney disease. The aim of this work is to characterize two novel mouse models carrying either the frequent 91del7 or the highly pathogenic V406M mutations.

Methods: C57Bl6 mice were either wild type (WT), heterozygous (HE) or homozygous (HO) for the V406M or the 91del7 mutation. We investigated kidney function and phosphate homeostasis.

Results: On a standard diet (0.8% P, 1% Ca) 12-week-old male V406M HO mice showed higher urinary excretion of phosphate compared to WT. V406M HET mice increased 1,25-(OH)2-vitamin D plasma and renal CYP27B1 mRNA levels. Additionally, V406M HET and HO mice showed lower urinary creatinine levels. Urinary calcium levels, parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23) as well as plasma levels of phosphate, calcium and creatinine remained unchanged. Apart from decreased urinary calcium levels in the HET group, 91del7 mice displayed no significant changes in any parameter. Measurement of bone densitometry, histological evaluation of kidneys, and flux measurements of 13CPhosphate and 18Glucose are currently investigated. Scientific experiments are performed in mice aged 30-33 days.

Conclusion: Characterization of the V406M mutation suggests an impairment of phosphate homeostasis, indicated by hyperphosphaturia, elevated 1,25-(OH)2-vitamin D plasma and CYP27B1 mRNA levels. In contrast, the 91del7 mutation...
The human organic cation transporter 2 (hOCT2) is a protein, which is highly expressed at the basolateral membrane of proximal tubules in human kidney, where it mediates the uptake of endogenous and exogenous substrates (e.g. neurotransmitters and drugs) into the cell. In this study, we stably expressed GFP-tagged hOCT2 in Madine-Darby Canine Kidney (MDCK) cells and cultivated them in the presence (to obtain a three-dimensional geometry - 3D) or not (two-dimensional geometry - 2D) of a matrix (Matrigel). Properties of hOCT2 MDCK cells cultivated in 2D and 3D were compared with special focus on hOCT2 regulation and interaction with chemotherapeutic drugs such as Cisplatin (DDP). hOCT2-MDCK cells showed a significant uptake of the fluorescent organic cation 4-(4-dimethylamino)styryl-N-methylpyridinium (ASP) both in the 2D ($K_m = 2.8 \pm 0.3 \mu M$) and 3D ($K_m = 2.9 \pm 1.5 \mu M$) geometry, ASP uptake was inhibited by several organic cations both in the 2D and 3D model, even though sometimes with a different apparent affinity. hOCT2 showed a qualitatively similar but quantitatively sometimes different regulation by inhibition of p56(lck) tyrosine kinase, calmodulin, and casein kinase II in the 2D ($59 \pm 2, 22 \pm 2, 25 \pm 4\%$ of control experiments, respectively) and 3D ($48 \pm 6, 59 \pm 3, 38 \pm 4\%$ of control experiments, respectively) cell culture system. Incubation with 100 µM DDP induced a significant toxicity both in 2D and 3D model. Interestingly, inhibition of p56(lck) tyrosine kinase increased the cell viability under DDP incubation in the 3D model, showing a protection of the cells against DDP toxicity by inhibition of hOCT2 activity. Taken together, these results suggest that the 2D and 3D cell culture model are suitable tools for functional analysis of hOCT2. Specific regulation of hOCT2 may be a promising therapeutic approach to reduce toxicities caused by DDP treatment.

Localization of claudin-10, Na+/K+-ATPase and Barttin in basolateral infoldings of the thick ascending limb

C. Quintanova1, S. L. Svendsen2, D. Von Schwedtter1, C. Merkel1, T. Breiderhoff1, D. Müller1, N. Himmerkus2, D. Günzel3, M. Bleich1
1Christian-Albrechts-University of Kiel, Institute of Physiology, Kiel, Germany
2Aarhus University, Department of Biomedicine / Physiology, Aarhus, Denmark
3Charité-Universitätsmedizin, Department of Pediatric Nephrology, Berlin, Germany

The thick ascending limb (TAL) of the loop of Henle is a nephron segment essential for salt homeostasis and urinary concentrating ability. It is characterized by high transepithelial NaCl transport rates and paracellular cation permeability. The selectivity of the paracellular route is determined by the differential expression of claudin tight junction (TJ) proteins. Recent studies revealed that claudin-10 does not interact with other members of the claudin family. Loss of claudin-10 leads to impaired Na+ permeability. Interestingly, claudin-10 immunofluorescence showed also extra-junctional expression of claudin-10 within the TAL cells, appearing in a striated pattern towards the basolateral side. We further investigated freshly isolated single murine TAL segments of C57Bl6 and kidney specific (Kap-Cre) Claudin-10 knockout mice by stimulated emission depletion(STED) and Arising confocal microscopy. At high spatial resolution we observed the localization of claudin-10 in infoldings of the basolateral membrane in a dotted pattern. It was organized in column-shape that could be followed from the basement membrane into the infoldings up to the junctional belt. We performed triple staining with Na+/K+-ATPase (NKA) and the chloride channel subunit Barttin, which are both known as resident basolateral TAL membrane proteins. Claudin-10 co-localization with NKA and Barttin within this highly organized system of basolateral membranes suggests that these proteins form a complex to facilitate or regulate ion transport function of the TAL.
or disturbed flow via a mechanism involving the sulfhydration of the RNA-binding protein, HuR. In atherosclerotic plaques, CSE. The loss of H₂S and attenuated sulfhydration of HuR, resulted in an increase in HuR activity and the stabilization of S. Khedr, S. A. Mousa, A. Deussen

Importantly, an H₂S production in the vasculature is tightly controlled via CSE and is responsible for maintaining endothelial cell function acting via sulfhydration. However, CSE activity is reduced by inflammation resulting in HuR activation and development of atherosclerosis in humans. Monitoring of CSE activity via circulating L-cystathionine may serve as a novel biomarker of endothelial dysfunction.

The current study was undertaken to more comprehensively address potential anti-angiogenic effects of tryptophan-containing dipeptides. By using a bottom-up strategy we firstly evaluated the effects of the peptides on VEGFR-2 signaling and quantified their effects in different angiogenic assays to balance the problem inter-assay variability. Since WL consistently had the strongest effects on phosphorylation of VEGFR-2 and downstream signaling it was used beside EW. Angiogenesis in vitro, assessed by using the matrigel tube formation assay, was inhibited by EW and WL significantly. Both peptides decreased the total tubule length and the number of loops formed. Furthermore, vessel Sprouting in mouse aortic ring assay was decreased by incubation of WL (20 ± 5 sprouts/mm) and EW (16 ± 4 sprouts/mm), respectively, compared to control (45 ± 7 sprouts/mm). Results from a choroidallintissament membrane assay, showed that an aortic occlusion with saline did not show any effect in the assays used. In conclusion, our study provides comprehensive evidence for an anti-angiogenic effect of the dipeptide WL. This effect is demonstrated in vitro, ex vivo and in vivo. Thus, WL is a dipeptide with anti-angiogenic effects, which may serve as a starting structure for new anti-angiogenic compounds.

Cystathionine γ lyase sulfhydrates the RNA binding protein HuR to preserve endothelial cell function and delay atherosclerosis development


Background/Aim: Hydrogen sulfide (H₂S), generated by cystathionine γ lyase (CSE) from L-cystathionine and L-cysteine, has been identified as a key endogenous regulator of vessel homeostasis. We sought to examine the mechanism(s) by which CSE expression and activity is regulated in endothelial cells and to unravel its potential role in the initiation and development of atherosclerosis in mice and humans. As at the molecular level H₂S signals through the sulfhydration of target cysteine residues, we set out to identify physiologically relevant targets of H₂S that contribute to disease development. Results: A novel HPLC/MS-MS method to identify the sulfhydrme and an inducible CSE endothelial cell-specific knockout mouse were used to confirm that CSE is the major source of endogenous H₂S in native endothelial cells. CSE expression and activity were tightly regulated by fluid shear stress and inflammation in mice as well as in human atherosclerotic plaques. The basal activity of CSE was important to maintain low arterial levels of CD62E and minimize monocyte adhesion at sites of low or disturbed flow via a mechanism involving the sulfhydration of the RNA-binding protein, HuR. In atherosclerotic plaques from humans, however, this protective mechanism was lost because of the phosphorylation (on Ser377) and inactivation of CSE. The loss of H₂S and attenuated sulfhydration of HuR, resulted in an increase in HuR activity and the stabilization of HuR-target mRNAs, e.g. CD62E and CTSS, both of which are linked with endothelial cell activation and atherosclerosis. Importantly, an H₂S donor was able to sulfhidrate HuR: decrease HuR binding to CTSS and decrease atherosclerosis development in a mouse model of partial carotid ligation. Finally, in mice and humans, plasma levels of L-cystathionine were found to correlate negatively with vascular reactivity indicating its potential use as a biomarker for vascular disease.

Conclusion: H₂S production in the vasculature is tightly controlled via CSE and is responsible for maintaining endothelial cell function acting via sulfhydration. However, CSE activity is reduced by inflammation resulting in HuR activation and development of atherosclerosis in humans. Monitoring of CSE activity via circulating L-cystathionine may serve as a novel biomarker of endothelial dysfunction.
Thus, AMPKα1 regulates endothelial cell proliferation and angiogenesis by maintaining the activity of the pentose phosphate cycle. Mechanistically, AMPKα1 phosphorylates calpastatin to decrease the proteolysis of HK1 which represents a novel mechanism to integrate endothelial angiogenesis and metabolism.

A 11-7

NADPH oxidase 4 is dispensable for perivascular adipose tissue mediated endothelial function in conductance arteries

P. Diaka-Nuhoglu, H. Langbein, C. Brunssen, H. Morawietz
University Hospital Cologne, Department of Internal Medicine II, Division of Vascular Endothelium and Microcirculation, Düsseldorf, Germany

Introduction: Brown-like PVAT encapsulating the aorta may release vasoactive factors which may promote beneficial vascular effects through the promotion of nitric oxide (NO) production. The main objective of the study was to find out whether NADPH oxidases 4 (Nox4) play a role in PVAT mediated vasodilation in a mouse model of hypercholesterolemia (Ldlr⁻/⁻). Therefore, we hypothesized that PVAT exerts a vasoactive effect via Nox4-generated H₂O₂.

Method: Thoracic aortic segments with or without PVAT were isolated from Ldlr⁻/⁻ and Nox4⁻/-Ldr⁻/⁻ mice. Aortic PVAT segments from mice and human PVAT from the left internal mammary artery were processed for isolation of total RNA and real-time PCR analysis. Vascular function of aortic segments from mice with and without PVAT was analyzed in a wire myograph.

Results: We have shown that Nox4 is expressed in murine and human PVAT. Furthermore, the expression of Nox4 mRNA is reduced in PVAT of Ldlr⁻/⁻ mice compared with wild type mice. Analysis of Pparγ1a as an indicator for beiging of PVAT, was unregulated in murine and human PVAT. Combination with 50mM potassium was not different between Ldr⁻/⁻ and Nox4⁻/-Ldr⁻/⁻ mice without PVAT. However, PVAT increased potassium induced contraction in Ldr⁻/⁻ mice but not in Nox4⁻/-Ldr⁻/⁻ mice. PE contraction was not different between Ldr⁻/⁻ and Nox4⁻/-Ldr⁻/⁻ mice. PE contraction was elevated by PVAT in Ldr⁻/⁻ and Nox4⁻/-Ldr⁻/⁻ mice. ACh-induced vasorelaxation was not different between Ldr⁻/⁻ and Nox4⁻/-Ldr⁻/⁻ mice and we could not observe changes in ACh⁻/⁻ induced relaxation by PVAT or catase. Also no changes in smooth muscle function was observed among the groups. Further analysis of arginase 1 but not adiponectin was upregulated in PVAT of Nox4⁻/-Ldr⁻/⁻ mice but not in Ldr⁻/⁻ mice.

Conclusion: PVAT had influence on contraction either with potassium or PE but no effect on vascular relaxation. Loss of Nox4 did not further alter endothelial function.

A 11-8

Cytochrome P450 reductase maintains normal endothelial function

P.F. Malacarne, A. Gajos-Draus, M. Siragusa, N. Müller, C. Raitu, B. Pflüger-Müller, K. Schröder, R.P. Brandes, F. Rezende

1Goethe-University, Institute for Cardiovascular Physiology, Frankfurt, Germany
2Goethe-University, Institute for Vascular Signaling, Frankfurt, Germany
3National Science Centre No. 2017/24/T/NZ5/00102, Poland, Poland

Background: Oxidative stress has been linked to vascular disease and cytochrome P450 enzymes (CYP) are capable of producing reactive oxygen species (ROS). CYP enzymes receive their electrons from cytochrome P450 reductase (POR), but the vascular importance of POR is largely unknown. We therefore aimed to identify the function of POR in the vascular system using a knock-out mouse approach.

Results: Endothelial-specific, tamoxifen-inducible POR knockout mice (ecPOR⁻/⁻) were generated and their vascular function under basal conditions and in vivo with antipodal gene deletion was analyzed. Under basal condition, ecPOR⁻/⁻ exhibited endothelial dysfunction: Aortic rings exhibited a lower relaxation to acetylcholine in organ bath experiments when compared to wild type [C50: 2.4±10⁻⁸ M (WT) and 6.2±10⁻⁹ M (ecPOR⁻/⁻)]. When treated with AngII, ecPOR⁻/⁻ show a significant increase in blood pressure [137±15 mmHg in WT, 152±17.5 mmHg in ecPOR⁻/⁻] which was accompanied by reduced heart rate and significantly increased peripheral resistance. In vivo treatment with AngI worsened endothelial dysfunction in ecPOR⁻/⁻ [EC50: 6.9±10⁻⁸ M (WT) and 1.9±10⁻⁷ M (ecPOR⁻/⁻)] in mesenteric artery. 4.8±10⁻⁸ M (WT) and 1.0±10⁻⁷ M (ecPOR⁻/⁻) in aorta, concentrations for acetylcholine. Moreover, nitric oxide production [detected as nitrite in plasma] was reduced in ecPOR⁻/⁻. Furthermore, endothelial cells isolated from ecPOR⁻/⁻ showed lower proliferation rates than the WT [65% less growth in ecPOR⁻/⁻]. Primary human aortic endothelial cells knockout of POR (CRISPR/Cas9 technology, POR⁻/-) showed significant lower nitrite production (abolished by NMMMA – L- Linoglycerolmonomethyl Aminine citrate) as compared to non-targeted control cells.

Conclusions: POR importantly contributes to enzyme endothelial function and maintains nitric oxide production.

A 11-9

A novel role for the “Regulator of G-protein signaling 5” in arterial bed-specific smooth muscle cell differentiation

C. Arnold, E. Demirel, M. Jäger, C. Stich, M. Hecker, T. Korf

1Physiology and Pathophysiology, University of Heidelberg, Cardiovascular Physiology, Heidelberg, Germany
2Medical Faculty Mannheim, University of Heidelberg, Center of Medical Research, Bioinformatic and Statistic, Mannheim, Germany

In healthy blood vessels, vascular smooth muscle cells (VSMCs) are in a quiescent state and contribute to the control of vascular tone and blood pressure. The adaptation to hemodynamic changes, e.g. during hypertension, alters the vessel wall architecture due to VSMC proliferation and migration resulting in arterial stiffening. This severely increases the risk for cardiovascular events. Thus, the regulation of VSMC differentiation is a promising target to prevent the deleterious consequence of arterial remodeling.

Numerous signaling pathways in VSMCs are dependent on G-proteins which are regulated by the regulators of G-protein signaling (RGS) that render G-protein α-subunits inactive. Our previous work has shown that RGS5, an endogenous inhibitor of Gα12/13, signaling controls blood pressure and arterial remodeling during hypertension in mice by promoting RhoA activity. RhoA is a well-studied GTPase known to drive arterial remodeling in response to hemodynamic changes. Moreover, RGS5 expression levels in mice were positively correlated with arterial diameter with the highest RGS5 expression in the thoracic aorta. Additionally, RGS5 overexpression in cultured VSMCs stimulated quiescence as shown by DNA microarray analysis which revealed an expression pattern of genes associated with the quiescent/contractile VSMC phenotype.

However, RhoA activity was increased under these conditions pointing toward the ambiguous role of RhoA in the context of VSMC phenotype regulation. Further, RGS5 decreased both VSMC proliferation as evidenced by the number of Ki67-positive nuclei and migration, as shown in lateral sheet migration as well as collagen gel invasion assays. Thus, we hypothesized that RGS5 expression increases with the number of VSMC layers to maintain quiescence. To address this, we cultured VSMCs in 3D multilayered VSMC monolayers. Under these conditions, RGS5 expression as well as RhoA activity were significantly increased which was abolished in the absence of RGS5. Moreover, our lab has shown that TGFB signaling was increased in VSMC organs which supported VSMC quiescence. Remarkably, loss of RGS5 in VSMC organs diminished Smad2/3 phosphorylation, thus pointing toward attenuated TGFB receptor signaling.

In conclusion, this points towards an arterial bed specific regulation of VSMC differentiation which is facilitated by RGS5-dependent TGFB receptor activation possibly mediated by RhoA.

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A 12-1

Twist kinetics and action potential parameters are independent of expressed myosin heavy chain isoform in human embryonic stem cell-derived cardiomyocytes


1Hannover Medical School, Molecular and Cell Physiology, Hannover, Germany
2Hannover Medical School, Neurophysiology, Hannover, Germany
3Hannover Medical School, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), Hannover Medical School-Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany

In this study, we have shown that human embryonic stem cell derived cardiomyocytes (hESC-CMs) for ~35 days on a stiff matrix leads to a switch of their myosin isoform expression from mixed αβ-myosin heavy chain (MyHC) protein towards pure ventricularα-myosin heavy chain (α-MyHC). Yet, 25% of the CMs still showed mixed MyHC expression. The α-MyHC expression is often estimated by analyzing the expression of the corresponding MYH6/MYH7-mRNAs. In this study we addressed the following questions:

i) How are α and β-myosin heavy chain protein expression related to the correspondingmRNA expression of individual hESC-CMs? ii) Is α-MyHC composition of single individual CMs associated with atrial/ventricular action potentials and different twitch kinetics?

To be able to analyze these different parameters for individual hESC-CMs and to retrieve the very same hESC-CMs in different experimental setups, we developed a very precise single cell mapping technique. With this technique we were able to record twitch contractions or action potentials of individual hESC-CMs followed by fluorescence in situ hybridization against MYH6 and MYH7-mRNAs and finally immunofluorescent staining of α-MyHC for the very same individual CMs. For that, hESC-CMs were cultivated for ~35 days either in suspension culture or plated on glass coverslips.

The majority of individual β-MyHC expressing hESC-CMs still had detectable variables of MYH6-mRNA. There was only a weak correlation between the MYHL7-mRNA fraction and β-MyHC fraction for individual CMs. The uptake
velocities of action potentials were faster in plated hESC-CMs compared to CMs in suspension culture, indicating maturation of voltage-gated sodium channels.

We saw that MYH6-MYH7 mRNA composition and αβ-MHC isoform expression varied in hESC-CMs and did not correlate closely. Contraction and action potential parameters were also variable and independent of MyHC isoform. This suggests that hESC-CMs with pure ventricular MyHC isoform do not fully switch to ventricular CM phenotype.

A 12-2

Estrogen-dependent modulation of cardiac titin properties

J. Kardel, S. Bongardt, M. Krüger

Hannover Medical University, Cardiovascular Physiology, Hannover, Germany

The sarcomeric protein titin is a main determinant of myocardial passive stiffness and diastolic function. Titin-based cardiomyocyte passive stiffness can be modulated by altered titin isoform expression or by posttranslational modification, e.g. by phosphorylation of the elastic I-band domains N2-B and PEVK. Altered titin properties have been associated with acute and chronic changes in left ventricular diastolic function, and sex-dependent differences in diastolic function in patients with hypertrophic cardiomyopathy have been suggested. However, whether modulation of titin properties occurs in a sex-specific and estrogen-dependent manner is still largely unknown. Here, we studied left ventricles of adult male, female and pregnant female rats (n=5 per group) and determined relative expression of cardiac titin isoforms (N2B, N2BA) using SYPRO-Ruby stained agarose-stabilized SDS-PAGE and found no significant changes between groups. Western blot analyses using phosphospecific antibodies demonstrated significantly increased phosphorylation levels of PKCδ in hearts from pregnant rats, but relative PKCδ-dependent phosphorylation at S1187 and S1202 in the PEVK region was not significantly different among groups. We did not observe sex-specific differences in PKA- and ERK1/2-dependent titin phosphorylation at S4010 in the N2B region. Taken together, our results suggest that in healthy adult rat hearts basal titin properties do not undergo sex-specific modification. We further studied acute estrogen-induced activation of titin-targeting kinases using cultivated adult rat cardiomyocytes treated for 30 min. with estrogen, the estrogen-receptor inhibitor fulvestrant, or a combination of both. Kinase activities were assessed by Western blot analysis. Estrogen-induced activation of AKT and ERK1/2, whereas levels of P-PKCδ remained unchanged. Incubation with fulvestrant alone, or with fulvestrant and estrogen, significantly increased the levels of activated AKT, ERK1/2, PKCa, and P-Tnl indicating increased PKA activity and PKCα translocation that partially inhibited the nuclear but not the membrane estrogen receptors, and that these G-protein coupled estrogen receptors mediated the observed estrogen-induced effects in adult cardiomyocytes. Experiments are currently performed to test this hypothesis and to analyze whether the acute estrogen-induced kinase activation results in titin modification and altered cardiomyocyte passive tension.

