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Austrian Physiological Society and
Life Sciences Switzerland (LS²) Physiology

Abstract Booklet

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PLENARY LECTURES

PL 01

Determinants of pulmonary immune homeostasis

Sylvia Knapp

Medical University of Vienna, Research Division Infection Biology, Vienna, Austria

Content

Organisms depend on their ability to adapt and adjust to perturbations of homeostasis such as environmental changes. The body's immune system undergoes frequent adaptations, often triggered by external threats like pathogens – but also during different developmental stages and upon aging. We focus on the pulmonary immune system and as such study the temporal changes of immunity, from the early fetal – postnatal interface to advanced age. I will discuss the importance and mechanisms of postnatal immune development, factors determining the successful establishment of immune homeostasis, and provide examples and underlying pathways that determine the impact of aging on lung immunity.

PL 02

Large language models and artificial intelligence in medicine and healthcare

Günter Klambauer

Johannes Kepler University Linz, Institute for Machine Learning, Linz, Austria

Content

Artificial Intelligence has made monumental strides, fundamentally transforming almost all domains of science and technology, including medicine and healthcare. In this keynote, we will journey through the evolution of Machine Learning, from its early beginnings to the rise of Deep Learning, and demystify the inner workings of Large Language Models (LLMs). By exploring groundbreaking applications in diagnostics, treatment planning, and patient care, we will uncover how AI reshaping the future of medicine. Join this talk as we delve into the possibilities and implications of AI in healthcare, offering a glimpse into what the near future holds.

PL 03

Modeling neural network pathology in cerebral organoids

Jürgen Knoblich[1,2]

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[2] *Medical University of Vienna, Department of Neurology, Vienna, Austria*

Content

Cerebral organoids derived from pluripotent human stem cells can recapitulate morphogenesis and cell fate specification in the fetal human brain. Our group uses stem cell derived 3D cultures to explore the mechanistic basis for neurodevelopmental disorders. To identify gene regulatory network alterations and cell fate changes common to specific diseases, we have developed metholodologies for CRISPR/Cas9 based screening in organoids. This has allowed us to assign loss-of-function phenotypes to dozens of chromatin regulators implicated in Autism spectrum disorder and identify common deregulated gene networks. We have modeled Tuberous sclerosis (TSC), a neurodevelopmental disorder caused by overactivation of the mTOR pathway and have identified a progenitor cell type responsible for tumor formation and brain pathologies in TSC patients. Our data suggest that these progenitor cells located in the caudal ganglionic eminence might be responsible for the generation of inhibitory interneurons that migrate into the cerebral cortex after birth in humans but not in mice. By analyzing neural network activity using extracellular recordings, we have identified characteristic phenotypes also seen in TSC patients during the interictal phase. Together, our results offer new insights into human brain development and the mechanistic defects that underlie neurodevelopmental disorders including Epilepsy or Autism.

PL 04

From the role of reciprocal synapses in the olfactory bulb to the discovery of fast heartbeat interoception: How to 'feel the pulse' within the brain

Veronica Egger

Regensburg University, Zoology, Regensburg, Germany

Content

Activity within the vertebrate olfactory bulb is strongly patterned by respiration-related slow oscillations and sensory-related gamma oscillations. Recently, our investigations of the reciprocal synapse between the principal mitral cells and the inhibitory granule cells in acute brain slices have led to the hypothesis that this microcircuit subserves odor perception by selectively synchronizing active olfactory receptor channels in the gamma range. In order to test such concepts in an intact network we had developed a perfused nose-brain preparation of the rat that is devoid of respiratory and cardiac modulation. Nevertheless, we observed apparently spontaneous slow oscillations of the local field potential

in this preparation that entrained mitral cell spiking and were sensitive to hypoxia, yet insensitive to blockade of neuronal spiking. These oscillations turned out to originate from the baroreceptive transduction of arterial pressure pulsations induced by the peristaltic perfusion pump in our setup, which happen to fall within the physiological range of heartbeat-induced pulsations. Various lines of evidence imply Piezo2 channels in mitral cells as the molecular mechanism. Parallel multi-electrode and heartbeat recordings in awake mice confirmed that a subset of OB neurons synchronized their spiking to heartbeat within 20 ms. Similar entrainment was observed in the hippocampus and prefrontal cortex. We propose that a network of interoceptive ‘heartbeat sentinel neurons’ can modulate perception and cognition, e.g. within the context of arousal.

TEACHING LECTURE

TL 01

Circadian rhythms and sleep

Henrik Oster

University of Lübeck, Institute of Neurobiology, Lübeck, Germany

Content

Sleep is a highly conserved behavior across the whole animal kingdom. Although sleep behaviors differ substantially between species, they share specific common characteristics: (i) regular 24-hour (circadian) occurrence, (ii) behavioral quiescence, and (iii) decreased responsiveness to external stimuli. Moreover, sleep is rapidly reversible and homeostatically regulated, i.e., it shows rebound effects after sleep deprivation. Sleep and wakefulness are coordinated by circuits comprising the brainstem, the hypothalamus, and the basal forebrain. Although humans spend about 30 % of their life asleep and extended sleep loss is fatal, its physiological function remains poorly understood.