A 12-3

A snake toxin phospholipase A2 homologue impairs excitation-contraction coupling and induces cell collapse in adult rat cardiomyocytes


1Hannover Medical School, Molecular and Cell Physiology, Hannover, Germany
2Hannover Medical School, Neurophysiology, Hannover, Germany
3Universidade de Costa Rica, Instituto Oódono Plácido, San José, Costa Rica

Venom from Bothrops asper snake contains high concentrations of Myostoxin-II (MII-II), a Lys-49 phospholipase A homologue lacking catalytic activity. MII-II is highly toxic for skeletal muscle cells but displays variable toxicity to other cell types. In skeletal muscle, MII-II triggers spontaneous intracellular calcium transients, disruption of the cell membrane, hypercontraction, and cell collapse leading to muscle necrosis. While biochemical (e.g. amino acid sequence, folding) and toxic properties (e.g. myotoxicity in mice, cytotoxicity on C2C12 cells) have been studied in the past, detailed effects on adult cells on a molecular and cellular level are not understood. Therefore, we tested whether intact cardiac muscle cells of adult rats constitute a target of MII-II and analyzed its effects on excitation-contraction coupling.

After anesthetizing the rat and confirming the anesthetized state, the heart was rapidly excised. Single cardiomyocytes were isolated by a mechanoreactionary procedure including collagenase digestion. Cells were loaded with calcium indicator FURA2-AS (Atto). Calcium transients and cell shortening (SL) changes were analysed under electrical stimulation (1 Hz) during a baseline period and after MII-II exposition (25 µg/mL) using either a standard extracellular solution (n=18 cells) or a calcium-free extracellular solution (n=17 cells). Sham control experiments (vehicle administration) were also recorded for separated groups of cells.

In standard extracellular solution, 25 µg/mL MII-II induced: a) slightly increased time to peak of calcium transients; b) lack of calcium transient after electrical stimulation; c) spontaneous calcium transients unrelated to electrical stimulation; d) cell to cell variability of calcium transients; and e) cell collapse. In calcium-free extracellular solution, 25 µg/mL MII-II induced: a) slow increase of intracellular calcium concentration; and b) cell collapse. Before cell collapse, MII-II did not induce major alterations of SL recordings neither in standard nor in calcium free solution.

Adult rat cardiomyocytes are a novel target of MII-II. In this cell model MII-II alters key variables of excitation-contraction coupling while it does not affect sarcromeric proteins as suggested by the unchanged SL recordings before cell collapse. Future experiments using agonists and antagonists of calcium handling proteins will reveal further insights into the molecular mechanism of cytotoxicity of MII-II.

A 12-4

Cardiogenesis-derived cells as a Model System for Myocardial Infarction

L. Sathöfer, J. Fandrey, T. Schreiber

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

Coronary heart disease is the most common cause of death in Europe. Although significant achievements in prevention and treatment of myocardial infarction have been made, there is a major threat. Future studies aiming to identify novel targets on the molecular basis of ischemia and reperfusion (I/R) damage to the myocardium are necessary to discover potential new targets to treat I/R injury.

Experiments using cardiogenesis-derived cells (CDCs) which are a heterogeneous primary cell culture created from murine neonatal hearts containing cardiomyocytes, endothelial cells, smooth muscle cells and stem cells, in order to simulate ischemia and reperfusion, oxygen-glucose deprivation (OGD; 0.2% O2/glucose-free medium) was applied for several time periods, followed by one hour of reperfusion (RP). In general, the hypoxic tissue response is governed by hypoxia-inducible factor-1α (HIF-1α). Therefore, additional experiments were performed with dimethyl-oxal-glycine (DMOG) which increases HIF-1α to boost the endogenous response during reperfusion.

Within the 6h of OGD a continuous increase in lactate dehydrogenase (LDH) release was observed, while 1h of reperfusion reduces cell death and LDH release. A significant reduction of LDH release was observed in 1h of reperfusion with DMOG. On the mRNA level expression of several genes was affected by OGD and subsequent RP. Among those genes were the connexin tissue growth factor (CtGF) and adrenergic receptor alpha 1b (Adra1b). The results are promising, because elevated expression levels of CtGF are inversely correlated with reduced infarct size, less hypertrophy and better function after ischemia and reperfusion. In contrast, the Adra1b expression remained at control level for 2h. After 4h OGD expression levels were reduced while RP had no effect.

In order to assess basic mechanism of I/R CDCs can serve as possible model system for MI. For a deeper insight, however, the effect of OGD on CDC’s needs to be compared with I/R in an in vivo model with respect to viability, RNA and protein level and the use of DMOG as a possible therapeutic agent during reperfusion.

A 12-5

Structural and Functional Assessment of Human Ventricular Myocardium

1Friedrich-Alexander University, Institute of Cellular and Molecular Physiology, Erlangen, Germany
2Ludwig-Maximilian University, Walter-Brende-Centre of Experimental Medicine, Munich, Germany

Question: The loss and remodeling of transverse tubules (t-tubules) as part of the phenotypic change in failing human cardiomyocytes (CMs) is gaining increasing recognition because of its pathophysiological importance in heart failure. It has been associated with defective excitation-contraction coupling, decreased contractility and impaired cardiac recovery. Thus, the transverse tubular system (t-system) is considered a potential therapeutic target to improve cardiac function and potential recovery pathways. Normal rabbit myocardium served as control. After 1-3 weeks in culture, slices were fixed with anaesthetizing the rat and confirming the anaesthetized state, the heart was rapidly excised. Single cardiomyocytes were isolated by a mechanoreactionary procedure including collagenase digestion. Cells were loaded with calcium indicator FURA2-AS (Atto). Calcium transients and cell shortening (SL) changes were analysed under electrical stimulation (1 Hz) during a baseline period and after MII-II exposition (25 µg/mL) using either a standard extracellular solution (n=18 cells) or a calcium-free extracellular solution (n=17 cells). Sham control experiments (vehicle administration) were also recorded for separated groups of cells.

In standard extracellular solution, 25 µg/mL MII-II induced: a) slightly increased time to peak of calcium transients; b) lack of calcium transient after electrical stimulation; c) spontaneous calcium transients unrelated to electrical stimulation; d) cell to cell variability of calcium transients; and e) cell collapse. In calcium-free extracellular solution, 25 µg/mL MII-II induced: a) slow increase of intracellular calcium concentration; and b) cell collapse. Before cell collapse, MII-II did not induce major alterations of SL recordings neither in standard nor in calcium free solution.

Adult rat cardiomyocytes are a novel target of MII-II. In this cell model MII-II alters key variables of excitation-contraction coupling while it does not affect sarcromeric proteins as suggested by the unchanged SL recordings before cell collapse. Future experiments using agonists and antagonists of calcium handling proteins will reveal further insights into the molecular mechanism of cytotoxicity of MII-II.

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A 12-6

The RyR2R420Q/+ Mutation Triggers Arrhythmogenic Ca2+ Events in Mouse Cardiomyocytes

R. López1, M. Fernandez-Tenorio1, R. Janicke2, A. Zahradníková jr.3, Y. Y. Wang2, J. - P. Benitah4, E. Zorio1, A. M. Gomez5, E. Niggli6

1Université Paris-Sud, UMR-S 1180, Châtenay-Malabry, France
2Hospital La Fe, Valencia, Spain
3Université Paris-Sud, UMR-S 1180, Châtenay-Malabry, France
4University of Bonn, Department of Cardiac Surgery, Bonn, Germany
5Université Paris-Sud, UMR-S 1180, Châtenay-Malabry, France
6Université Paris-Sud, UMR-S 1180, Châtenay-Malabry, France

Question. L-Adrenergic receptor (L-AR) stimulation of cardiac muscle is known to enhance Ca2+ release from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR). In pathological conditions, hypersensitivity of RyR2 and/or SR Ca2+ overload could contribute to and potentially induce arrhythmogenic Ca2+ signals. We hypothesize that at the cellular level, generation of arrhythmogenic Ca2+ waves in the heterozygous RyR2R420Q+/- knock-in (KI) animal may result from a disturbed balance in the SR Ca2+ uptake/release homeostasis. Methods. Mice carrying the RyR2R420Q+/- mutation were challenged to epinephrine/caffeine treatment and their cardiac electrical activity was monitored via electrocardiography. In isolated cardiomyocytes, cytosolic Ca2+ waves, luminal SR Ca2+ content and Ca2+ sparks were studied in detail with fluorescent probes with confocal microscopy under L-AR stimulation. Spark-to-spark delays were analyzed to investigate RyRs Ca2+ sensitivity. Results. Epinephrine treatment induced bidirectional ventricular tachycardia (BVT) in the KI mice but not in L-AR-ternates. KI cardiomyocytes showed higher L-AR Ca2+ frequency with longer duration and small differences in amplitude. Wave occurrence was absent in WT cells while in the KI cardiomyocytes was observed in 10%. Isoproterenol treatment increased occurrence of waves much more in the KI cells (59.3% in WT and 90% in KI cells). Wave latency was identical in control conditions (WT and KI cells > 10s), but L-AR stimulation significantly shortened the delay to spontaneous Ca2+ wave initiation, much more in KI cells (WT, 6.3±3.5s vs. KI, 1.8±1.1s, meanscD). The intra-SR Ca2+ concentration at the wave generation threshold was significantly lower in the KI cells (meanSD of JF-5EX WT, 0.58±0.17 vs. KI, 0.40±0.18). We found a leftward shift of the Ca2+ spark-to-spark delay in KI myocytes, which was further shortened in both cell types upon L-AR stimulation, suggesting a sensitization of the mutated RyR2 channel. Conclusion. The heterozygous RyR2R420Q+/- animal recapitulates features of the human catecholaminergic polymorphic ventricular tachycardia. On the cellular level the increased RyR activity resulted in a Ca2+ leak with a higher and earlier spontaneous Ca2+ wave occurrence upon L-AR stimulation. The enhanced Ca2+ sensitivity of the RyR2R420Q+/- channels suggests a gain-of-function due to the mutation, which is further pronounced during L-AR stimulation.

A 12-7

Titin turnover and synthesis in cultures of embryonic and adult rat cardiomyocytes

E. Müller1, S. Bongard1, M. Krüger1, S. Kötter2

1Heinrich-Heine-Universität Düsseldorf, Cardiovascular Physiology, Düsseldorf, Germany
2Heinrich-Heine-Universität Düsseldorf, Institut für Herz- und Kreislaufphysiologie, Düsseldorf, Germany

Introduction: Titin is the biggest protein known with a size of 3-3.7 MDa and the third filament system in the sarcomeres of striated muscle cells. Due to its gigantic size disintegration and degradation of the titin filament are major challenges for cardiomyocyte protein turnover, and the precise mechanisms involved in these processes are still largely unknown. In this project we studied titin turnover during sarcomeric de- and redifferentiation processes using long term cultivation of adult rat cardiomyocytes (ARC). We further tested the involvement of autophagy in titin turnover by modulating autophagic activity in cultivated embryonic rat cardiomyocytes (ERC).

Methods. ERCs were treated for 24 hours with the autophagy inhibitor chloroquine or proteasome inhibitor MG132 and ubiquitination levels were analyzed by Western blot. ARCs were cultivated in medium M199 containing 1% FBS for up to 20 days and analyzed by immunofluorescence staining with antibodies detecting different titin isoforms within the z-disc, the I-band and the M-line as well as actinin. Furthermore, we stained for E3-ligases and polyubiquitin as markers of intracellular protein turnover mechanisms.

Results. During cultivation, ERCs lost their rod shape, built extensions and sarcomeric structures were gradually decreased. After 15 days nearly all cells had lost their sarcomeres. These structures were rebuilt in the following days but without entirely regaining the parallel sarcomere organization observable in freshly isolated ARCs. After 16 days almost all cells had regained sarcomeric structures. We did not detect differential domain specific degrading and appearing of titin antibody signals, suggesting that titin isoforms are degraded in one. Furthermore, we observed increasing numbers of ubiquitin containing aggregates in the dedifferentiating cells which occurred simultaneously with the loss of sarcomeres. In addition, staining for E3-ligases revealed partial sarcomeric patterns. Inhibition of autophagy or the proteasome in cultured ERCs resulted in specific titin NKG- or K44-polyubiquitination, indicating participation of both, autophagy and the proteasome in titin degradation.

Conclusion. Taken together, our data indicate that titin is rapidly degraded and re-integrated during sarcomere turnover and that both major protein quality control mechanisms contribute to titin homeostasis.

A 12-8

Generation of transgenic ES cells and mouse lines enabling the inducible overexpression of Cx43

P. Niemann1, M. Hesse1, C. Geisler1, S. Grünberg1, P. Freitag1, D. Malaín1, W. RöII1, B. K. Fleischmann1

1University of Bonn, Institute of Physiology I, Bonn, Germany
2University of Bonn, Department of Cardiac Surgery, Bonn, Germany

Gap junctions (GJs) are a critical component of the functional syncytium of the heart and play a central role for cardiac function regulating the rapid and coordinated excitation of cardiomyocytes (CMs). Ventricular GJs are mainly composed of the transmembrane protein Connexin 43 (Cx43). Loss of Cx43+ CMs upon myocardial infarction results in a dramatic decrease of cardiac conduction velocity within the scar tissue and a rise in incidence of ventricular tachyarrhythmias (VTs). In order to explore novel strategies for the prevention of such VTs and to test in particular the impact of Cx43 (over)expression in CMs and cardiac myofibroblasts in healthy and infarcted hearts we have generated inducible and conditional embryonic stem (ES) cells and mouse lines overexpressing Cx43.

A DNA fragment composed of the murine Cx43 3’DNA, the GSSG2A DNA-sequence and the mCherry cDNA wasCre- mediated under the control of the CAG promoter in the Rosa26 A6 vector from Addgene. Next, G4 mouse ES cells were co-transfected with this construct and zinc finger nucleases. Several clones were established by homologous recombination within the Rosa26 locus and for six of them the kanpyrom was determined. Single integration of the Cre inducible transgene was assessed by qPCR and Southern blot analyses. Suitable ES cell clones were transduced with an AAV-Cre virus and western blot (WB) experiments and immunostainings were performed to analyze transgene expression. Since HeLa cells do not express endogenous Cx43, they were used to test the functionality of the construct. For this purpose HeLa cells were transduced with the Cx43-GSSG2A-mCherry plasmid, patched and dialysed with fluorescence dyes to examine whether intercellular communication through GJs occurs.

Following Cre virus transduction, WB analyses and immunostainings revealed inducible expression of the exogenous Cx43 protein. Murine blastocysts were injected with cells of appropriate ES cell clones and two Rosa26Cre-CAG-Cx43-mCherry mouse lines were created. Thus, we have generated an inducible and conditional mouse model (over-)expressing murine Cx43 and the fluorescent marker mCherry. We are planning to cross the mouse with different MerCreMer mouse lines in order to explore cardiac and functional consequences of Cx43 overexpression in heart and other organs.

A 12-9

Insulin regulates cardiac contractility in mice with cardiomyocyte-restricted depletion of protein kinase B

D. Miltos1, S. Gödecke1, F. Funk2, J. Schmitt2, A. Gödecke1, A. Heinen1

1Heinrich-Heine-Universität Düsseldorf, Institut für Herz- und Kreislaufphysiologie, Düsseldorf, Germany
2Heinrich-Heine-Universität Düsseldorf, Institut für Pharmakologie und Klinische Pharmakologie, Düsseldorf, Germany

Introduction: Protein kinase B (Akt) is involved in glucose homeostasis as it plays a central role in the insulin signalling pathway. In addition, there is evidence that Akt-mediated insulin signalling affects cardiac calcium handling and function, but the described functional effects are contradictory, and the underlying mechanisms are unclear. Here, we aim to investigate 1) the potential inotropic action of insulin, and 2) the role of Akt in this process.

Methods: Experiments were performed in isolated perfused hearts of mice with cardiomyocyte-restricted depletion of Akt-isoform 2 (KO), or wild type (WT) mice. Knock out and WT mice were treated with insulin (0.1, 1.0, 10 µg, i.p.). arid cardiac function was analysed on day 7. After stabilization, isolated hearts were treated with insulin (6 nM, 10 min), and cardiac function was analysed before (BL) and during insulin (INS) treatment. In addition, in vivo effects of insulin were investigated in anesthetized and conscious WT mice. Male mice were treated with insulin (0.1, 1.0 µg, i.p.), and cardiac function was assessed before (BL) and 10 min after insulin (INS) treatment by echocardiography including speckle-tracking based strain analysis. Data are means?SD. Statistics: Two-way RM ANOVA followed by post hoc test.

Results. Insulin showed improved basal cardiac function in KO compared to WT (dp/dtmax = 319±253 vs 317±230; dp/dtmin=261±254 vs 257±236; dp/dtmax = 317±444 vs 320±435 min). In KO mice, insulin strongly improved development pressure (107±7 vs 82±7 µmHg) and dp/dtmax = 341±254 vs 340±254 min) and dp/dtmax = 415±321 vs 415±321 min). In contrast, insulin had no effect on WT mice in cardiac function. In vivo experiments confirmed these findings as insulin did not affect ejection fraction (EF) in WT (INS: NS (65.9±4.9% vs BL: 69.7±2.6%), but enhanced EF by 30% in KO mice (71.3±10.8% vs 54.7±11.2%). Similarly, strain analysis revealed compromised basal radial strain peak in KO compared to WT (28.7±3.2% vs 36.2±2.5%). Insulin treatment improved radial peak strain in KO (31.7±3.8% vs BL), but not in WT mice (32.3±3.2% vs BL). Interestingly, strain rate after insulin was improved in KO compared to WT during diastole, but was comparable during systole.
Conclusion: A cardiomyocyte-restricted loss of Akt1 and Akt2 impairs cardiac function. Furthermore, insulin improves cardiac function in Akt1/2 knock out mice suggesting a regulatory role of insulin on cardiac contractility by an Akt-mediated signaling.

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A 12-10
Unbiased screen of the cardiac proteome and effect of spermidine therapy on titin properties in a rat model of heart failure with preserved ejection fraction (HFpEF)

F. Koser1, M. Abdellatif1, C. Türk1, M. Krüger1, S. Sedej2, W. A. Linke1

1University Hospital Münster, Institute of Physiology II, Münster, Germany
2Medical University of Graz, Division of Cardiology, Graz, Austria
3University of Cologne, Institute for Genetics, Cologne, Germany

Background: Despite high public-health importance, the pathomechanism of HFpEF remains elusive and specific therapy is lacking. A key feature in HFpEF patients is diastolic dysfunction, caused in part by altered stiffness of the cardiomyocyte protein titin. Good patient-mimicking animal models of HFpEF are useful, to elucidate the impact of age, gender and comorbidities on HFpEF progression. The ZSF1 rat is an established model of HFpEF induced by metabolic syndrome, the most prevalent cause and comorbidity of HFpEF. Polyamines have pleiotropic effects mimicking those of calcic restriction; thus, spermidine may have beneficial effects on hearts of ZSF1 obese rats, and whether proposed regulators of titin phosphorylation/stiffness in HFpEF are affected by spermidine treatment.

Methods & Results: Eight-week-old female and male ZSF1 and WKY rats were fed with a Purina diet up to the onset of the HFpEF phenotype in the ZSF1 obese rats at around 20 weeks of age. Gene ontology enrichment analysis of the cardiac proteome, phosphoproteome and acetylation of ZSF1 obese vs. lean or ZSF1 obese vs. WKY rats by quantitative mass spectrometry revealed proteins mainly associated with metabolic terms to be regulated in ZSF1 obese rats. In contrast, postulated ‘key features’ of HFpEF (inflammation, oxidative stress) were not altered. We identified 3 kinases (CaMKIIδ, ERK2, PKA) and 3 phosphatases (PP1, PP2A, PP5) known to modify titin phosphorylation, but only CaMKIIδ expression was significantly increased in female ZSF1 obese vs. WKY rats. Many phosphosites were identified in titin, of which less than 10 were significantly altered in both studies. Immunoblot-based quantitation of expression/activity of titin-targeting kinases (ERK1/2, PKG, PKC) and phosphatase PP5 demonstrated no alteration in spermidine-treated vs. non-treated ZSF1 obese hearts. Total-titin and site-specific titin phosphorylation in N2Bus or PEVK were also unaffected by spermidine treatment.

Conclusions: Pathways previously suggested to define HFpEF pathophysiology and proposed regulators of titin phosphorylation/stiffness were unaltered in hearts of ZSF1 obese rats. This model could therefore be sub-optimal to mimic human HFpEF. HFpEF pathophysiology and preventive effects of spermidine treatment may relate to metabolic alterations independent of inflammation and oxidative stress.

A 12-11
Hypertrophic Cardiomyopathy: unequal allelic expression and contractile imbalance from cell-to-cell for myosin-binding protein C and cardiac troponin I mutations

V. Burkart1, J. Beck1, D. Allgäu-Nebeling1, A. Radacoac1, B. Piep1, C. dos Remedios2, D. Hilfiker-Kleiner1, V. D. V. Jolanda1, J. Montag4
1Hannover Medical School, Molecular and Cell Physiology, Hannover, Germany
2University of Sydney, Sydney, Australia
3Hannover Medical School, Clinic of Cardiology and Angiology, Hannover, Germany
4VU University Medical Center, Amsterdam, Netherlands

Hypertrophic Cardiomyopathy (HCM) is mostly caused by heterozygous mutations in myosin binding protein C (cMyBP-C, MYBPC3), β-myosin heavy chain (β-MyHC, MYH7), cardiac troponin T (cTnT, TNNT2) or cardiac troponin I (cTnI, TNNI3). A common mechanism why different functional effects of different mutations induce a similar HCM phenotype is unclear. Our previous studies on ventricular cardiomyocytes (CMs) from HCM-patients with β-MyHC-missense mutations revealed highly variable force generation and Ca2+-sensitivity among the individual CMs and unequal fractions of mutated vs. wildtype β-MyHC-mRNA in individual CMs. This allelic imbalance from CM to CM seems to be due to burst-like, stochastic transcription of MYH7 Independent for the two alleles. Here we asked whether this holds also true for HCM-mutations in cMyBP-C and in cTnI.

We studied cardiac tissue from an HCM-patient with nonsense mutation c.927-2A>G in MYBPC3 and from another HCM-patient with missense mutation R145W in cTnI. Force measurements on CMs from both patients at physiological activation levels showed significantly larger variability of force from cell to cell compared to donor CMs. Some CMs generated forces like donor CMs while others had different force levels. CMs with cTnI-mutation had reduced Ca2+-sensitivity while for CMs with MYBPC3-mutation an increase was found. Immunofluorescent labelling of cMyBP-C revealed 35% smaller and significantly more variable intensity and patchy fluorescent labelling for patient’s CMs compared to donor CMs, suggesting varying levels of cMyBP-C. Western blot data suggested nonsense-mediated mRNA decay for the mutated allele and haploinsufficiency. For the cTnI mutation relative expression of mutated and wildtype mRNA revealed different fractions of both, mutant and wildtype TNNT2 mRNA in individual CMs, ranging from essentially only mutant to only wildtype mRNA. RNA-fluorescence in situ hybridization revealed many nuclei with no active transcription sites (81% for donor and 95% for patient CMs). For cTnI evidence suggests that cell-to-cell allelic imbalance of wildtype and mutated mRNA is caused by burst-like transcription of TNNI3. For cMyBP-C it is unclear whether burst-like transcription causes varying amounts of wildtype protein from cell to cell. In both cases the resulting contractile imbalance among neighboring CMs in the myocardium may well contribute to development of HCM-typical cellular/myofibrillar disarray, hypertrophy and fibrosis.
POSTER SESSION B

B 01 | Circuit Neurophysiology

B 01-1
Effects of activin A on hippocampal network activity
M. Dahlmanns1, J. K. Dahlmanns2, F. Zheng3, C. Alzheimer1
1University of Erlangen-Nürnberg, Institute of Physiology and Pathophysiology, Erlangen, Germany
2University 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 carbocchiole (25 µM) and monitored by means of field potential recordings in CA1 stratum oriens and CA3 stratum radiatum. Superfusion of slices with carbocchiole induced robust oscillations in the theta range in both regions of wt slices, whereas no such drug effect was observed in dnActRIB slices. Only at a higher carbocchiole 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 hippocampus cultures, in which network activity was monitored with the calcium fluorescent dye Fluo-5AM. 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.

B 01-2
Impaired Processing of Self-Generated Sounds in a Mouse Model of Schizophrenia
Presidoposis
B. P. Rummel1, A. J. Gogos2, T. Sigurdsson3
1Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt am Main, Germany
2Columbia University, Department of Physiology and Cellular Biophysics, New York, NY
3Columbia University, Department of Neuroscience, New York, US

Studies in humans and animals models have consistently shown that auditory responses to sounds caused by self-generated actions are attenuated. In contrast, patients suffering from schizophrenia show reduced attenuation of such sounds. However, the causes of this sensory processing deficit in schizophrenia are not well understood. To address this, we examined neural responses to self-generated sounds in Df(16)A+/- mice, which model the strongest known genetic risk factor for schizophrenia, the 22q11.2 microdeletion. Df(16)A+/- mice (n=9) and their wild-type (WT) littermates (n=9) were trained to press a lever which triggered a sound, while the identical sound was also randomly delivered independent of the animal’s behavior. During this task, responses of auditory cortex (Acx) neurons to these sounds were recorded using silicon probes. In WT animals, responses of Acx neurons to self-generated sounds were robustly attenuated in amplitude, compared to randomly occurring stimuli. In contrast, responses of neurons in Df(16)A+/- mice showed reduced attenuation compared to WT littermates. No differences were found in responses to random sounds between genotypes and we observed that general movement-related attenuation of auditory sounds to be similar between genotypes. Interestingly, we found neural activity that preceded the self-generated sound to be specifically significantly reduced in putative interneurons in Df(16)A+/- animals suggesting anticipatory movement-related activity. These findings demonstrate that the 22q11.2 microdeletion, a major risk factor for schizophrenia, causes impaired attenuation of self-generated sounds similarly to what is found in patients, and reveal a possible cellular basis for this sensory deficit.

B 01-3
Caloric restriction improves in vivo cortical function in aged mice
N. Asapavumumas, N. Fröhlich, C. Lerdkrai, E. Zirдум, O. Garaschuk
Eberhard 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 status between 3 and 18 months of age 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, indicative of 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.

B 01-4
Non-canonical axon morphologies gate information flow in neuronal ensembles
A. Hodapp1, M. E. Kaiser1, M. Klump1, A. Draugn1, Y. Yanovsky2, M. Engelhardt3, C. Thomes1, M. Both1
1Heidelberg University, Institute of Physiology and Pathophysiology, Heidelberg, Germany
2Heidelberg University, Institute of Neuroanatomy, Medical Faculty Mannheim, Mannheim, Germany
3Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt am Main, Germany

Neurons receive multiple synaptic inputs at their dendrites, which integrate within the somato-dendritic compartment and generate action potentials (AP) once voltage threshold is reached at the axon initial segment. Canonically, the axon of pyramidal cells is located next to the soma but we have recently shown that in about 50% of CA1 pyramidal cells the axon originates from a basal dendrite rather than from the soma. This axon-carrying dendrite (AcD) might constitute a privileged input channel in this subset of cells. Additionally, previous work in vitro has shown that during sharp wave-ripple complexes (SPW-R) CA1 pyramidal cells are recruited in a peculiar way: their somatic action potential initiates abruptly from baseline resembling an ectopically generated spike. Here we asked whether the anatomical feature of AcD cells underlies the selective activation of CA1 pyramidal cells during SPW-R.

To test our hypothesis, we performed extra- and intracellular electrophysiological recordings as well as immunofluorescent staining in acute hippocampal mouse brain slices. Interestingly, excitatory input to axon-carrying dendrites remains efficient even during the strong perisomatic inhibition that accompanies SPW-Rs and prevents other pyramidal cells from firing. Thus, only AcD cells are able to fire APs during the events. In line with our previous data, AP waveforms resemble ectopically generated spikes. Multicompartment modelling of single cells confirms that in somatic recordings of AcD cells, APs initiate abruptly from resting membrane potential when excitatory and inhibitory inputs arrive with a certain spatio-temporal configuration. Intraacellular administration of picrotoxin diminished perisomatic inhibition, recruited more cells into SPW-R and shifted ectopic AP waveforms towards classical APs. In summary, AcD cells are selectively recruited during SPW-R activity while the firing probability of other neurons is abruptly reduced. In contrast, responses of neurons in Df(16)A+/- mice show reduced attenuation compared to WT littermates. No differences were found in responses to random sounds between genotypes and we observed that general movement related attenuation of auditory sounds to be similar between genotypes. Interestingly, we found neural activity that preceded the self-generated sound to be specifically significantly reduced in putative interneurons in Df(16)A+/- animals suggesting anticipatory movement-related activity. These findings demonstrate that the 22q11.2 microdeletion, a major risk factor for schizophrenia, causes impaired attenuation of self-generated sounds similarly to what is found in patients, and reveal a possible cellular basis for this sensory deficit.
B 01-5

Stable behavioral state-specific mesoscale activity patterns in the developing cortex of neonates N. Mojtahed1, Y. Kovachuk1, A. Böttcher2, O. Garaschuk1
1Eberhard Karls University of Tübingen, Institute of Neurophysiology, Tübingen, Germany
2Eberhard Karls University of Tübingen, Werner Reichardt Centre for Integrative Neuroscience, Tübingen, Germany

Endogenous neuronal activity is a hallmark of the developing brain. In rodents, a handful of such activities were described in different cortical areas from a microscopic perspective but is still lacking. Here we combined large-scale in vivo Ca2+ imaging of the dorsal cortex in awake neonatal mice with advanced mathematical analyses to reveal unique behavioral state-specific maps of endogenous activity. These maps were remarkably stable over time within and across experiments and used patches of correlated activity with little hemispheric symmetry as well as stationary and propagating waves as building blocks. To specifically address the role of motor behavior, the maps recorded during motion and rest were almost inverse, with sensory-motor areas active during motion and posterior-lateral areas active at rest. The retrosplenial cortex engaged in both resting- and motion-related activities, building functional long-range connections with respective cortical areas. Together, these data provide an unprecedentedly complete view on the endogenous network activity and set the stage for future inactivation studies probing its exact function in orchestrating the early development of the mammalian brain.

B 01-6

Amplitudes and propagation of cortical spreading depolarization (CSD) in adult rats are influenced by Calcitonin gene-related peptide (CGRP) F. Gimeno-Ferres1, F. Richter1, R. Bauer2, A. Lehmenkühler1, H. - G. Schalb1
1University Hospital Jena, Institute of Physiology I / Neurophysiology, Jena, Germany
2University Hospital Jena, Institute of Molecular Cell Biology, CMB-Center for Molecular Biomedicine, Jena, Germany

It is known from the literature that CGRP plays an important role in migraine and that CSD is an effective preventive treatment against migraine pain. CGRP is able to increase neuronal excitability. Further it has been shown that a CSD 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 influences 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) parameter and the parameters of CSD in the treated cortex to untreated 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 microelectrodes (distance 5-8 mm) 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) were measured with a micropipette filled with Corning IE-190 ion exchange. In the untreated area, CSD was elicited by a microinjection of 1 nL KCl (100 µM, 500 µs up to 1 s, depth 1200 µm) into the grey matter at intervals of 30 min. In the area 100 µL of CGRP at concentrations from 10^{-8} M to 10^{-3} M (only one concentration per experiment) were applied topically and left there for three hours. In both cortical areas [K+]c was measured. 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^{-3} M to 80 % of controls; 10^{-4} M to 70 % of controls; untreated to 85-90 % of controls) and slowed the propagation velocity (10^{-3} M from 3.0 to 2.6 cm/s; 10^{-4} M from 2.4 to 2.2 cm/s). Rarely spontaneous CSDs were observed originating from the CGRP-treated area. In a few other rats, CSD induced focal ictal activity after 2-3 hours of application that did not spread into the untreated cortex. Focal ictal activity occurred at intervals of 8-10 min and was accompanied by burst transients 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.

B 01-7

The contribution of medial prefrontal cortex neurons to spatial working memory P. Voge1, S. Duvarci, T. Sigurdsson
Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt, Germany

Spatial working memory (SWM), the ability to store and update spatial information in the short term, is an essential feature of goal-directed behavior. Lesion and inactivation studies suggest that the medial prefrontal cortex (mPFC) plays a key role in the execution of SWM tasks. However, little is known about the temporal structure of mPFC involvement during these tasks, that is, when precisely activity of encoding, maintenance, and/or retrieval. In the present study we addressed this issue by recording from and optogenetically silencing mPFC pyramidal neurons in a temporally specific manner while mice performed a non-match-to-sample T-maze task. This task decomposed SWM in three phases (sample, delay and choice phase) that were assumed to capture all memory stages that have to be passed for successful task performance (encoding, maintenance, and retrieval). Once animals had learned the task, testing sessions began in which the 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. Further, 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 vertical basal ganglia 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 01-8

Influence of chemogenetic G-protein coupled receptor modulation of medial prefrontal cortex subregions on attention and waiting impulsivity V. van der Veen1, P. Steele-Perrins1, K. Kilonzo1, S. Schultz1, M. Jendryka1, B. Liss1, A. Pecek2, W. Nissen1, D. Kätzel3
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 decision making. Lesion studies in rodents have implicated the anterior cingulate cortex (ACC) and infralimbic cortex (IL) in impulse control, and the ACC and the prelimbic cortex (PL) in sustained attention, however the extent of lesions and adaptation effects evoked by them are difficult to delineate. Therefore, we sought to clarify the specific role of the different subregions of the ACC 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-Exclusively-Activated-by-Designer-Drugs (DREADDs), specifically expressed inexcitatory cells in distinct mPFC subregions of mice. The mice were trained and tested on the five choice serial reaction time task (SCSRTT). 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, counterbalancing 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 Gq imaging, but not in an untransfected region, proving the capability of DREADDs to activate excitatory cells in vivo. In the SCSRTT, we found that activating Gq signalling in the ACC by application of CNO decreases premature responding when impulse control is challenged but activation of Gq signalling in the IL does not show such an effect. Activating Gq signalling 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. Kilenzi1, B. van der Veen1, J. Teutsch, S. Schultz, D. Kätzel1
1Universität Ulm, Applied Physiology, Ulm, Germany

Cognitive dysfunction remains an unmet 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 assoziated 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 (DMTP), task. Targeted knock-down of
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 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**


1Goethe University Frankfurt, Institute of Neurophysiology, Frankfurt am Main, Germany
2University 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, unconditioned 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 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 at the time of the US omission. 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 fear extinction and reveal a crucial role of DA neurons in this form of learning. Current work is 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. Mytyluk, T. Korokkov

1Max Planck Institute for Metabolism Research, Neural Circuits and Behavior Group, Cologne, Germany
2University 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 LH-projecting dopaminergic and GABAergic VTA cells we optogenetically manipulated these pathways in several behavioural 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.
significantly suppressed, indicating successful NMDAR dysfunction at these synapses. In contrast, mossy fiber LTP in CA3 was left unaffected indicating NMDAR specificity of patient autoantibodies. These results indicate that patient-derived autoantibodies against NMDAR injected stereotactically into the hippocampus impair NMDAR-dependent synaptic plasticity, and lead to a distinct NMDAR-dependent behavioral phenotype.

B 02-2
Increased theta-resonance behavior in CA1 pyramidal neurons during blood-brain barrier-dysfunction-induced epileptogenesis
J. Shin1, K. Pagiazitis2, N. M. Simon3, J. Pagiazitis1, J. Shin2, N. Farassa1, K. M. Costa1,3, J. Roepel1
1Albert Einstein College of Medicine, New York, US
2Max Planck Institute for Brain Research, Frankfurt am Main, Germany
3National Institute on Drug Abuse, Baltimore, US

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 electrophysiologically studying the BBBd-impacted rat hippocampus following a cortical phototoxotoxias. 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 theta-activity through intrinsic subthreshold oscillations and resonance (increased voltage response) at theta-frequencies. While all pyramidal neurons display theta-resonance at hyperpolarized potentials (<-70mV, Ithetamediated), the expression of resonance at depolarized potentials (D-Res, >-65mV, Ithetamediated) depends on the Ithetaratio and is observed only in 30% of neurons. The remaining 70% of cells can express subthreshold resonance if the Ithetaratio is modulated. To elucidate the mechanisms contributing to seizures and theta-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 change between groups at subthreshold potential, the perithreshold excitability was significantly increased in D-Res neurons after BBBd. D-Res neurons revealed a more hyperpolarized action potential threshold together with a slice-increase in input resistance and maximal impedance. The higher number of resonant-neurons together with the increased excitability is in favor of the higher transmission of theta-oscillations in BBDb-treated animals. Our data provide evidence that BBBd-induced changes in intrinsic excitability of CA1 pyramidal neurons contribute to epileptogenesis and power increase in theta-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
1University of Leipzig, Carl-Ludwig-Institute for Physiology, Leipzig, Germany
2Leipzig University, Medical Faculty / Carl-Ludwig-Institute of Physiology, Leipzig, Germany
3University of Copenhavn, Center for Translational Neuromedicine, Kobenhavn, Denmark

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) degeneration, 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 blockade of voltage-activated K+ channels, which can cause an increase of neuronal activity could be beneficial to the SMA phenotype in vertebrates, we injected recently SMA-Δ7 mice with 4-AP (1mg/kg, i.p.). The treated SMA mice displayed slightly increased lifespan and bodyweight compared to vehicle-treated SMA mice. Furthermore, 4-AP treated SMA mice show an improvement in motor behavior at the end stage of disease, resulting in better weight control and ability to walk. Strikingly, proprioceptive synapses and innervated NMJ in SMA animals were significantly improved in number and function compared to untreated mutant littermates, with no rescue of vulnerable motor neurons, suggesting that 4-AP either prevents synaptic degeneration or induces sprouting. In vivo study reveals that sufficient increase of neuronal activity by 4-AP may have a beneficial long-term effect on motor function for SMA patients by improving central and peripheral synaptic connectivity.

B 02-4
Transient electrophysiological impairment of surviving dopamine neurons is associated with a temporary motor deficit in a 6-OHDA Parkinson Model
L. Kovacheva7, 1, J. Shin2, N. Farassa1, K. M. Costa1,3, J. Roepel7
1Goethe University, Institute for Neuropsychophysiology, Frankfurt am Main, Germany
2Max Planck Institute for Brain Research, Frankfurt am Main, Germany
3National Institute on Drug Abuse, Baltimore, US

Parkinson disease (PD) is the second most prevalent neurodegenerative disorder and is characterized by the accelerated loss of dopamine neurons (DA) in the substantia nigra (SN). It is currently unclear to what degree surviving DA neurons homeostatically adapt their electrical activity en route to cell death during PD, which might partially protect or accelerate their degeneration. To investigate this issue, we used a single-hi intra-striatal 6-OHDA mouse model to study surviving DA SN neurons at different timepoints post-lesion. By monitoring spontaneous motor behavior of the 6-OHDA and ACSF-infused control mice for 3 months post-surgery, we identified two distinct phases. During the first few weeks after lesion the 6-OHDA infused mice displayed a strong ipsilateral turning deficit compared to ACSF-infused mice (impaired phase). Thereafter, from the course of 2 weeks this turning deficit completely disappeared (recovered phase). To explore the functional activities of surviving DA SN neurons during these two behavioral states, we initiated in vivo multi unit extracellular recordings combined with justicaelular labeling for immunohistochemical characterization and localization. By focusing on the medial SN, we found that the firing frequencies and patterns of surviving DA neurons recorded during the recovered phase were not significantly different from DA SN neurons from ACSF-infused controls. In contrast, the activity of DA SN neurons from 6-OHDA infused mice recorded during the impaired phase, displayed a significant 7-fold reduction in burst rate (control: 0.31 ± 0.14, n=9; 6-OHDA: 0.04Hz ± 0.02, n=9), while their mean frequency of firing was not affected (control: 5.4Hz ± 0.51, n=9; 6-OHDA: 4.13Hz ± 0.59, n=9). Via in vitro whole cell recording we were able to explore the intrinsic electrophysiological dynamics of the surviving DA neurons. In synaptic isolation, in vitro, the DA neurons presented with a 4-fold increase of CV only during the impaired phase (impaired phase: control 6.4% ± 0.6, n=14, 6-OHDA 27.7% ± 4.5, n=31; recovered phase: control 10.2% ± 1.5, n=12, 6-OHDA 9% ± 3.2, n=33). This transient electrophysiological phenotype, which might be based on shift in activity-dependent calcium-loading during the early post-lesion phase, was in line with the observed behavioral temporal dynamics in our model. Our next step would be to further explore the mechanisms behind the functional development in the surviving DA SN neurons.

B 02-5
Molecular and cellular pathophysiology of Episodic ataxia 6
P. Kouvermann1, Y. Kolobkova5, V. Untere1, C. Falke1
1Forschungszentrum Jülich, Zelluläre Biophysik, ICS-4, Jülich, Germany
2University of Copenhagen, Center for Translational Neuromedicine, Kobenhavn, Denmark

Episodic ataxia 6 (EA6) is an inherited human disease, which is characterized by combined ataxia and epilepsy and which has been associated to mutations in the glial excitory amino acid transporter 1 (EAAT1) [1, 2, 3, 4]. A proline to arginine substitution at position 290 (P290R) of EAAT1 results in severe form of EA6 [1; 6]. EAAT1 also functions as an anion channel [5], and in works on heterogeneous expression systems demonstrated that P290R impairs the glutamate transport rate, while increasing channel activity [6]. To understand how these functional changes result in ataxia, we developed a transgenic mouse model (S1a3P290R+).
Slt1a3P290R/+ 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 Slt1a3P290R/+ mice indicated that Bergmann glial cells undergo apoptosis during the second week of life. We studied internal [Ca²⁺] in Bergmann glial cells using fluorescent lifetime imaging with the Ca²⁺-sensitive dye MDAE [8] and found reduced values of [Ca²⁺] in transgenic mice at ages prior to apoptosis. Whole cell recordings of glutamate-evoked Ca²⁺ currents from Bergmann glial cells from young mice (P9-14) showed that the peak currents of Bergmann glial cells from mutant mice were highly increased. Cell-attached recordings of Purkinje neuron simple spiking activity showed that spike firing of Purkinje neurons from transgenic mice is impaired and that inhibition of spiking through climbing fiber signaling is considerably prolonged, probably due to an impaired glutamate clearance in the tissue. We conclude that P290R mutation triggers glial apoptosis via increased Ca²⁺ efflux, resulting in the loss of glutamate glutamate clearance and in ataxia and progressive cerebellar hypoplasia.


B 02-6

The relevance of the ER-located enzymes PDI and PERK relating to the Huntington-mediated ER-stress in SH-SYSY cells

C. Sänger, P. Kranz, K. Göpelt, M. Baumann, U. Brockmeier, E. Metzen
Universität Duisburg-Essen, Institute of Physiology, Essen, Germany

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 regulate ER stress. However, prolonged ER stress finally leads to apoptotic 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 under conditions caused by mHTT in a neuroblastoma cell model 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. Wärn1, D. Franz2, M. Rohde1, V. Neubert1, M. Paap2, S. Perl1, C. Niemann1, F. Plocksties1, 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 dIz2 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. We hypothesized that DBS may influence basal synaptic GABAergic transmission and synaptic plasticity finally leading to reset the inhibition of basal ganglia output nuclei.

To test this hypothesis, bipolar DBS-electrodes were bilaterally implanted in the entopeduncular nucleus (the homolog of the medial globus pallidus of primates) of dt(sz) and control hamsters. After short-term bilateral DBS (3 Hz, 130 Hz, 50 µA) in awake hamsters, 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 same conditions, and in the absence of the glutamate 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. Sömmes1, S. Spieß1,2, R. Mustafa1,2, K. Kojer1, C. Litke1,2, J. Koch1, M. Orth1, B. Liss1, R. Parlatò1,2
1Ulm 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 nuclei of patients which interfered in turn 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 huntingtin (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 HD human HD patient skeletal muscle biopsies and fibroblasts at different stages (pre-symptomatic, early HD and late HD) were analyzed to monitor nucleolar activity and integrity over time. The analysis was carried out by immunofluorescence with specific nucleolar markers as well as confocal imaging and a 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. Schulze1, S. Müller1, X. Gu1, S. Lukas1, B. Hannes1, R. Till1, R. Marco1, T. Kirschstein1, R. Köhling1, F. Schulze1
1Rostock 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 casin 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 KCa2.2 and also of KCN1 and HCN3 channels. Therefore, we postulated 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.
B 02-10
Reduced olfactory perception to Isoamyl Acetate in Streptozotocin-Induced Diabetic female rats

B. 02-10

Hyperpolarizations in SN DA neurons, and afforded their full protection from degeneration in vivo in a neurotoxin PD mouse model (chronic MPTP/probenecid). In conclusion, Cav.2.3 emerges as a novel target for combating Ca\(^{2+}\)-dependent neurodegeneration in PD.

B 02-11
Cav.2.3 channels trigger selective dopaminergic neuron loss in Parkinson's disease

B. 02-11

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 mostly spared. The mechanisms underlying this age-dependent and region selective loss are unclear. However, activity-related metabolic stress and dysfunctional Ca\(^{2+}\) 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 Ca\(^{2+}\) levels which are thought to impart mitochondrial stress and render these neurons more vulnerable to degenerative stressors. This stressful Ca\(^{2+}\)-driven mode of action distinguishes SN DA from resistant VTA DA neurons. Particularly in distal dendrites of SN dopaminergic neurons, these Ca\(^{2+}\) transients are sensitive to inhibitors of L-type voltage-gated Ca\(^{2+}\) channels (LTCCs), such as isradipine. Consistent with a role for activity-related Ca\(^{2+}\) signals in triggering neuronal demise in PD is epidemiological evidence, correlating use of LTCC blockers with a reduced risk for developing the disease later in life. But a recent phase-III clinical trial with isradipine with its potential for neuroprotection in PD patients was negative, and in PD animal models, there is no agreement regarding their role in the neuroprotection by LTCC inhibition. We combined single cell molecular techniques, brain slice patch-clamp recordings and Ca\(^{2+}\) imaging with pharmacological and genetic tools to analyze the role of all brain voltage-gated Ca\(^{2+}\) channels (VGCCs) for DA neuronal viability in PD. We identified Cav.2.3 R-type Ca\(^{2+}\) channels, which have yet to be studied in the context of PD, as a new key regulator of SN DA neuronal viability in mouse SN DA neurons, and they were upregulated during aging, in contrast to LTCCs. Plasma membrane Cav.2.3 protein was higher in SN DA than in VTA DA neurons. Moreover, Cav.2.3 KO reduced activity-associated somatic Ca\(^{2+}\) transients and Ca\(^{2+}\) dependent after-hyperpolarizations in SN DA neurons, and afforded their full protection from degeneration in vivo in a neurotoxin PD mouse model (chronic MPTP/probenecid). In conclusion, Cav.2.3 emerges as a novel target for combating Ca\(^{2+}\)-dependent neurodegeneration in PD.

B 02-12
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

B. 02-12

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 Th17/IL-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. Therefore, 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 and 60 days of exposure. One group was treated with saline (control), and the other 5 groups were treated with 2-Hep (which is a blocker of IL-17A) at different concentrations (5 x 10^(-5) to 5 x 10^(-4)). After the 60 days of exposure, we analyzed the IL-17A levels in the hippocampus and the remaining 6 were processed for immunohistochemistry (against IL-17A or IL-17RA and GFAP, Iba-1, NeuN or CD3).

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 IA2066919 to H.S.V and IN221417 to S.R.A.

B 03 | Sensory Physiology

B 03-1
Autoregulation as a potential pathway of miRNA-host-gene interaction linked to neuropathic pain development

B. 03-1

MicroRNAs (miRNAs) are 22-25bp non-coding RNA fragments that are implicated in mRNA regulation via degradation or translational inhibition. Currently, ~1'900 miRNA genes have been experimentally found or predicted by potent computational miRNA prediction algorithms. Potential mRNA::miRNA 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 to establish 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
Computational analysis of miRNAs genomic location revealed that the majority of miRNAs were located within intronic regions of protein coding genes across species. The microCOS predictions algorithm predicted that >60% of intragenic miRNAs target their host genes with a higher frequency and prediction score compared to the IRM data. Host genes subjected to pathway analysis revealed a number of potentially neuropathic pain-related pathways. RNA sequencing revealed a number of dysregulated intragenic miRNAs as well as host genes. Significant correlations for intragenic miRNA-host gene interactions were identified.

Conclusion

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 potential underlying autoregulatory mechanisms of intragenic miRNAs to their host genes, which was conserved across species. These results 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. Rühe
t et al.

RWTH Aachen University, Institute of Physiology, Aachen, Germany

The intracellular injection of RN-1734 at 500 µM into the knee joint reduced the responses of C-fibers of the normal joint to mechanical stimulation (control 214±55 APs/15 s, after 3 hours 53±30 APs/15 s) and nociceptors (control 215±30 APs/15 s, after 3 hours 74±42 APs/15 s) mechanical stimulation. In line with this, the lower mechanical thresholds in the receptive fields increased. The responses of nociceptive A-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 intracellular application of the TRPV4 agonists 4αPDD, GSK 1016790A, and RN-1747 did not consistently alter the responses of A- 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 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 03-3**

**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. Schaible

University Hospital Jena, Institute of Physiology I / 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 mNm) or noxious (40 mNm) 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 A-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, 4αPDD, 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 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 possible 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 postero lateral thalamic nucleus, parietal cortex, central nucleus of the amygdala and affective hub 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 oscillatory 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 coherences 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. Ebersberger1, C. König1, A. Eitner1, R. Wetzker2, H. - G. Schaible1
1 Jena University Hospital, Institute of Physiology/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 P2Eγ receptor subtype EP3 which counteracts nociception during inflammation. Therefore, EP3 receptor activation could limit the sensitizing effect of PGE2 but how the EP3 receptor interferes with EP2 and EP4-dependent sensitization signaling networks is not clarified.

We used the whole cell patch clamp method to analyze TTX-resistant sodium currents of primary sensory neurons (DRGs). Stimulation of EP3, 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 antagonist. 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 control DRG protein lysates, no decrease of either EP2 or EP4 dependent activation of PKA and Erk1/2 by concomitant EP3 activation could be observed. However, EP3 was proofed to be 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 EP2 dependent sensitization in DRGs of PI3Kγ−/− mice but was maintained in neurons carrying a kinase-dead version of PI3Kγ. PI3Kγ, activated by Gαi subunits of G-protein coupled receptors, can activate phosphatidylserine dehydrogenases in a kinase-independent manner and thereby providing 5′AMP for AMPK-activated Kinase (AMPK) activity. Indeed, an inhibitor of AMPK activity could block the EP3-dependent inhibition of EP2-induced sensitization.

While inhibition of EP2 activation through EP3 is lost in PI3Kγ−/− DRGs, its inhibitory capacity on selective EP4-induced sensitization was still intact. EP4 is known to strongly undergo internalization processes and this subunit could also recruit G-protein-coupled receptor kinase 2 (GRK2) to enable it. Inhibition of GRK2 diminished EP3 dependent receptor desensitization 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. Hawksins
University of Oxford, Oxford, UK

Melanopsin (Opn4) expressing intrinsically photosensitive retinal ganglion cells (iRGCs) 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 iRGCs in image forming vision. iRGCs are not a homogenous population of cells but rather are comprised by various subgroups with distinct physiological functions. To-date, iRGC subgroups are defined by morphological criteria, which are not free of ambiguity. This inhibits the rigorous study of individual subgroups in physiological experiments. To date, these individual iRGC subpopulations and identify specific molecular markers, we performed an in-depth analysis of the 804 iRGCs (Opn4-positive) cells contained in a published single-cell RNA-seqencing dataset (Rheinme et al, 2018). Using the k-nearest neighbors classification algorithm we were able to identify six genetically distinct clusters of iRGCs by a data-driven approach. Subsequently, we identified candidate molecular markers that were not only specific for each iRGCs cluster but showed high specificity against all classes of non-intrinsically photosensitive retinal ganglion cells. To independently validate these bioinformatically obtained markers, we performed immunostaining on retinal cross sections collected from iRGC reporter mice. Indeed, immunostaining against the identified markers (e.g. Kvl2, Atdoc) labeled distinct subpopulations of iRGCs, which will 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 iRGC subgroups.

Taken together, the identified markers will facilitate the study of the physiology of the distinct iRGC subpopulations. Moreover, the present dataset of differentially expressed genes may provide a starting point for studying iRGC-subtype specific signaling processes.

Loss of adult-born juxtaglomerular neurons after their functional integration in the mouse olfactory bulb

K. Li1, X. Su, Y. Kovalchuk, S. Wang, K. Li, O. Garaschuk
1 Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tübingen, Germany

In the adult mammalian subventricular zone, newborn cells are generated throughout life. Subsequently, progenitors migrate through the rostral migratory stream (RMS) into the olfactory bulb (OB), where they differentiate into local interneurons: granule cells and juxtaglomerular neurons (JGNs). According to previous immunohistochemical data, only a half of the newcomers survive over the longer time periods, whereas the other cells die soon after their arrival. It has long been postulated that the death is caused by the “failure to integrate” into the pre-existing neuronal circuitry. To directly test this hypothesis, keratinus encoding a ratelomatic Ca2+ indicator Twitch-2B was injected into the RMS to label migrating neuroblasts. Functional integration of Twitch-2B-positive adult-born JGNs was assessed by their response to odorant stimulus and their survival/death was monitored in vivo through a cranial window by means of two-photon microscopy. Our data showed that some adult-born JGNs were eliminated after showing responsiveness to the odorant stimulus, and there was no significant difference between eliminated and surviving adult-born JGNs in terms of the morphal amplitude and area under curve of their odor-evoked responses, suggesting that survival/death of adult-born JGNs is independent of their functional integration into the OB circuitry. However, it turned out that subsequently eliminated and surviving adult-born JGNs have different patterns of spontaneous activity. To test whether the morphology of adult-born JGNs is predictive for their future fate, we labeled JGNs with a mixture of retroviruses encoding mCherry (red), Venus (green) and Cerulean (blue) fluorescent proteins, thus giving each particular cell its own color identity, and longitudinally imaged their morphology. Surprisingly, the development of the surviving JGNs was accompanied by a significant reduction of their dendritic complexity after they reached final destination, but so far these morphological changes could not predict the cell’s fate.

Taken together, these data refute the “failure to integrate” hypothesis and suggest that activity-dependent survival/death of adult-born JGNs depends on their spontaneous rather than sensory-driven activity.

The role of endogenous activity for the development of adult-born neurons in the mouse olfactory bulb

K. Li1, X. Su1, Y. Kovalchuk1, O. Garaschuk1
1 Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tübingen, Germany
2 Eberhard Karls University of Tübingen, Institute of Physiology, Department of Neurophysiology, Tübingen, Germany

Olfactory bulb (OB) is continuously supplied with new neurons, generated in the adult subventricular zone and reaching the destination layers in the OB after long-distance migration in the rostral migratory stream (RMS) and in the OB proper. The mechanisms underlying the migration, morphogenesis and survival of these adult-born interneurons are not well understood. Here, we studied in vivo the roles of endogenous and sensory-driven neuronal activity for the development of adult-born juxtaglomerular neurons (JGNs). To modify the endogenous activity of adult-born JGNs we overexpressed Kv1.2 or Kir2.1 potassium channels via viral injection into the RMS and used two-photon Ca2+ imaging in awake mice to monitor spontaneous and sensory-driven Ca2+ signals as well as cell morphology in control and genetically-modified JGNs. Kv1.2/kir2.1 overexpression modified spontaneous Ca2+ signaling of adult-born cells and significantly impaired their migration, morphogenesis, odor-evoked responsiveness, and survival rate. Interestingly, adult-born JGNs in odor-deprived hemibulbs displayed normal migration and morphology, thus stressing the importance of endogenous activity for these processes. Further, our results showed that CREB expression was down-regulated in Kv1.2- and Kir2.1-overexpressing JGNs. Taken together, our results demonstrate that the endogenous but not sensory-driven activity plays a key role in regulating migration, morphogenesis and early-phase survival of adult-born OB interneurons and identify an important role of Kv1.2/kir2.1 in the mentioned above developmental processes. Further, the data identify a critical role of the CREB signaling pathway for early development of adult-born JGNs.
B 03-10
The soluble epoxide hydrolase attenuates pathological vascularization by preventing astrocyte loss in a retinopathy of prematurity model in mice
J. Hu 1,2, S. I. Bibili 1,2, J. Wittig 1,2, J. Lin 3, S. Zukunft 1,2, H. - P. Hammes 3, R. Popp 1, I. Fleming 1,2
1Goethe-University, Institute for Vascular Signalling, Centre for Molecular Medicine, Frankfurt, Germany
2German Centre for Cardiovascular Research (DZHK) partner site RheinMain, Frankfurt, Germany
3University of Heidelberg, 5th Medical Department, University Medicine Mannheim, Mannheim, Germany

Retinopathy of prematurity (ROP) is a leading cause of childhood blindness. Several signalling pathways have been implicated in the process of ROP but a novel druggable target is the cytochrome P450 (CYP) soluble epoxide hydrolase (sEH) pathway. The latter enzymes play a crucial role in biological processes by generating bioactive epoxides and diols from endogenous polyunsaturated fatty acids (PUFAs). This study aimed to characterize the effects of the newly identified sEH-derived w-3PUFA diol, 19,20-dihydroxyoctadec-cis-5,8,11,14-eicosatetraenoic acid (DHDHP) in a mouse ROP model.

ROP in mice was achieved by placing pups in 75% oxygen for 5 days from postnatal day (P) 7 until P12, before moving animals back to room air for an additional 5 days. This procedure resulted in the development of avascular areas that were significantly larger in retinas from sEH-/- mice than in their wild-type (WT) littermates at P17. Avascularization was accompanied by angiogenesis in the form of characteristic vascular “tufts” which were more evident in retinas from sEH-/- mice. Although there was no difference in vascular obliteration, a greater decrease in the astrocyte network was observed in sEH-/- mice after exposure to high oxygen compared to that in WT littermates at P8. Mechanistically, hyperoaemia depleted 19,20-DHDHP by affecting sEH activity. 19,20-DHDHP reduction increased levels of cholesterol in the mitochondrial membrane which disturbed the presenilin-1/-/membondial homolog 1 (MTCH1) complex. The loss of the PS-1/-MTCH1 complex resulted in mitochondrial dysfunction, subsequent activation of caspase 3 and cleavage of the DNA binding motif of PARP1 leading to mitochondrial (mt) DNA damage. Administration of 19,20-DHDHP preserved the PS-1MTCH1 complex in the outer mitochondria membrane and prevented PARP1 cleavage and mtDNA damage in astrocytes exposed to hyperoaemia in vitro. Moreover, intravitreal injection of 19,20-DHDHP to sEH-/- mice at day 7 significantly prevented PARP1 cleavage, mtDNA damage and astrocyte loss induced by exposure to 75% O2 on day 8, and reduced both the avascular area as well as vascular tuft formation on P17.

Our data indicate that the sEH-dependent generation of 19,20-DHDHP from w-3 PUFA is required for retinal astrocyte survival and preservation of mitochondrial membrane and DNA integrity under hypoxic conditions.

B 04 | Hypoxia and Hyperoxia

B 04-1
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 neurological 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 synaptogenesis 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 developed an in vitro neurosphere system to study changes in expression of synaptogenic factors using qPCR. To investigate the involvement of HIF-2a in specific cell populations, the formation of synaptic contacts and the expression of synapse regulating molecules will be studied in hippocampal neuron-astrocyte co-cultures. Two further approaches will explore the role of HIF-2a 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.

B 04-2
Hypoxia enhances endothelial ICAM-1 expression through upregulation of arginase-II and mitochondrial oxidative stress
X. Liang, P. Arulampalam, 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, HIF-1a, HIF-2a, and ICAM-1 expression, whereas no change in the expression of VCAM-1 and E-selectin was observed. Similar effects were obtained in cells treated with a hypoxia mimetic Dimethylsulfoxylglycine (DMOG), Silencing HIF-1a, but not HIF-2a, 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-1a-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.

Application and effects of artificial oxygen carriers in pO2-controlled kidney cell culture
A. Stokovs1, M. Cantore1, J. Fandrey, K. B. Ferenz 2
1University of Duisburg-Essen, Institute of physiology, Essen, Germany
2University of Duisburg-Essen, CeNIDE, Duisburg, Germany

Kidneys donated for transplantation that experienced 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) compared. In addition, we established a cellular system in which we measured and controlled the pericylic O2-fraction (O2). 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.

As several target genes of HIF-1a accumulation was detected after 4 hours of hypoxia, the hypoxic phase of all further experiments was set to 4 hours. The O2 measurement was successfully established and a steady decreasing O2 was observed reaching the target O2 concentration of 1%. Starting at this baseline of 1% O2 the application of 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 pericylic O2-fraction (O2). 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.

Combined, we expect to gain deep knowledge of the role of HIF-2a during physiological and pathological processes in synaptic transmission.
10 (β-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 HIF-2α KO mice. Our data suggest that additional knock-out of HIF-2α might reverse the disease ameliorating effect of myeloid HIF-1α knock-out in experimental colitis. Therefore, HIF-2 seems to have antagonistic functions to HIF-1 in stimulated myeloid cells.

**B 04-4**

Role of hypoxia on lipid signaling

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 inflamed 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 (BMMDs).

Methods: We are studying MEFs and BMMDs from mice with wild type and altered sphingolipid metabolism regarding hypoxia response and sphingolipid levels under normoxic (N2, 21% O2) and hypoxic conditions (HDX, 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 HIF-1α RNA expression and on specific HIF-1α target genes of BMMDs, 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.

**B 04-5**

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 (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 myeloid HIF-1α ameliorates dextran sodium sulfate (DSS)-induced colitis in mice. Based on this work, initially were worked with HIF-1α−/− x HIF-2α−/− mice (WT) and Ly2-Cre/HIF-1α+/- x HIF-2α−/− 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 pro-inflammatory cytokine interleukin-10 (β-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 HIF-2α KO mice. Our data suggest that additional knock-out of HIF-2α might reverse the disease ameliorating effect of myeloid HIF-1α knock-out in experimental colitis. Therefore, HIF-2 seems to have antagonistic functions to HIF-1 in stimulated myeloid cells.

**B 04-6**

Targeted manipulation of nuclear transport processes inhibits hypoxia signaling pathways and 3D spheroid growth of cancer cells

F. K. Kesy*, M. von Fallouis1, Y. Landsman2, R. Depping1
1University of Löbeck, Institute of Physiology, Löbeck, Germany
2University Medical Center Schleswig-Holstein, Clinic for Radiotherapy, Lübeck, Germany
3Karyopharm Therapeutics, Newton, US

The nucleocytoplasmic transport of macromolecules is critical for both cell physiology and pathophysiology. The main receptor for nuclear export of proteins is exportin 1 (XPO1). The activity of XPO1 is inhibited by selinexor (KPT-335), a selective inhibitor of nuclear export which is currently tested in advanced phase 2 and 3 clinical studies. Selinexor covalently binds to cytostatin-52 in the cargo-packet pocket of XPO1 and blocks its function. XPO1 plays an important role for the cellular adaptation to reduced oxygen availability by controlling the nuclear transport of hypoxia-inducible factors (HIF). More than 1000 genes are regulated by HIF which are involved in a wide variety of cellular processes including angiogenesis, cell proliferation, metabolic reprogramming and haematopoiesis. Due to the fact that HIF target genes also encode proteins involved in critical aspects of cancer progression, cell survival and metastasis, HIF-dependent signaling pathways are exploited by cancer cells during the progression of many solid tumors. In tumor research, three-dimensional (3D) tumor spheroid culture models represent a powerful tool recapitulating in vivo microenvironmental properties of cultured tumor cells ex vivo. Unlike two-dimensional (2D) cell culture models, 3D tumor spheroids cells grow with zones of cellular heterogeneity providing gradients of nutrients and oxygen and a high physiological relevance.

In this study, we evaluated the physiological activity of selinexor on the hypoxia response pathway in 2D monolayer and 3D tumor spheroid culture models of MCF-7 breast cancer cells. Selinexor treatment reduced HIF-3 transcriptional activity in luciferase reporter gene assays and expression of the HIF-1 target gene solute carrier family 2 member 1 (SLC2A1) in quantitative real-time (qRT)-PCR experiments. MCF-7 breast cancer cells formed regularly shaped tumor spheroids in different 3D generation models. Incubation with selinexor inhibited the formation of 3D tumor spheroids and caused remarkable structural changes. Moreover, selinexor treatment reduced viability of cells cultured in 3D culture conditions. For the first time, we demonstrate the effect of specific XPO1 inhibition on the hypoxic response on the molecular level in 2D and 3D culture models of MCF-7 cells.

**B 04-7**

Transcriptional regulation of androglobin and calcium-mediated androglobin cleavage

T. W. Koshi, M. Suarez Alonso, A. Keppner, D. Hoogewijs
University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular System, Fribourg, Switzerland

The author has objected to a publication of the abstract.
Results: Lung tissue MDA, GPx and HIF-1α resulted at comparable values. SOD tended to increase in both HBO groups but did not reach a significant value (P > 0.05). Interestingly, Nrf2 expression was found to be significantly decreased (P = 0.006) session-NBO group for Nrf2 expression was also notable.

Introduction: Hyperbaric oxygen (HBO) administrations are known to interact with antioxidant/antioxidant and inflammatory pathways of living organisms. To avoid oxygen toxicity, HBO is generally administered intermittently with short air respiration intervals at airtight every half an hour. In this experimental study, the effect of continuous HBO exposure on 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 kept 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, 20:40-60 or 60:40-20 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-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) measurements.

Discussion: Lung tissue TNF-α and IL-1β levels tended to decrease with HBO exposure but reached only significant values in the 30:30-30 and 20:40-60 min groups when compared with controls (P = 0.02 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 group. Interestingly, the oxidative activity of the Sph-exposed rats (10 mM) considerably increased by 50% in the 40:40-20 and 30:30-30 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 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. Jägers1, J. Fandrey, E. Guibins2, K. B. Ferenz1

1University of Duisburg-Essen, University Hospital Essen, Institute of Molecular Biology, Essen, Germany
2University of Duisburg-Essen, University Hospital Essen, Institute of Physiology, Essen, Germany
3University of Cincinnati, Department of Surgery, Cincinnati, US
4University of Duisburg-Essen, CENIDE, Duisburg, 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 almost anoxic 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 potential of Sph-based oxygen carriers to (i) eliminate hypoxic areas in infected lungs and to (ii) reach high local Sph concentrations in the inflamed tissue and in the biofilm. Lung oxygen carriers were synthesized and loaded with 10 mM Sph. Particle size was controlled using dynamic light scattering while the 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 1 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 107 pg. aeruginosa 782.

B 04-11 Cardiopulmonary effects of the vasodilator relaxin on rats in normobaric hypoxia

U. Köhler1, H. Al-Ahmed2, J. Jägers1, A. Salameh1, B. Raßler3, S. Öter4, U. Kowalleck5

1University of Duisburg-Essen, Carl-Ludwig-Institute for Physiology, Leipzig, Germany
2Universitätsklinikum Leipzig, Institut für Pathologie, Leipzig, Germany
3Heinrich-Heine-University Düsseldorf, Department of Undersea and Hyperbaric Medicine, Düsseldorf, Germany
4University of Duisburg-Essen, University Hospital Essen, Institute of Physiological Chemistry, Essen, Germany
5University of Duisburg-Essen, University Hospital Essen, Institute of Physiology, Essen, Germany

Relaxin (RLX), known as a pregnancy hormone, is an effective systemic vasodilator and is involved in remodeling of the extracellular matrix. Previous studies showed that hypoxia exposure for several hours leads to an impairment of left ventricular pump function and – as a result of pulmonary hypoxic vasoconstriction – the development of pulmonary edema. RLX is known to have positive effects on hemodynamic parameters in acute heart failure. Therefore, we expected that RLX might improve the hypoxia-induced depression of left ventricular pump function. Furthermore we hypothesized that RLX might reduce pulmonary hypoxic vasoconstriction and thus would attenuate or even prevent pulmonary edema. Experiments were performed on 52 rats splitted in 6 groups. Normoxic and hypoxic (10% NiO2) control groups received 0.9% NaCl-infusion over 24 hours. We chose 3 doses RLX (RLX D1=3μg/kg, D2=15μg/kg, D2=75μg/kg) to define an optimized dose. All doses were administered to hypoxia-exposed animals. Additionally, RLX D1 was also given to a group of normoxic rats. After 24 h, hemodynamic measurements were performed, using left and right heart catheterization. Heart and lung samples were obtained for histological analyses.

First results show, that RLX slightly improved left and right ventricular pump function compared to hypoxic controls. LV dP/dt max in dimension of left ventricular contractility was increased by RLX D2, but remained significantly lower than in normoxic controls. In contrast, right ventricular contractility was re-increased to control level with RLX D1 and D2. All groups of RLX-infused rats showed moderate pulmonary edema while normoxic controls did not. In contrast to hypoxic controls, the edema in RLX-treated rats occurred predominantly in the upper lung lobe, while there was significantly less edema in the lower lobe. We suggest that the vasodilator RLX abrogated hypoxic pulmonary vasoconstriction resulting in pulmonary overperfusion. The following increase in pulmonary blood pressure may lead to liquid accumulation in the upper lung lobe due to gravity effects. In conclusion, the results show that RLX may improve hemodynamic function under hypoxic conditions but cannot prevent pulmonary edema.

B 04-12 Prevention of decompression illness: recently developed albumin-derived perfluorocarbon-based nanocapsules prove effectiveness on a clinical, biochemical and histological level

D. Mayer1, F. Guerriero2, C. Gausche3, A. Kreczy4, M. Ljubkovic5, K. Mayer1, M. Kern1, K. B. Ferenz1

1REGIO MED Klinikum Coburg, Department of Gastroenterology, Coburg, Germany
2University of Split, School of Medicine, University postgraduate study program Evidence-based medicine, Split, Croatia
3Université de Bretagne Occidentale, Laboratoire ORPHÉE, Brest, France
4REGIO MED Klinikum Coburg, Department of Pathology, Coburg, Germany
5University of Split, Department of Physiology, Split, Croatia
6University of Duisburg-Essen, Institute of Physical Chemistry, Essen, Germany
7University of Duisburg-Essen, University Hospital Essen, Institute of Physiological Chemistry, Essen, Germany
8University of Duisburg-Essen, University Hospital Essen, Institute of Physiology, Essen, Germany
9University of Duisburg-Essen, CENIDE, Duisburg, Germany

Decompression illness (DCI) is caused by the formation of nitrogen bubbles in blood and tissues. Perfluorocarbons with extraordinary gas dissolving capabilities failed in clinical routine because of side-effects associated with added emulsifiers. Albumin-derived perfluorocarbon-based nanocapsules (A-AOCs) combine good tolerability with the ability of transporting surplus nitrogen to the lung and improving oxygen supply in small vessels. The aim of this study was to assess whether A-AOCs could protect against DCI.

Thirty seven Wistar rats were treated with A-AOCs (n=12), albumin nanocapsules filled with neutral oil (A-0-0, n=13) or serum albumin (A-0-0, n=13) before a simulated air dive in a hyperbaric chamber (Compression: 100 kPa/min up to 1,000 kPa, maximum for 35 min, decompression: 100 kPa/min, plateau at 200/160 kPa, 10 min at 130 kPa). 11 rats injected mice were infused 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. Oxygen carriers with or without loading of Sph showed a medium diameter of 140 nm. The carriers were stably loaded with high amounts of Sph as the supernatant was free of any Sph. While Sph-free oxygen carriers showed no antimicrobial effect, Sph-loaded oxygen carriers killed S. aureus 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, in instance, cystic fibrosis mice.
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 of lactate, transaminases, creatine kinase, myoglobin, D-dimer-levels, lactate dehydrogenase were measured in plasma.

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 Gq protein inhibitor FR900359 is a strong pulmonary vasorelaxant ex and in vivo
A. Seidinger1, G. König1, E. Koslinska1, 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. Therefore, we have identified the depsipeptide FR900359 (FR) as a strong and specific pharmacological 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 isotropic 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 intratracheal application of FR (2.5 µg per mouse) with subsequent serotonin injection (3 mM, i.v.) by a 1 F Miller catheter.

Isometric force measurements revealed that a single dose of FR (1 µM) induces a strong vasorelaxation in murine pulmonary arteries after pre-constriction with several QG-dependent agonists. While FR was able to almost fully reverse serotonin pre-constriction (0.5 µM, 79.2 ± 2.5 % relaxation), endothelin (ET, 3 nM) and U46619 (U, 0.1 µM)-dependent constrictions were only reduced by 71.8 ± 1.2 % and 52.2 ± 3.0 %, respectively. 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 vasodilatory effect of FR with that of drugs currently used in human pulmonary hypertension (PH) and found that FR was superior (74 ± 2.8 % (n=7) at 10 µM FR vs 8.8 ± 2.9 %, n=5 (bosentan), 27.5 ± 3.9 %, n=7 (iloprost), 34.6 ± 3.5 %, n=6 (sildenafil), all p<0.001 vs FR. Apart from the prominent vasodilatory effect in pulmonary arteries we can also show that FR is a powerful relaxant of smaller intrapulmonary arteries as found in lung slices (83 ± 1.2 %, n=7) and the isolated perfused lung model (88 ± 6.46 %, n=7). 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 Gq 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 QG-dependent and QG-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. Matthey1, G. König1, B. K. Fleischmann1, D. Wenzel1
1University of Bonn, Institute of Physiology 1, Bonn, Germany
2University of Bonn, Research Training Group 1873, Bonn, Germany
Gq 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 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. ER (5 µg/ml) or vehicle were applied via the intratracheal route ex vivo. Airway 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 vivo effects of FR, a cell growth assay using human lung fibroblasts (HFL1) was used. After starving a time period 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 (3.9±0.4 cmH 2 O/mL n=9 (FR) vs 9.2±1.9 cmH 2 O/mL n=9 (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 lungs revealed that FR treatment decreased collagen deposition (3.1±0.2 µm²/µm perimeter basal membrane (pbm), n=11 (FR) vs 3.7±0.2 µm²/µm...
Genetic ablation of the newly identified androglobin leads to primary ciliary dyskinesia

A. Keppner1, S. Santambrogio2, P. Engeler1, K. Steiner1, D. Hoogewijs1

1University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular systems, Fribourg, Fribourg, Switzerland
2University of Zurich, Institute of Physiology, Zürich, Zürich, Switzerland

The author has objected to a publication of the abstract.

TGF-ß1 disturbs airway epithelia integrity and decreases claudin 3 in tight junctions via activating SMAD 2/3 proteins.

C. Schilling, R. Lischbaum, M. Frick, P. Dietl, O. Wittekindt

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-ß1). However, its effect on epithelial barrier function is poorly elaborated. Herein we investigated the effects of TGF-ß1 on TJ in lung epithelia.

Human bronchial epithelial cells (hBEpC) were cultivated under air-liquid conditions (ALI). To elaborate TGF-ß1 effects on epithelial barrier properties, we cultivated hBEpC with or without TGF-ß1 for different time points before harvesting TJ proteins for immunoblotting and immunofluorescence experiments. Moreover, to elucidate TGF-ß1 induced changes in protein abundance, we performed proteomics analysis and compared the results with a reference dataset. A novel approach to investigate the effect of TGF-ß1 on the claudin family, was a tissue microarray (TMA) based on a cohort of lung biopsies from patients with different lung diseases. Our data support our hypothesis that caveolae within ATI cells are potential mechanosensors. Our findings indicate that activation of ATI cells by P. Engeler1, K. Steiner1, D. Hoogewijs1

1University of Fribourg, Department of Endocrinology, Metabolism and Cardiovascular systems, Fribourg, Fribourg, Switzerland
2University of Zurich, Institute of Physiology, Zürich, Zürich, Switzerland

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Human bronchial epithelial cells (hBEpC) were cultivated under air-liquid conditions (ALI). To elaborate TGF-ß1 effects on epithelial barrier properties, we cultivated hBEpC with or without TGF-ß1 for different time points before harvesting TJ proteins for immunoblotting and immunofluorescence experiments. Moreover, to elucidate TGF-ß1 induced changes in protein abundance, we performed proteomics analysis and compared the results with a reference dataset. A novel approach to investigate the effect of TGF-ß1 on the claudin family, was a tissue microarray (TMA) based on a cohort of lung biopsies from patients with different lung diseases. Our data support our hypothesis that caveolae within ATI cells are potential mechanosensors. Our findings indicate that activation of ATI cells by
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 (from 5 kPa to 2 GPa), TGF-β1 (5 ng/ml) and/or 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 fibric changes underlying the pathophysiology of IPF. In addition, 10-4A cells allow for selective genetic editing to specifically address signaling pathways in IPF development or induced reporter gene expression for high throughput screens.

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B 05-09

P2X4 is activated in an autocrine fashion upon exocytosis by ATP stored in lamellar bodies

G. Fölsch, V. Winkelmann, L. Bareis, L. Staudenmaier, E. Hecht, C. Ziller, K. Ehinger

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 cells. 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 intracellular compartments of the alveolar lumen by exocytosis. Regulated secretion via exocytosis entails a sequence of highly regulated steps leading to secretion of 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) best known for its prominent role in endocytosis, but not exocytosis. To delineate the function of Dnm2 for LB exocytosis, we infected primary ATI1 cells with adenoviruses expressing either wt or mutant Dnm2. Mutants targeted the different functional domains of Dnm2, including Dnm2-K44A, Dnm2-S61D, Dnm2-T65A and Dnm2-T141A (point mutations in the GTPase domain), Dnm2-K89SA, Dnm2-R72A (point mutations in the GTPase activating domain) and Dnm2-ΔPRD (removed prolin rich domain). We used high-resolution live 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.

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B 05-10

Establishing a Co-Culture model of pulmonary fibrosis

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

1Ulm University, 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 distal lung fibrosis. 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 physiological 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 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 fibroblast 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 (Alveosia 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.

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B 05-11

Secretion of pulmonary surfactant from alveolar type II cells depends on dynamin 2

E. Wirsching, M. Poppi, T. Felder, N. Hobist, P. Dietli, M. Frick

1Ulm University, Institute of General Physiology, Ulm, Germany
2Avedix, ARTORG Center for Biomedical Engineering Research, Bern, Switzerland

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 epithelial type 2 (ATII) cells and used in to 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). Dnm2 is 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 ATI1 cells with adenoviruses expressing either wt or mutant Dnm2. Mutants targeted the different functional domains of Dnm2, including Dnm2-K44A, Dnm2-S61D, Dnm2-T65A and Dnm2-T141A (point mutations in the GTPase domain), Dnm2-K89SA, Dnm2-R72A (point mutations in the GTPase activating domain) and Dnm2-ΔPRD (removed prolin rich domain).

We used high-resolution live 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.

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B 05-12

Cystic fibrosis transmembrane conductance regulator (CFTR) loss as a new mechanism of edema formation and therapeutic target in lung infection


1Charité - Universitätsmedizin Berlin, Institute of Physiology, Berlin, Germany
2German Heart Center Berlin, Berlin, Germany
3Duke University, Department of Medicine, Neurology, and Neurobiology, Durham, USA

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 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 Cl concentration ([Cl]) inhibition. The CFTR sensitive with no lysine kinase 1 (WNK1) by tyrphostin or WNK463 replicated the effect of CFTR inhibition on endothelial permeability, while WNK1 activation by lemongrzoide blocked the effects of CFTR inhibition. In line with endothelial leak, inhibition of CFTR also increased endothelial Ca²⁺ ([Ca²⁺]) release, an effect that was again replicated by WNK1 inhibition and attenuated by lemongrzoide. Endothelial Ca²⁺ 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 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 through intracellular Cl accumulation, inhibition of Cl-sensitive WNK1 and subsequent disinhibition of TRPV4, ultimately resulting in endothelial Ca²⁺ influx and vascular barrier failure.

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our data provide clear evidence that K<sup>ir</sup> channels modulate synaptic as well as intrinsic properties of GABAergic cells in the hippocampus.

B 06-3
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, Ca<sup>2+</sup>-activated K<sup>+</sup> 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<sub>A</sub> receptor blocker gabazine (GZ) induces epileptiform activity acutely. 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 concomitant incubation 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-CA1 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 inhibited by the KCa2.2 blocker UCL 1684, demonstrating involvement of KCa2.2. We conclude that KCa2.2 protein degradation by the proteasome and not by lysosomes is involved in the generation of epileptiform afterdischarges.

B 06-4
pH dependent activation of TALK1 channel
S. Postic<sup>1</sup>, W. - H. Tsai<sup>1</sup>, S. - B. Yang<sup>1</sup>, M. Slak Rupnik<sup>2</sup>
<sup>1</sup>Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan
<sup>2</sup>Medical University Vienna, Physiology/Center for physiology and pharmacology, Vienna, Austria

To determine the influence of K<sup>ir</sup> channels on GABAergic inhibition we performed patch-clamp recordings of either pyramidal cells or P<sub>VC</sub> interneurons in acute hippocampal slices of mice. We modulated the channel activity by bath-applying either the K<sup>ir</sup> channel opener diazoxide (300 µM) to mimic the gain-of-function mutations underlying DEND syndrome or the K<sub>ir</sub>-channel blocker tolbutamide (500 µM) to test its therapeutic potential. Measurements of miniature inhibitory post-synaptic currents (mIPSCs) in pyramidal cells revealed that activation of K<sub>ir</sub> channels by diazoxide diminishes spontaneous release of GABAergic vesicles (Fig. 1 A-C). However, closing the channels with tolbutamide reversed this effect. In contrast, tolbutamide itself only slightly increases mIPSCs release, indicating that the channels are mainly closed under resting conditions (Fig. 1 D). When recording from P<sub>VC</sub> interneurons, we found that diazoxide decreases the membrane resistance and hyperpolarizes the membrane towards the K<sup>+</sup> equilibrium potential. Thus,

Two-pore domain potassium (K<sub>2P</sub>) channels play an important role in cellular electrical excitability and are regulated by diverse physiological stimuli including voltage, temperature, lipids, protons and certain permeant ions (e.g. Rb<sup>+</sup> and Cs<sup>+</sup>), as well as pharmacological compounds. The TALK-2 K<sub>2P</sub> 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 ataxia and ventricular arrhythmias their gating mechanisms are widely unexplored.

Here, we present novel pharmacological activators like 2-APB, Ordastine, DCPIB and three other Negative Charged channel opener diazoxide. In contrast to other K<sub>2P</sub> channels (e.g. TREK-1) that were activated by both LC-CoA and PIP<sub>2</sub>, we observed that the TALK-2 K<sub>2P</sub> channel was activated robustly by LC-CoA, but showed only a slight activation by PIP<sub>2</sub>. In addition to the observations described above, we discovered another interesting difference of the TALK-2 channel compared to the remaining members of this family. In K<sub>2P</sub> channels all stimuli are thought to finally converge at the selectivity filter representing the principal gate in K<sub>2P</sub> 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 MTS-reagents. Unlike all other K<sub>2P</sub> channels, we report here that the non-activated TALK-2 channels are insensitive to inhibition by the large QA ion Tetratetrametilammonium (TPA). However, TPA sensitivity increased when the channel was activated by 2-APB but not when activated by the permeant ion Rb<sup>+</sup>. Suitable observations were made testing for MTS-TEA modification of an introduced pore cysteine. However, the inhibition by smaller QA ions was similar with and without polarity pointing towards a structural constriction at the pore entrance of the channel. These findings identify a unique structural gating behaviour within the K<sub>2P</sub> channel family, rising interesting questions towards further gating mechanisms and structural diversity in K<sub>2P</sub> channels.
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 the 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 R233M 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 in the neutral to alkaline range did not influence the intrinsic properties of the 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.

B 06-5
Biophysical characterisation and interplay of voltage-gated calcium currents and A-type potassium currents in substantia nigra dopaminergic neurons
A. Rotta, T. Trusch, J. Liss
University of Applied Psychology, Ulm, Germany

The characterisation of voltage-gated Ca²⁺ channel (Cav) currents and of Ca²⁺-sensitive, voltage-gated A-type K⁺ currents in dopaminergic (DA) neurons of the substantia nigra (SN), and their possible functional interplay is of particular interest as the underlying ion channels (Cav and Kv4.3/KChip) both have been linked to viability of SN DA neurons in health and disease. While both these currents are present in SN DA neurons, a comprehensive dissection of the individual contributions of distinct Cav channel subtypes to voltage activated Ca²⁺ currents, as well as their impact on A-type Kv4.3 currents is missing. We are biophysically characterising Cav and A-type currents in SN DA neurons from coronal mouse brain slices at 33°C using whole-cell patch clamp techniques. We used suitable voltage step protocols and blocked all other confounding ion channels to isolate voltage activated Ca²⁺ currents. To dissect contributions of distinct Cav channels, we apply respective Cav-blockers, and we also analyse transgenic mice. Neurons were filled with neurobiol tracer during the recording to confirm the SN DA phenotype (tyrosine hydroxylase (TH) positive) with immunohistochemical staining afterwards. Only neurons positive for TH are included in the analysis. Data are given as mean ± SD, p-values referring to Mann-Whitney-U-Test.

In our preliminary recordings SN DA total bulk Ca²⁺ currents had a maximal conductance of 16.2 ± 0.6 nS at -11.6 ± 1.4 mV (n = 10) and a voltage dependence of steady-state activation (V⁰) at -47.0 ± 0.3 mV with a slope of 7.67 ± 0.03 (n = 10). The Ca²⁺ channel Cav-channel inhibitor ZD43 (10 µM) blocked 74 ± 3% of activated Ca²⁺ currents at -42 ± 2 mV (n = 6, p < 0.0001). With the L-type Cav blocker iridaside (10 µM) we blocked roughly 40% of current evoked at -40 mV (n = 4) and with 1 µM iridaside only 25% (n = 5). When we analysed bulk Ca²⁺ currents in SN DA neurons from Cav2.3 (voltage-gated R-type Cav) KO and WT mice, we noticed that the bulk current in KO mice was about 50% smaller than in the WT (n = 7 WT, n = 3 KO, p = 0.017). We are currently further addressing the effects of distinct Cav blockers in SN DA neurons from WT, Cav2.3 KO and Cav3.1 KO mice. Moreover, we are analysing biophysical properties and Ca²⁺-sensitivity of A-type Kv4.3 currents in SN DA neurons, in the absence and presence of Cav blockers, to quantify the effect of individual Cav channels on these currents.

B 06-6
ICAM-1 mediated cell-cell adhesion of A549 lung cancer cells to endothelial cells depends on the activity of the K⁺ channel KCa3.1
E. Buik, M. Rieke, H. Schiller, A. Schwab
University of Muenster, Institute of Physiology II, Muenster, Germany

Non-small cell lung cancer (NSCLC) is one of the leading causes of cancer related deaths worldwide. The main reason for this poor outcome is due to the early development of metastases. An important event within this metastatic process is cell-cell adhesion between cancer and endothelial cells. Previous studies identified the Ca²⁺-activated K⁺ channel KCa3.1 to be involved in the progression of lung cancer cells. Here, we investigated whether KCa3.1 plays a role in cell-cell adhesion of A549 NSCLC cells to HMEC-1 endothelial cells and which is the underlying mechanism. To estimate cell-cell adhesion between A549 and HMEC-1 cells, we applied single cell force spectroscopy to measure the adhesion force. The role of KCa3.1 channels was assessed pharmacologically (KCa3.1 channel blocker minicaps) or using siRNA for KCa3.1 in A549 or HMEC-1 cells. Applying minicaps, the activation force of A549 to HMEC-1 cells increased by 49%. Silencing of KCa3.1 either in A549 cells or HMEC-1 cells increased the adhesion force by 23% or 34%. The elevated adhesion force is due to increased expression of ICAM-1 in both cell lines when KCa3.1 channels are silenced. In the presence of blocking ICAM-1 antibody, the KCa3.1-dependent strengthening of cell-cell adhesion is abolished. Since ICAM-1 expression may be due to an increased production of reactive oxygen species (ROS), we also performed ROS-measurements using the nonfluorescent ROS indicator dichlorofluorescein diacetate (DCH2D). ROS in A549 cells exposed to TRAM-34 or senicapoc is increased by up to ~30%. Similar effects were observed for HMEC-1 cells. ROS production depends on the mitochondrial membrane potential. We therefore tested whether KCa3.1 channel expression is regulated in mitochondria. Immunostaining and immuno blotting of isolated mitochondria confirm the expression of mitochondrial KCa3.1 channels in A549 and HMEC-1 cells.

In conclusion, we found that KCa3.1 channels regulate ICAM-1 dependent cell-cell adhesion between A549 lung cancer and HMEC-1 endothelial cells. The localization of KCa3.1 channels in mitochondria of A549 cells suggests that the production of ROS could be a regulator between A549 and HMEC-1 cells.

B 06-7
The ‘cap’ provides a necessary structural requirement for the stabilization of the K⁺ flux gated open state of K2P channels
K. S. Vlaming1, S. Rinne1, A. Kope1, J. Leonard1, M. Bedoya1, M. Schewe1, A. K. Kiper1, T. Baumann1, W. W. Gonzalez2, B. de Groot3, N. Decher1
1Pädiatrische Universitäts-Medizin, Institute for Physiology and Pathophysiology, Vegetative Physiology, Marburg, Germany
2Max-Planck-Institute for Biophysical Chemistry, Biomolecular Dynamics Group, Göttingen, Germany
3University of Talca, Center for Bioinformatics and Molecular Simulations, Talca, Chile

K+ channels have a unique extracellular ‘cap’ structure, formed by two MT-P1-linkers, that creates two extracellular tunnel-like ion portals (EIP) leading towards the selectivity filter (SF). We have previously described a novel mutation in the KCNKn gene encoding the K+ channel TASK-4 which was identified in a patient with severe cardiac conduction disorder. This mutation is located within the EIP at position 88 (G88R), right above the SF, introducing a gain-of-function to channel gating. In the current study we characterized and utilized this mutation to investigate the role of the unique ‘cap’ structure on the conductivity and gating of K+ channels. Single-channel and macro-patch clamp recordings in the inside-out configuration of Xenopus oocytes showed that the conductivity of the G88R mutant increased from 46 pS to 113 pS and the open time by a factor of three. Modulation of K+ currents, as well as their impact on A-type Kv4.3 currents is missing.

In the current study we characterized and utilized this mutation to investigate the role of the unique ‘cap’ structure on the conductivity and gating of K+ channels. Single-channel and macro-patch clamp recordings in the inside-out configuration of Xenopus oocytes showed that the conductivity of the G88R mutant increased from 46 pS to 113 pS and the open time by a factor of three. Modulation of K+ currents, as well as their impact on A-type Kv4.3 currents is missing. Introduction and studying different amino acids at position 88 led to the conclusion that the biophysical property of the residue at this particular site is a crucial requirement for the stabilization of the selectivity filter. In summary, our data extend our understanding of selectivity filtered K+ channels.

B 06-8
The mono-ADP-ribosyltransferase ARTD10 regulates the voltage-gated ion channel Kv1.1 through PKCδ
Y. Tian1, P. Verheugd2, P. Tripathi3, D. Komming3, D. Wiemuth3, B. Falkenburger3, B. Lüscher1
1RWTH, Physiology, Aachen, Germany
2RWTH, Biochemistry and molecular biology, Aachen, Germany
3RWTH, Neurology, Aachen, Germany

All excitable cells contain tetrameric voltage-gated K⁺ channels. Kv1.1 K⁺ channels, fast inactivation occurs when the cytoplasmic N-terminus of an associated Kvb1 subunit enters the channel cavity and blocks the pore of the Kvα1.1 subunit. Modulation of Kvα1.1 by Kvb1 is subject to several regulatory mechanisms. For example, phosphorylation of Kvα1.1 at serine 446 increases peak current amplitude, and increases the extent of inactivation. It has been shown that activation of protein kinase Hrk (PKCδ) indirectly leads to de-phosphorylation of serine 446, and thereby reduces the extent of inactivation of Kvα1.1. PARP10 also named ARTD10 is an enzyme mediating mono-ADP-ribosylation of substrate proteins. This involves the transfer of ADP-ribose from NAD⁺ onto substrates. PKCδ has been identified as potential substrate of ARTD10 through protein microarray analysis. In HEK293 cells stably expressing ARTD10, we observed that ARTD10 reduced Kv1.1 protein abundance and PKCδ kinase activity. Since PKCδ indirectly leads to de-phosphorylation of Kvα1.1, a larger fraction of Kvα1.1 was phosphorylated in these cells, and patch clamp indicated that Kv1.1 current amplitude was indeed increased and inactivation enhanced. These results indicate that Kvα1.1 is regulated by ARTD10 in vitro.

To investigate the significance of this regulation in neurons, we isolated primary hippocampal neurons, in which Kv1.1 is the predominant voltage gated K⁺ channel. OUL35 is a potent and selective inhibitor of ARTD10, which decreased peak current
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**

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 (1) to 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 K⁺ (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 and 100 mV 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 currents amplitudes in a concentration-dependent manner, i.e. amplitudes at -200 mV were reduced by 10 mM Ba²⁺ to 26 % of control values, whereas 1 mM Ba²⁺ 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 -180 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.


### B 06-10

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

**A. S. Schmalzl, 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 potassium channel hKv1.3_V388C opened a new pathway (σ-pore) behind the central o-pore. At potentials more negative than -100 mV, where the central o-pore would be normally closed, a large inward current through the o-pore appeared being carried by different cations like Na⁺, Li⁺ or Cs⁺ 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 K⁺ (145 mM KF, 2 mM MgCl₂, 10 mM HEPES and 10 mM HEPES). To simultaneously measure current through both o- and o-pores 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 asCTX, KTX and AqTx blocked the o-porewith 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 external side of the channel like PAP-1 blocked currents through both the o- and the o-pore with high affinity similar to wildtype (Kᵢ = 2 mV). The phenylalkylamine blocker verapamil and its derivative nonverapamil acting also from the external side of the channel reduced currents through both pores with the same affinity and similar to wildtype. Methoxyverapamil and acamproverapamil could also reduce both currents, but showed 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.

B 07-2
The melastatin-like Transient-Receptor-Potential-7 protein regulates Cyclooxygenase-2 expression in human myeloid leukemia cells
S. Hampel, W. Nadjafi, K. Höltig, M. Fratelli1, L. Addington1, D. Steinritz1, V. Chubanov1, I. Boekhoff, A. Breit, R. Greil, T. Gudermann1, S. Zierer1
1Ludwig-Maximilians Universität München, Wieder Straub Institute of Pharmacology and Toxicology, München, Germany
2Paracelsus Medical University Salzburg, Department of Medicine III, Salzburg, Austria

We recently acknowledged the ubiquitously expressed melastatin-like transient-receptor-potential-7 protein (TRPM7) as a modulator of immune system homeostasis. TRPM7 combines a cation channel, conducting calcium, magnesium and zinc, with a serine/threonine α-kinase, phosphorylating i.e. annexin A1, myosin II and Smad2. TRPM7 has been implicated in the growth of 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) often highly express 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 signalling cascades. Genetic inactivation of the TRPM7 kinase by introducing a point mutation at the active site of the kinase (K1648R) in hap1 cells revealed that the reduction of 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 chronic 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.

B 07-4
Uncovering the nuclear-coding domain from the channel gate in HCN2 channels
S. Yüksel1, M. Kondapuram1, T. Schwabe1, T. Zimmer1, M. Lelle1, A. Schweinitz2, M. Bonus2, H. Gohike1, R. Schmauder1, J. Kusch1, K. Benndorf1
1University Hospital Jena, Institute of Physiology II, Jena, Germany
2Heinitz Heinrich-Universität Düsseldorf, Institute for Pharmacological 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 disk located between 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 (AAAAS) between S6 and the first helix (A) of the CL to uncouple pore and CNBD-CL portion. The AAAAS was formed by one, two, three, four or five glycines following S441: 441-G, 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: (1) The S6 helix bundle is a functional gate. (2) Already a single additional glycine destroyed the cAMP-induced enhancement 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:

B 07-3
Interactions between opposite subunits stabilize autoinhibition in HCN2 channels
M. Kondapuram1, S. Yüksel1, C. Sattler1, B. Frie2, G. Golihke1, R. Schmauder1, K. Benndorf1, J. Kusch1
1Universitätsklinikum Jena, Institut für physiologie II, Jena, Germany
2Heinitz Heinrich-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, Cell, 2017) and on MD simulations, we hypothesized a charged 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 wildtype mHCN2 (WT) and mutant channels 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]sat. 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 stabilize to function 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.

B 07-5
2',3'-cyclic-nucleotide 3'-phosphodiesterase: An unexpected role as HCN channel modulator
M. A. Komadoski1, S. Rinne1, M. Wailesci1, L. Nowack1, A. K. Kiper1, K. S. Vowinkel1, P. Hundebøe3, S. Meut1, N. Decher1
1Philips-University of Marburg, Institute of Physiology and Pathophysiology, Vegetative Physiology, Marburg, Germany
2University of Muenster, Institute of Neuropsychophatology, Muenster, Germany
3Institute of Neurosciences, Department of Neurology and Institute of Translational Neurology, University Hospital Muenster, Muenster, Germany

Question: Hyperpolarization-activated cyclic-nucleotide gated (HCN) channels play a major role in generating neuronal rhythm (“pacemaker”) activity. Upon membrane hyperpolarization HCN channels mediate a slowly depolarizing current, described as i.v in the brain. Trafficking or gating of HCN channels are modulated by different types of molecules like 3,5'-cAMP, PiP2 and the auxiliary subunit Trip8b. Using a split-ubiquitin yeast-two-hybrid screen with HCN2 and a human brain cDNA library, we have identified the oligodendrocyte/myelin membrane associated enzyme 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP), which has previously been linked to multiple sclerosis (MS), as a novel HCN channel modulator. Although the enzymatic activity of CNP – the catalysis of 2',3'-cyclic-AMP to 2'-AMP – has been shown in vitro, the physiological function of this enzyme in brain tissues remains not fully understood. Since HCN channels play an important role in neuronal pacemaker activity, an interaction partner modulating HCN channels could influence important (patho-)physiological processes. Therefore, the interaction between CNP and HCN channels might be of major relevance.

Methods and Results: Immunohistochemistry experiments with HEla cells showed a co-localization of CNP and HCN at the plasma membrane. Furthermore, PCR analysis with cDNA of oligodendrocyte progenitor cells and mature oligodendrocytes confirmed the expression of CNP and HCN, respectively also of HCN1, HCN2 and HCN4. Strikingly, we were also able to show an i. c. current in mature oligodendrocytes. The modulatory effect of CNP on HCN channels was analyzed by surface expression assays in Xenopus oocytes and HEla cells, which indicated a significant decrease of HCN2 surface expression. Consistent with this observation, two-electrode voltage-clamp recordings in oocytes revealed a selective reduction of HCN1, HCN2 and HCN4 current amplitudes by CNP, while current amplitudes of other ion channels where not affected. In addition, inside-out macropatch-clamp measurements in oocytes were utilized to probe the role of 2',3'-cyclic-AMP on the voltage-dependence of HCN2 channel activation.
Conclusion: Our data suggest that CNP modules 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. Havryl1, 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 calcitrol. 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 any eukaryote genome including human. In yeast, a single kinase (Kci1) 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]methyl]-9H-purine-2,6-diamine (TNP), decreased the basal Pi-uptake in OK cells in a dose-dependent manner. 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. Kortzak, P. Hidalgo1,2  
1Forschungszentrum Jülrich, Institute of Complex Systems (ICoS), Jülrich, 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 VACCs cell surface expression, however, the mechanisms underlying their intracellular transport are poorly understood. Conclusively, CaV1.2 channel consists of a large CaV0 pool-forming subunit and two accessory subunits the β- and γ-subunit. CaV0.2 is mandatory for targeting the channel complex to the plasma membrane. We here studied the post-endocytotic 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 CaV0.2 by generating a CaV0.2-concanavalin 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/adrin 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 mounted 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-BASIC 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 (I Na), which were prevented 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. This purinergic reaction and the bile acid induced currents, which were potentiated by ATP, were not abolished by previous application of the unspecific P2-inhibitor Suramin, but by the specific P2X-inhibitors 8-Bromo2'-deoxyadenosine and Parodoxine. However, the bile acid induced currents in the knockout cell line were not affected by previous application of different P2-inhibitors. Interestingly our immunocytochemical data suggest that normal rat cholangiocytes can be divided into subgroups by size and that BASIC is mainly expressed in smaller cholangiocytes, which are linked to P2X4-mediated signal pathways. According to these data we propose a functional cell model which includes a possible 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. Klapaerlschuck, S. Markwardt  
ML University Halle, JB Institute for Physiology, Halle, Germany  
Cells release ATP in many ways. One of them is via the cell volume-sensitive anion channel VRAC induced by activation of sphingosine-1-phosphate (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 P2X2, P2X7 and P2Y12 receptors.

B 08 | Novel Techniques and Molecular Analysis of Channels

B 08-1  
Effect of ProTocrin-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. ProTocrin-II (Pro-ToIII) is a 30-residue disulfide-rich peptide isolated from the Thianthema pruniensis tarantula 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 cooperativity in gating. 14-3-3 protein mediates this coupled gating and this protein is believed to be involved in dipoles. It is not known if dimerization of Nav1.7 ion channel affects its gating. We investigated the effect of ProTx-II in dipole transfected Nav1.7 cells as dipole inhibits dimerization of α-subunits. Whole-cell patch clamp technique was used to assess the pharmacological effect of ProTx-II on Nav1.7 ion channel. Continuous perfusion technique was applied to investigate the effect over time as ProTx-II binds slowly to the ion channel. A two pulse voltage protocol was applied to assess the effect of ProTx-II, first pulse at maximum activation and the 2nd at half voltage (Vhalf) of activation. We found that ProTx-II inhibits Nav.1.7 and reduces peak current with high potency. Dipoles treatment significantly reduced the effect of ProTx-II on Nav1.7 ion channel. This study further highlights the role of dimerization in modulating the sodium channel gating and shows that even sodium channel pharmacology is affected by their dimerization.

B 08-2
Studying coassembly of voltage-gated sodium channels by concatemer formation
P. Neter1, I. Coburger1, S. H. Heinemann1, F. Stumpff1
1Jena University Hospital, Jena, 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 range 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 crosstalk between them. This has far-reaching consequences for Na+ function as such and also for the interpretation of Na+-mediated human diseases. Using rat skeletal muscle sodium channel expressed in HEK293 cells we demonstrated that Na+ currents obtained from SR121585-transfected cells showed a significantly increased IC50 compared to WT currents. Continuous perfusion technique with SR121585 was applied to investigate the effect on Nav1.7 ion channels over time as SR121585 is a slow-binding drug. A two pulse voltage protocol was applied to assess the effect of SR121585, first pulse at maximum activation and the 2nd at half voltage (Vhalf) of activation. We found that SR121585 inhibits Nav1.7 and reduces peak current with high potency. SR121585 treatment significantly reduced the effect of SR121585 on Nav1.7 ion channel. This study further highlights the role of dimerization in modulating the sodium channel gating and shows that even sodium channel pharmacology is affected by their dimerization.

B 08-3
A “receptophore” model for local anesthetics binding site in cardiac ion channels
W. González1,2, G. Núñez-Vivanco1, D. Ramírez2, A. K. Kiper3, S. Rinné3, M. Bedoya1, L. Sánchez1, J. C. Márquez-Montesinos1, M. Reyes-Parada2, Y. Varoz-Yarovsky2, N. Decher2
1University of Talca, Center for Bioinformatics, Simulations and Modelling (CEBSM), Talca, Chile
2Universidad Autónoma, Instituto de Ciencias Biomédicas, Santiago, Chile
3Universidad de Chile, Facultad de Medicina, Santiago, Chile
Intoxication with local anesthetics may provoke arrhythmias by interaction with cardiac ion channels such as Nav1.5, K1.5 and TASK-1. While Na+, K1.5, K1.5 and TASK-1 channels have different sequences and structures, they share local similarities in the architecture of the binding site for local anesthetics. Using computational polypharmacology and multi-target/structure-based methods such as Geomfinder (http://cheminfo.biomedcentral.com/articles/10.1186/s13321-013-0131-9), MultiDock (http://mordred.chem.ox.ac.uk/MultiDock), and Multibind (http://bioinfo3d.cs.tau.ac.il/MultiBind) we searched for local anesthetics Binding Site Similarities (BSS) between Na1.5, K1.5 and TASK-1 channels, converting 3D coordinates of proteins into easy to compare patterns. We report a “receptophore model” revealing features of the cardiac ion channels that are essential to ensure optimal interactions with the local anesthetics. This knowledge of promiscuous drug action will help in the multi-target drug design of novel cardiac ion channels modulators that may simultaneously interact with Na1.5, K1.5 and TASK-1. This approach might yield innovative anti-arrhythmic compounds having a more promising risk–benefit ratio than currently available drugs in clinic.
Birds can shake too: wet chicken shakes depend on the cold and menthol receptor

Cyclic nucleotide-gated channels (CNG) constitute the last step in the signal transduction cascade of photoreceptors and olfactory sensory neurons, translating sensory stimuli into electrical signals. Ligand binding to these 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-fcGMP), 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-fcGMP). FRET efficiency was determined by measuring the decrease of donor fluorescence, allowing in this way the quantification of ligand binding. The efficiency of P1-fcGMP to activate CNGA1-eGFP channels was close to that of the physiological ligand cGMP. The potency of P1-fcGMP 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 compared the natural shaking of water-sprayed chickens with the effect of C1 administration. 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 (TRPM7) ion channel is the main molecular transducer of low temperatures in mammalian skin. From the multitude of TRPM7 agonists, only one is known to trigger WDS in rodents. As avian TRPM7 is cation-intensive, it was not possible to test the role of TRPM7 in shaking or feather ruffling in birds. The novel TRPM7 agonist 1-disopropylphosphorylethylamine (Cryoamin, C1) made this possible. We compared the natural shaking of water-sprayed chickens with the effect of C1 administration. Human, rat and chicken TRPM7 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. 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 TRPM7 orthologs tested were robustly activated by C1 (10 µM) and these calcium transients were abolished by the TRPM7 antagonist AMTB (1 µM). C1 activated inwardly-rectifying currents with a reversal potential close to 0 mV. In both rat and chicken culture, the currents also led to the TRPM7-specific inwardly-rectifying current. In rats, intraprettrial injections of C1 (33 mg/kg) evoked WDS and jumping/escape behavior with a shorter onset time compared to cGMP (1 mg/kg).

In chickens we first investigated 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 similarities between the shaking behaviors recorded in rats and chickens exposed to the novel TRPM7 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.

Lysosomes are cell organelles with important roles in energy metabolism, antigen processing and digestion of macromolecules via hydrolytic enzymes, and changes in lysosomal function are associated with severe human diseases. Lysosomes differ from other organelles within the endosomal pathway in their high chloride concentration ([Cl\(^-\)] = 27.9 µM P1-fcGMP, H\(_0\) = 2.1 vs. EC\(_{50}\) = 33.5 µM cGMP, H\(_0\) = 2.6). We analyzed in great detail potential sources for errors, such as FRET to labelled ligands bound unspecifically to the membrane or FRET between adjacent subunits. We also took into account effects such as absorption of the donor fluorescence by unbound acceptor molecules and effects of changes in the donor fluorescence introduced by gating and changes in the local environment of the eGFP tag. In case of CNGA1 channels, results corrected for these effects suggest that each membrane step has a considerable contribution to channel activation. In addition, we were able to characterize binding to modulatory subunits in heterotetrameric channels. By exploiting FRET our approach overcomes technical limitations of the conventional (confocal) patch-clamp fluorescence and allows monitoring ligand binding to individual subunits specifically.

To better understand the molecular interaction of ASIC1a with the basic neuropeptide Big Dynorphin, we set out to identify the binding site of the peptide on 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.
H1 voltage-gated proton channels are proton-specific ion channels with unique properties. For example, they are massively activated in human sperm where they are necessary for maturation and motility, hence essential for conception. Voltage-gated proton channels are strongly inhibited by Zn2+ and two histidine residues, H140 and H193, were found experimentally to be essential for Zn2+ binding. However, the two histidines are too far apart to coordinate simultaneously one Zn2+ in between. It was thus hypothesized that two Zn2+ binding sites can be formed between pairs of equivalent histidine residues (H140-Zn-H140 and H193-Zn-H193) at the interface of a H1 homodimer. The consequent experimental measurements were also 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 H1 homodimer 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.
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-lightening 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 laparoscopy and injected with 1 ng cRNA encoding for human claudin-5, or Phos-tag-free water as controls, respectively. After 3 days, oocytes were devitellinized and clustered in pairs of claudin-5-expressing and control oocytes as follows: cldn5-cldn5 (n=33), cldn5-ctrl (n=20), and ctrl-ctrl (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 × (measured width/2))

Results: After injection of cRNA, 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 cldn5-cldn5, cldn5-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 to claudin-5. The injection of claudin-5 cRNA 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:

B 09-2

Claudin 1 expression is induced by GATA3 in basal cells of human airway epithelia

R. Lochbaum, C. Schilpp1, P. Braubach, M. Frick1, P. Dietl1, O. H. Wittekind1
1Ulm University, Institute of General Pathology, 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 human airway epithelium 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 (hTEC). Transepithelial electrical resistance (TEER) was measured to determine the paracellular permeability. Semiquantitative 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 (Cldn1) promoter by GATA3. Overexpression and silencing of GATA3 were performed to further investigate its impact on controlling Gldn1 expression. Human lung slices were analyzed for GATA3 and Cldn 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 hTECs. GATA3 is localized both in differentiated and basal cells, with higher amount in the latter ones. Basal cells of hTECs 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 agrees with Cldn1 localization in hTEC epithelia. Evidently GATA3 enhances Cldn1 expression in human airway epithelia 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.

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B 09-3

Elevated apical surface liquid volume disturbs paracellular permeability in NCI-H441 epithelia

N. Schade, R. Lochbaum, C. Schilpp, P. Dietl, O. H. Wittekind
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-typic air-liquid 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 epithelial metabolic distress activates AMP activated protein kinase (AMPK) that induces 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 cells remained at ALI conditions. Epithelial permeability was quantified by measuring transepithelial electrical resistance (TEER) as well as the apparent permeability coefficient for 4kDa dextrans. Expression level of claudins and AMPK were measured by semi-quantitative RT-PCR.

Compared to ALI cultured 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 TJ. Hence we investigated changes in claudin expression levels of LLI versus ALI cultured epithelia. We observed an overall increase in claudin expression in cells exposed to LLI. We further detected elevated expression of AMPK, in LLI cultured 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.

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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 owing to 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 catastrophe. Although many of the cellular and humoral factors involved in the activation and control of the immune system in ARDS have been identified, 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 Lipopolysaccharide (LPS) in the presence of response-modulating cytokines, enzyme inhibitors or signalling molecule receptor blockers. Breakdown of the epithelial barrier integrity is monitored by impedance 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-lane 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 gap catastrophe as the functional backbone of immune response activation. Relevance and implications of this type of bistability 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. Leng, T. Fandrey, T. Schreiber
University of Duisburg-Essen, Institute of Physiology, Essen, Germany
Ischemia 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 recovery. 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 the role of HIF-2α during neural development is poorly understood.

To investigate the impact of HIF on neural regeneration, we established a murine neurosphere culture for wildtype (WT) and HIF-2α-knockout (KO) mice. 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. It 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 neurospheres 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, neurospheres migrated significantly less in general, but not different between the genotypes. Additionally, mRNA analyses showed a strong effect on the expression of genes involved in neurogenesis. Here, genes like Ngn1, which has a protective function against astrogliosis, NeuroD1, which is important for neuron differentiation, or Glut1, 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. Ernesti1, R. Depping2, M. Ranjarji1
1Department of Ophthalmology, University Hospital Schleswig-Holstein, Lübeck, Germany
2Institute of Physiology, University Lübeck, 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 immunotherapies are based on the fact that both PDR and wet AMD are associated with hypoxia as a stimulatory factor for endothelial and retinal neovascularization (ONV, 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 (s FLT-1), a high-affinity tyrosine kinase receptor for VEGF-A [6]. Release of sFLT-1 follows by binding of VEGF-A in a spatial proximity to the sprouting vessels leading EC (tip cell) forms a local VEGF-A gradient around the 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 hypoxic and normoxic 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 HRMEC, 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 hepatic sulfite protectoglycan-containing matrigel 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. Reichert1, J. Mayer1, C. Zehm2, T. Tokai1, F. Lange3, S. Balltrusch1, S. Ibrahim1, R. Köhling1, F. Lange1, M. Georg1, M. Kurt1, J. Ernesti1
1Rostock University Medical Center, 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 mtDNA defects are widely known to have a negative impact on lifespan and tissue integrity. The present study asks the question whether activity isolated and specific point mutations in the mitochondrial genome can have observable effects on global life parameters such as lifespan and cognitive function. Herein, we examined the effect of single nucleotide polymorphism (SNP) in the complex IV gene.

Methods: We characterized the cimoplastic mouse strain C57BL/6J-mtHDI carrying an electron transport chain complex IV mutation that leads to an altered cytochrome c oxidase subunit III. Using MitoXO2 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 fission-related gene Fat4, these data confirmed that the aging mtHDI 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 mtHDI 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 lifespan.

B 09-8
Chemogenetic control of acute H2O2 production in cells with D-amino acid oxidase (DAO)
N. Mueller1, K. Noack1, P.F. Malacarne1, F. Rezende1, K. Schröder1, R.P. Brandes2
1Goethe-University Frankfurt, Vascular Research Centre, Frankfurt, Germany
2University of 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 (DAO) was overexpressed by a lentiviral system. This enzyme converts D-amino acids into amino acids and produces H₂O₂ as a byproduct. Subsequently, H₂O₂ production was increased by providing D-Alanine (D-Ala). Treatment with D-Ala (1-100µM) led to an accumulation of H₂O₂ as measured by chemiluminescence with Luminol/HRP and Amplex red®. PE-Glylated catalase (250U/mL) reduced this signal by approx. 50% whereas the DAO inhibitor 4h-isox-[3,2-b]isoxazole-5- carboxylic acid (1µM/L) completely blocked H₂O₂ production. DAO-derived H₂O₂ (1mM/L, D-Ala, 10min) had intracellular signaling function as it increased oxidation of peroxide-3 and phosphorylation of p38 MAP kinase.

Conclusion: The DAO 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 selective transplantaion 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 Enterococcus 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 that had to be treated. With this setup, we wanted to untangle the influence of IM on CRC and the immune signaling pathways under tumour 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 azoxin (0.1% O2) or hypoxia (3% O2) for up to 8 hours to simulate tumour hypoxia and the colitogenic 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. Kritschke1, X. von Amein1, I. Korovina1, A. Neuwirth1, B. Sprott2, B. Wieloch2, T. Chavakis2, A. Drüssel3
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 nodule in PAS stained sections (Smith et al., 1994). In order to study a hematopoietic-specific deletion of HIF2α, Vav-cre (Stadtmauer et al., 2005) mice were crossed with Hif2fl/fl (Gruber et al., 2007) mice. Uteromites from Vav-Cre-Hif2αfl/fl and hematopoietic Hif2α deficient Vav-Cre-Hif2αfl/fl mice were used in the experiments. We found that 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. Together, these findings result from an up-regulation of the apoptosis inducer FasL in Hif2α-deficient mice (assessed by qPCR and Western blot). Consistently, pharmacological inhibition of FasL by an intravitreal injection of a FasL-blocking antibody (at P14 of the OIR) reversed the reduced pathological neovascularization due to hematopoietic-specific Hif2α deficiency. Taken together, our experiments identified that hematopoietic cell HIF2α contributes to pathological retina angiogenesis. Our findings not only provide insights regarding the complex interplay between immune cells and endothelial cells in hypoxia-driven retinal neovascularization but may also have implications for novel therapeutic approaches in proliferative retinopathies.


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

A. Jatto1, K. Brechtel1, A. Zieseniss, D. M. Kachtschinski2
1University Medicine Göttingen, Institute for Cardiovascular Physiology, Göttingen, Germany
2Universitätsmedizin Göttingen, Institute for Cardiovascular Physiology, Göttingen, Germany

Inhibition of the prolyl-4-hydroxylase domain (PHD) enzymes is thought to induce tissue protective effects for example in case of ischemia. Cells with a progenitor- or stem cell-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, ather, 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 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 expression analysis a significant difference comparing control and rox treated Sca1+ cells was found. Chondrogenesis, osteogenesis and adipogenesis could successfully be induced in the Sca1+ population supporting a mesenchymal origin. The isolated Sca1+ population reacts to acute and chronic hypoxia or rox treatment by induction of selected Hif1α target genes including Epo expression. A co-culture of bone marrow derived macrophages (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.

B 09-12
miR663 Prevents Epo inhibition caused by TNF-α in Normoxia and Hypoxia

M. Ozkurt1, T. Heilwig-Bürgel1, R. Depping2, S. Kabaderi1, R. Ozyurt1, A. Karadag1, N. Erkasap1
1Eskişehir Osmangazi University, Physiology 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 find out which miRNAs 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 microRNA array study with TNF-α (0 ng/ml) treated cells and array results were confirmed by qRT-PCR. We transfected 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 cells with Epo dependent UT-7 cells. If miR663 mimic prevents the Epo-repression by TNF-α more UT-7 cells would survive. Percentages 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 HEK293 cells raised apoptosis of Epo dependent UT-7 cells and transfecting HepG2 cells with miR663 mimics decreased apoptosis of UT-7 cells.

Further in-vivo experiments will perhaps blaze a trail for miR663 as a novel treatment option for anemia seen in chronic inflammatory diseases. This study is supported by The Scientific and Technological Research Council Of Turkey (TÜBİTAK, no: 2165729), from the Eskişehir Osmangazi University Scientific Research Projects Committee (no: 2017-1635), Turkey and from Erwin-Riesch-Stiftung, Germany.

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

T. C. Stamm1, A. Heine1, E. Lachmann, S. Göddeke, A. Göddeke
1Heinrich-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 isoform-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 isoform-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-hydroxy tamoxifen injections for 5 consecutive days, and cardiac mitochondria were isolated by differential centrifugation 15 min after in vivo insulin (3 U/kg., i.p.) or vehicle (−/−) treatment. ADP stimulated (state 3), resting (state 4), and uncoupled respiration (OCR) were measured using complex I substrate pyruvate/glutamate/malate. In addition, respiratory control ratios (RCR) were calculated as state 3/state 4.
Results: Depletion of AKT1 isoform reduced RCR by 19% compared to WT (4.39±0.46 vs 5.45±0.95; p=0.018); depletion of AKT2 by 15% (4.65±0.51; p=0.034). No difference in ADP-stimulated state 3 respiration (WT: 392±58 nmol/mg/min, iCM-Akt1: 378±53 nmol/mg/min, iCM-Akt2: 367±22 nmol/mg/min), and uncoupled respiration (WT: 362±51 nmol/mg/min vs. 378±53 nmol/mg/min; p=0.132). Western blot analysis demonstrated that insulin initiated AKT1 and AKT2 translocation to mitochondria.

Conclusion: AKT1 as well as AKT2 isoforms are required to maintain mitochondrial respiration. Furthermore, stimulation of the AKT pathway by insulin regulates mitochondrial respiration in an isoform-specific manner. These data suggest a regulatory link between insulin/AKT signalling and mitochondrial oxidative phosphorylation.

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B 10 | The endothelium in health and disease

B 10-1 Differential adaptation of endothelia to acute and chronic changes in pressure
J. Fels
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, very little is known about the molecular and cellular effects of hydrostatic pressure 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 hydrostatic pressure. While chronic pressure elevations lead to endothelial cortical stiffening caused by apical F-actin formation, short-term pressure exposure stiffens endothelia via an increase in myosin contractility. Pressure-dependent myosin activation could be prevented by application of blockers for mechanosensitive ion channels (GmαT-4, amiloride). Additional knockdown studies showed the epithelial sodium channel ENaC as endothelial pressure sensor. During the early response to pressur, endothelial barrier functions remained stable. In contrast, chronic pressure exposure induced a decrease in endothelial electrical resistance as well as increased leukocyte transmigration. 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 quantity of interest, however, can be determined directly 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 U/ml). 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. M. Warwicke1, S. Seuter1, R. P. Brandes1
1Goethe-Universität Frankfurt, Institut für Kardiovaskuläre Physiologie, Frankfurt am Main, Germany
2CPI, Cardio-Pulmonary Institute, Frankfurt am Main, Germany

Question: Is the regulation of gene expression by 1,25-dihydroxyvitamin D₃ via changes in 3D chromatin structure conserved across cell types?

Background: 1,25-dihydroxyvitamin D₃ (1,25D) mediates most of its signalling through the vitamin D receptor (VDR). VDR is a nuclear receptor which acts as hetero-dimer with the retinoic acid receptor (RXR) and binds the vitamin D response element (VDRE). VDRE binding in the promoter regions of many genes results in a context-dependent gene induction or inhibition. 1,25D changes the transcriptome of monocytes, in some cases acting on sets of genes organized into topologically associating domains (TADs). 1,25D also modulates the transcriptome 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 realize 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 (VDRE), showed no specific motif enrichment.

Conclusion: The present data suggest that, throughout many cell-types, 1,25D alters the expression of genes organized in 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. Bibili1, J. Hu¹, R. Randriamboavonjy1, B. Fishtalherr1, M. S. Leisegang2, A. Weigert1, A. Papapetropoulos1, R. P. Brandes1, F. Sigala1, J. Wittig1, I. Fleming1
1Goethe University, Institute for Vascular Signalling, Centre for Molecular Medicine, Frankfurt am Main, Germany
2Goethe University, Institute for Cardiovascular Physiology, Frankfurt am Main, Germany
3Goethe University, Institute of Biochemistry I, Faculty of Medicine, Frankfurt am Main, Germany
4Biomedical Research Foundation of the Academy of Athens, Clinical, Experimental Surgery and Translational Research Center, Athens, Greece
5National and Kapodistrian University of Athens Medical School, First Propedeutic Department of Surgery, Vascular Surgery Division, Athens, Greece
6Goethe University, Functional Proteomics, SFB 815 Core Unit, Frankfurt am Main, Germany

Background/Aim: The expression of the cystathionine γ lyase (CSE) in the endothelium is regulated by blood flow. CSE generates polysulfides (H₂Sₙ) which sulfhydrate endothelial proteins to preserve endothelial function and inhibit atherosogenesis. The aim of the present study was to investigate the consequences of the CSE on the endothelial cell sulfhydrom.

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 sulfhydrom of cultured human endothelial cells exposed to laminar flow versus static conditions or flow plus a polysulfide donor, more than 300 sulfhydrated proteins where identified. A significant number of peptides were intergrins, with β3 integrin the most highly sulfhydrated 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 integrin sulfhydration in the CSE-deficient endothelial cells reduced its surface expression and its ability to interact with Go13 and inhibit RhoA activity. This, in turn, resulted in a significant attenuation of shear stress-induced signaling and vasodilatation. The effects were reversed in the presence of a polysulfide donor, SG1002, or with the adventitio nal overexpression of CSE, which were able to induce β3 integrin sulfhydration and preserve mechanotransduction in endothelial cells lacking CSE. A similar mechanism was evident on human atherosclerotic samples, in which reduced CSE activity impaired β3 integrin sulfhydration and enhanced RhoA activity. The loss of sulfhydration also prevented endothelial cell alignment and elongation in response to flow and elevated systolic blood pressure. In humans with reduced CSE activity and impaired flow-induced vasodilatation, per os administration of a H2S supplement, elevated circulating H2S levels and normalized the flow-mediated dilatation, compared to the placebo. These results, were coupled with increased β3 integrin re- sulfhydration of the circulating blood cells of the treated patients.

Conclusions: CSE-derived H2S contributes to vascular homeostasis by sulfhydrating 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.

VasoRin suppresses osteo-/chondrogenic transdifferentiation of vascular smooth muscle cells via inhibition of TGFB1 signaling

T. T. Luong1, M. Estepa1, B. Boehme1, B. Pieske1, F. Lang1, K. - U. Eckardt1, J. Voelkl1, I. Alesutan4

1Chinante – Universitätsmedizin Berlin, 2Boehme, 3B. Pieske, 4F. Lang, 5 - U. Eckardt, 6J. Voelkl, 7I. Alesutan

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 epigenetic genetic control mechanisms of NOS3 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 decoy oligodeoxyribonucleotides (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 viscosimeter. NOS3 expression was analyzed by quantitative RT-PCR and Western blot.

Results: 1) Using a decoy ODN mimicking a putative STAT binding motif in the promoter of the human EC, reduced phosphate-induced mRNA expression and activity and, thus, of osteo-/chondrogenic transdifferentiation of HAoSMCs, were all inhibited by addition of vasoRin to the cell culture medium. Furthermore, phosphate treatment suppressed VASN mRNA expression in HAoSMCs. Additionally, with vasoRin did not modify phosphate-induced TGFB1 mRNA expression, but blunted the phosphate-induced TGFB1-dependent signaling, osteo-chondrogenic transdifferentiation and calcification of HAoSMCs. Conclusion: VasoRin expression is suppressed by TGFB1 or phosphate in VSMCs. VasoRin supplementation inhibits TGFB1-dependent osteo-chondrogenic transdifferentiation and calcification of VSMCs. Thus, vasoRin may represent a potential therapeutic 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. Pflieger-Müller, 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 (e.g. inflammation, cancer). One of the best-characterized endocannabinoids, incubation of murine (mAoSMC) and human aortic smooth muscle cells (HaAoSMC) 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 G12/G13 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 by its anti-inflammatory actions. The effects were even more pronounced in human NaR1 knockout cells, whereas both AEA and NaR4 knockdown completely abolished cell

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 epigenetic genetic control mechanisms of NOS3 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 decoy oligodeoxyribonucleotides (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 viscosimeter. NOS3 expression was analyzed by quantitative RT-PCR and Western blot.

Results: 1) Using a decoy ODN mimicking a putative STAT binding motif in the promoter of the human EC, reduced phosphate-induced mRNA expression and activity and, thus, of osteo-/chondrogenic transdifferentiation of HAoSMCs, were all inhibited by addition of vasoRin to the cell culture medium. Furthermore, phosphate treatment suppressed VASN mRNA expression in HAoSMCs. Additionally, with vasoRin did not modify phosphate-induced TGFB1 mRNA expression, but blunted the phosphate-induced TGFB1-dependent signaling, osteo-chondrogenic transdifferentiation and calcification of HAoSMCs. Conclusion: VasoRin expression is suppressed by TGFB1 or phosphate in VSMCs. VasoRin supplementation inhibits TGFB1-dependent osteo-chondrogenic transdifferentiation and calcification of VSMCs. Thus, vasoRin may represent a potential therapeutic target to reduce the progression of vascular calcification during hyperphosphatemic conditions such as chronic kidney disease.

B 10-8 Bicarbonate-activated soluble adenyl cyclase (ADCY10) controls cell cycle via phosphatase 2A in human umbilical vein endothelial cells

W. W. Waranush1, 2, M. Moskop1, 2, A. Das1, 2, Y. Ladiiov, 2, F. V. Härtel1, N. Noll1

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-) and can form CaM-P in nearly all cell compartments. ADCY10 is involved in a variety of pathophysiological processes including cell cycle control in tumor cells. However, 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 bicarbonate (24 mM HCO3-; Pco2 of 40 mmHg, pH 7.4) which caused a three-fold increase in cell proliferation compared to cells cultured at ambient Pco2 (≈ 0.3 mmHg, pH 7.4) after 72 h. Inhibition of ADCY10 by 10 μM I247, a specific pharmacological inhibitor of ADCY10 (optimum concentration tested in pilot experiments), completely abolished cell
proliferation. In addition, ambient Pco phase 2 function as documented by attenuated sprout length in the spheroid outgrowth assay. Additional protein subunits of -to-mitotic phase transition. Whereas, inhibition of ADCY10 (ambient Pco or 10 µM KH7) reduced the cellular cyclin B1 content, indicating that ADCY10 is involved in the G₂ phase transition. This indicates that only PKA is a downstream target of ADCY10- phase to mitosis.

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

B 10-9
NoxO1 – more than a subunit of Nox1?
T. Schader1,2,3, C. Reschke1,2,3, J. Graumann1,2,3, K. Schröder1,2,3
1Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Germany
2Max Planck Institute for Heart and Lung Research, Kerckhoff Institute, Bad Nauheim, Germany
3DEZNI, Deutsches Zentrum für Herz-Kreislauf-Forschung, Partner Site Rhine-Main, Germany
4CPI, Cardio-Pulmonary Institute, Frankfurt, Germany

NoxO1 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 NoxO1 forces the formation of tip cells and thereby enhances angiogenesis in mice. Recently we found that knockout of NoxO1 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, NoxO1 expression exceeds the expression of the other subunits of the Nox1 complex. Therefore, we hypothesize that NoxO1, via its two SH3 domains, not only binds to the Nox1 complex but may interact with other proteins as well. We show that NoxO1 interacts with CCNY (also BioID2) from A. se rolitus to the Chromatin of Nox1, generating NoxO1-BioID2. The NoxO1 fusion protein revealed 80% of the activity compared to wild type NoxO1 in terms of superoxide formation via Nox1, as well as similar intracellular localization at the plasma membrane. By proximity-dependent biotin labeling and mass spectrometry analysis we identified Erbin (ErkB2-interacting protein) as a major interaction partner of NoxO1. Western blot after biotin labeling and proximity ligation assays confirmed this result. Erbin binds and stabilizes up-hosphorylated ErkB2 which heterodimerizes with EGF-receptor, ErB3 and ErB4. Consequently, Erbin acts as a negative regulator of EGF signaling. Overexpression of NoxO1 reduced phosphorylation of Erbin and subsequently EGF-induced phosphorylation of ERK1-2 and AKT.

We conclude that Erbin is a newly identified interaction partner of NoxO1. Binding of NoxO1 to Erbin prevents phosphorylation of Erbin, which potentially increases the efficiency of the protein to bind up-phosphorylated ErkB2, which prevents the activity of e.g. EGF-receptor. Consequently, EGF-induced ERK and AKT activation is reduced upon NoxO1 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
J.A. De1,2, R. Kőlpi1,2,3,4, I. Wittig1,4, J. Heidler1,4, S. Günther1,4, M. Looso5, M. S. Leisegang1,2,3,4, R. P. Brandes1,2,3,4
1Goethe University, Institute for Cardiovascular Physiology, Frankfurt, Germany
2Max Planck Institute for Heart and Lung Research, Kerckhoff Institute, Bad Nauheim, Germany
3Max-Planck-Institute for Heart and Lung Research, ECPPS Bioinformatics and Sequencing Facility, Bad Nauheim, Germany
4German Center of Cardiovascular Research (DZHK), Frankfurt am Main, Germany
5CPI, Cardio-Pulmonary Institute, Frankfurt am Main, Germany

Background: Long non-coding RNAs modulate chromatin remodelling complexes and thereby gene expression. The mechanisms governing the recruitment of these complexes to gene-specific promoters are largely unknown. It was previously shown that the lncRNAs Xist, HOTAIR and Kcnq1ot1 are required for the function of the Polycomb Repressive Complex 2 (PRC2). We previously identified the IncRNA MANTIS as a crucial component of the endothelial SW1/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 hypothesised that a specific network of IncRNAs 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 spheroid outgrowth assay. Additional protein subunits of endothelial SW1/SNF were identified by mass spectrometry and one of them, Double PHD Fingers 2 (DPP2), was also found to up-hold endothelial function. RNA-IP-pulldown was employed to identify novel RNA interaction partners of different chromatin remodelling proteins such as EZH2, BRG1, BRM, SMARCA5 and BAF170. This technique recovered (among others) the IncRNAs EPHA1-A51, CAAC14-A51, MALAT1 and NEAT1, which have been implicated in endothelial function. Importantly, there was a higher degree of IncRNA-profile overlap between the SW1/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 IncRNAs alter endothelial functions like proliferation and migration.

Conclusions: HUVEC contain a significant number of functionally important IncRNAs which interact with chromatin remodelling complexes. ChIPseq will reveal whether this interaction results in altered targeting of the complexes.

B 11 | Cellular Physiology

B 11-1
Cyclin Y is expressed in platelets and modulates outside-in integrin signaling
A. Kyselova, J. Antínez, I. Fleming, V. Randriambavonjy
Goethe University, Institute for Vascular Signalling, Frankfurt, Germany

Cyclin Y (CCNY) is a member of the cyclin family that was originally identified as a protein that interacts with cyclin-dependent kinase 14, and is involved in cell cycle regulation. Interestingly, CCNY has been detected in neuronal cells suggesting that it may have proliferation-independent actions. The aim of the present study was to investigate the possible expression of CCNY in platelets and to characterize its putative function.

CCNY was found to be expressed in platelets. In order to assess the function of CCNY in platelets, mice allowing global and conditional knock-out of CCNY were generated using CrispR/Cas9 technology by introducing a premature Stop codon in exon 1 of CCNY. CCNY deletion resulted in a reduction in the circulating platelet number but the agonist-induced platelet aggregation was comparable in platelets from wild-type and CCNY-/- mice, as was agonist-induced α and dense granule secretion. However, CCNY-/- platelets demonstrated enhanced adhesion to fibronectin and collagen as well as an attenuated spreading and clot retraction, indicating an alteration in “outside-in” integrin signaling. The phenotype was accompanied by a significant reduction in the agonist-induced tyrosine phosphorylation of the J3 integrin in CCNY-/- platelets. Stimulation of platelets with thrombin led to ubiquitination and membrane translocation of CCNY where it probably plays a role as an adaptor protein for the binding of a tyrosine kinase to the cytoplasmic tail of the J3 integrin.

Thus, CCNY is present in anucleated platelets where it is involved in the regulation of integrin-mediated outside in signaling.

B 11-2
The role of the secreted modular calcium binding protein 1 in platelet function
F. A. Delgado Lagos1,2, A. Kyselova1,2, V. Randriambavonjy3, B. Fisslthaler1, M. Siragusa1, I. Fleming1
1Goethe-Universität, Institute for Vascular Signalling, Frankfurt am Main, Germany
2German Centre of Cardiovascular Research (DZHK), Frankfurt am Main, Germany
3CPI, Cardio-Pulmonary Institute, Frankfurt am Main, Germany

The secreted modular calcium binding protein 1 (SMOC1) is a matricellular protein essential for development and angiogenesis that interferes with TGF-β signaling. Although SMOC1 expression is known to be regulated by microRNA-223 (miR-223), which is enriched in platelets, nothing is known about the expression or potential function of SMOC1 in platelets. Here we report that SMOC1 is expressed in platelets from healthy humans and mice and that its expression is increased in diabetic platelets, probably as a consequence of a decrease in miR-223 levels. To elucidate the role of SMOC1, functional assays were performed in mice lacking wild-type SMOC1 or SMOC1-deficient platelets from diabetic individuals and miR223-deficient mice. In conclusion, SMOC1 regulates platelet function by increasing thrombin activity. It is tempting to speculate that the increased expression of SMOC1 underlies platelet hyper-responsiveness to thrombin in individuals with diabetes.
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 (LTCC: Cacna1c, Cacnb2, Cacna2d1). To analyze if mRNA levels were not altered, miR-221 mimic reduced Cacnb2 and Cacna1c mRNA levels. As Cacna1c is the pore-forming ion channel, a potential link between GSE1 and the differentiation and maturation of megakaryocytes was assessed using a PF4-Cre x GSE1 mRNA model. The megakaryocyte-specific knockout of GSE1 resulted in a significant increased number of WIF positive megakaryocytes in the spleen as well as the bone marrow, whereas the platelet numbers in the peripheral blood were decreased. Downstream signaling analysis using RNA-seq and proteomics revealed a modulation of several Gfi1b/LSD1 downstream targets, affecting for example cytoskeleton remodelling. Taken together these data indicate that GSE1 and its downstream products seems to be important in the physiological megakaryopoiesis.

B11-4
Insulin-like growth factor 1 and insulin have different effects on macrophage polarization influencing cardiac regeneration after myocardial infarction

L. Poppe-van Strainmand, P. Panjwani, A. Spychala, R. Niederlof, A. Gödecke
Hochschule-Universität Düsseldorf, Institut für Herz- und KreislaufPhysiologie, Düsseldorf, Germany

Question Insulin-like growth factor 1 (IGF-1) is an anabolic hormone which activates similar signal transduction processes as insulin. In previous studies we have shown that short-term IGF-1 treatment for 3 days after myocardial infarction (MI) improved cardiac function, reduced scar size and increased capillary density in mouse hearts. The protective effect of IGF-1 was lost in mice without a knock-out of the IGF-1 receptor in myocardial cells, showing that neutrophils and/or macrophages (MΦ) are responsible for this effect. IGF-1 promoted macrophage polarization towards an M2-like phenotype in vitro and in vivo. In order to analyze the phenotype of IGF-1 polarized macrophages and to evaluate if IGF-1 is specific, this study investigates the effect of IGF-1 and insulin on macrophage polarization.

Methods Bone marrow derived cells were isolated from mice, cultivated with macrophage colony-stimulating factor (mCSF) (10 ng/ml) to enrich macrophages (M0), 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 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.

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B11-5
miR-221/222 in cardiac electrical remodeling

M. Knörr, S. Binas, U. Klöckner, S. Rabe, S. Mildenberger, M. Gekle, B. Schreier, C. Grossmann
Martin Luther University of Halle-Wittenberg, Julius Berlin 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 of cardiac remodeling 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. Cacna2d1 3-UTR was not affected. This indicates that miR-221/222 directly target Cacna1c and Cacnb2. Additionally, when Cacna2d1 mRNA levels were not altered, miR-221 mimics reduced Cacnb2 and Cacna1c mRNA levels. As Cacna1c is the pore-forming LTCC subunit, we analyzed 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 mRNAs 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 KCl (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.
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B 11-7

Rhein, a novel Histone Deacetylase (HDAC) inhibitor with antifibrotic potency
D. Monteiro Barbosa,1, 2, 3, P. Fahlbusch,1, 2, D. Herzfeld de Wizza,1, 2, S. Jacob,1, 2, U. Ketteler,1, 2, M. Kröger,1, R. Al-Hasani,1, 2, D. M. Ouwens,2, S. Hartwig,1, 2, S. Lehr,1, 2, J. Kolzka,1, 2, B. Knebel,1, 2
1Heinrich-Heine University, Medical Faculty, Institute for Clinical Biochemistry and Pathobiochemistry, Düsseldorf, Germany
2German Center of Diabetes Research Partner, Düsseldorf, Germany
3Heinrich-Heine University, Medical Faculty, Institute for Cardiovascular Physiology, Düsseldorf, Germany
4Heinrich-Heine University, Medical Faculty, Department of Cardiovascular Physiology, Düsseldorf, Germany

Myocardial 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 mechanism, maladaptation due to excessive production of extracellular matrix accelerates cardiac dysfunction. Hence, anti-fibrotic treatment reducing fibroblast-to-myofibroblast transition and abrogation of pro-fibrotic intercellular communication is of utmost importance. The ruthbalm antirrhainone Rhein, a drug already established as treatment for osteoarthritis, has been reported to display beneficial properties in the setting of fibrotic pathologies of other organs. However, its relevance for the treatment of cardiac fibrosis and the exact mechanism have not been elucidated yet.

The aim of this study, was to prove whether Rhein administration could interfere with fibroblast-to-myofibroblast transition and dissemination of fibrotic signals in the pathophysiological setting of myocardial fibrosis. Compositional characterization of primary human ventricular cardiac fibroblast secretomes subjected to hypothesis revealed a modulatory shift of secretory products in association to differential regulation of profibrotic pathways. Rhein administration mitigated this hypothesis-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 G2/M cell cycle phase and in line with decreased proliferation. Furthermore, Rhein was shown to reduce cellular sensitivity to exogenous TGFβ1 and to intracellularly abrogate TGFβ/SMAD signaling. Mechanically, Rhein-mediated effects were linked to HDAC-dependent increase of p53 and SMAD7 abundance. Finally, Rhein was demonstrated to abolish collagen contraction in response to TGFβ1 associated to inhibited cardiac fibroblast-to-myofibroblast transition, thus functionally proving its anti-fibrotic property. In conclusion, this study identifies Rhein as a novel potent HDAC inhibitor and provides evidence that Rhein may contribute to the treatment of cardiac fibrosis as anti-fibrotic agent.

B 11-9

Vitamin D modulates the three-dimensional chromatin structure
S. Seuter,1, T. M. Warwick,1, M. Schulz,1, A. Neme,2, C. Carlborg,1, R. P. Brandes1
1Goethe University Frankfurt, Institute for Cardiovascular Physiology, Frankfurt, Germany
2University of Eastern Finland, School of Medicine, Institute of Biomedicine, Kuopio, Finland

Background: CTGF (CCCTC-binding factor) is a transcription factor that plays a major role in chrogenin organization by forming chromatin loops. The DNA loop can increase transcription by bringing enhancers and promoters into close vicinity, but it can also insulate different functional topologically associating domains (TADs) from each other. Often, CTGF 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)2D3) significantly altered the binding of CTGF to approximately 2,100 genomic regions across nuclear TADs. VDR-binding could be shown by ChIP-seq experiments, showing that CTGF/CHIA-PET (Chromatin Interaction Analysis Using Paired-End Tag Sequencing) data, around 500 1,25(OH)2D3-sensitive TADs have been identified and were further segregated into different functional classes by pathway analysis and machine learning methods. Using CRISPR/Cas9 gene editing to knock out CTGF in THP-1 cells with different guide RNAs we generated lines with a 87-95% reduction of protein expression. The cells did not show any significant signs of reduced viability or other morphological changes, even though CTGF has been described to be essential for cell growth and differentiation. In line with our previous knock-down results using DsiRNAs, the CTGF knock-out led to an increase (CAMP) as well as decrease (ALOX5) of vitamin D target gene expressions. HidChIP, a combination of H4-C and ChiP, has been used to study cohesin-tethered chromatin loops in THP-1 cells using an antibody against the cohesin subunit SMCP, CTFC co-localizes at many loop anchor sites, confirming that CTGF and cohesin work together in the generation of TADs. Interestingly, most SMCP loop anchor sites were located in regions of open chromatin (determined by FAIRE-seq (Formaldehyde Assisted Isolation of Regulatory Elements)) and in the lobe of a number of vitamin D target genes SMCP also co-localized with VDR. Conclusions: Vitamin D likely modulates the CTGF- and cohesin-dependent 3D-chromatin architecture, which may be a mechanism to fine-tune the expression of VDR target genes.

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 deacetylase 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 biology so far. We hypothesized 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 interaction. 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 proteosomal 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. This 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 11-10

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. Pompisch, J. Baumann, K. Goepeit, M. Baumann, E. Metzen
University of Duisburg Essen, 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-oxo-7,8-dihydrooxoguanine glycosylase (OGG1) can remove 8-oxoG preferentially opposite cytosine to initiate BER, thereby preventing DNA damage. Furthermore, treatment with the Mut-T-Homologue1 (MTH1) inhibitor TH588 was reported to induce apoptosis by accumulation of 8-oxoGTP in the nucleotide pool. Therefore, we hypothesized that inhibiting OGG1 should 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 MTT assays and reduced proliferation in long-term survival assays after α-MSH treatment. Stress providing factors like exposure to hypoxia, irradiation and tert-butylyhydroperoxide 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 results in another cell line, 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, 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 msfGFP.

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-speckles.

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