Timing of the sleep-wake cycle is controlled by endogenous circadian clocks. At the cellular level, these clocks are built from interlocked transcriptional-translational feedback loops of clock genes and proteins driving tissue-specific transcriptional programs that translate time-of-day information into physiological functions. The SCN is the central structure of the mammalian circadian system. It synchronizes circadian clocks in all tissues with each other and the external time via the autonomous nervous system, rhythmic behaviors (like sleep or food intake), and hormones (e.g., glucocorticoids or melatonin).

Remarkably, sleep and circadian rhythm disruption share very similar pathologic endpoints. Moreover, both processes are mutually influencing each other. This has raised the notion that alterations in circadian clock function– either centrally or at the level of peripheral tissues – may mediate the deleterious effects of sleep loss.

SYMPOSIA

S 01 | Non-canonical signaling and function of hemoglobin, nitric oxide and circulating blood-cells in cardiovascular pathophysiology

S 01-01

Nitrosation of CD36 regulates endothelial function and serum lipids

Brant Isakson

University of Virginia.edu, Charlottesville, USA

Content

During obesity, endothelial cells (ECs) become lipid laden leading to endothelial dysfunction. We demonstrate endothelium downregulates caveolin-1 (Cav1) in mouse and human in response to obesity. Using an EC-specific Cav1 knockout mouse, we find mice are hyperlipidemic regardless of diet, but retain endothelial cell function. Whereas initially this was thought to be due to Cav1 mediate endocytosis, we find instead the mice have significantly increased nitric oxide (NO) in response to the lack of Cav1. The presence or absence of NO toggled inversely EC lipid content and plasma lipid in mice. We found the fatty acid translocase CD36 was directly nitrosated by endogenous NO at the same cysteines that are palmitoylated on CD36. The nitrosation of CD36 prevented it's trafficking to the plasma membrane and decreased lipid uptake. The physiological effect of this mechanism was a reliance on NO for endothelial function. This work suggests that CD36 nitrosation occurs as a protective mechanism to prevent EC lipotoxicity.

S 01-02

Red blood cells and their role in cardioprotection

Petra Kleinbongard

Institut für Pathophysiologie, Essen, Germany

Content

Red blood cells (RBCs) are known to participate in the regulation of the cardiovascular system. These mechanisms include the synthesis and export of nitric oxide (NO). RBC nitric oxide synthase (RBC NOS) is involved in RBC deformability through s-nitrosylation of RBC cytoskeletal proteins, and RBCs contribute to the regulation of circulating NO metabolites and blood pressure. During myocardial ischemia/reperfusion, RBC NOS bioactivity is cardioprotective. This cardioprotective effect appears to be controlled by the enzyme arginase, which is known to reciprocally regulate the availability of NOS substrate L-arginine. RBCs from diabetic mice and humans are characterized by increased

arginase activity, decreased NO production, and increased reactive oxygen species production due to NOS uncoupling. Transfer of such diabetic RBCs to an isolated heart preparation subjected to ischemia/reperfusion increases infarct size. Remote ischemia conditioning (RIC), a cardioprotective intervention in which short ischemia/reperfusion cycles are applied to tissues or organs remote from the heart, elicits a cardioprotective signal and reduces infarct size in all species tested to date, including humans. The cardioprotective effect of RIC can be transferred to isolated recipient heart preparations exposed to ischemia/reperfusion with plasma and its derivatives or to circulating platelets from human volunteers with RIC, thereby reducing infarct size. There is preliminary evidence that RBCs also contribute to the cardioprotective humoral transfer of RIC.

S 01-03

The ambivalent role of circulating immune cells during myocardial ischemia and reperfusion in myocardial injury or cardioprotection

Florian Kahles

University Hospital Aachen, Department of Cardiology, Aachen, Germany

Content

Myocardial infarction (MI) is a leading cause of mortality worldwide. While the mortality rate from MI has been declining, the overall number of deaths continues to rise, and survivors face significant challenges, including the risk of heart failure. The ischemic damage inflicted on the heart requires a delicate balance between debris removal and the formation of a functional scar to ensure effective cardiac repair. Current therapeutics have improved outcomes, yet the high mortality and morbidity associated with post-MI heart failure underscore the need for a deeper understanding of the underlying pathophysiology. A promising area of research highlights the critical role of circulating immune cells in orchestrating the inflammatory and reparative processes after MI. Ischemic injury initiates a biphasic accumulation of inflammatory and reparative monocytes/macrophages in the myocardium. This accumulation is influenced by various mechanisms, including enhanced hematopoiesis in the bone marrow and spleen, recruitment from the bloodstream, local proliferation, cell death, and cellular egress. Monocytes/macrophages and also other leukocytes are key players in cytokine production, proteolysis, efferocytosis, oxidative stress management, and angiogenesis, all of which are crucial for balancing the inflammatory and reparative phases. However, this balance can be disrupted by underlying conditions such as atherosclerosis, leading to impaired infarct healing and subsequent heart failure. This talk will delve into the complex role of circulating immune cells in MI, emphasizing their dual function in both exacerbating injury and facilitating healing, and will explore potential therapeutic strategies to optimize infarct healing and prevent heart failure.

S 01-04

Cardioprotective effects of platelets in the event of cardiovascular disease

Gemma Vilahur

Barcelona, Spain

Content

Traditionally, platelets have been recognized for their essential role in hemostasis and thrombosis, making them key contributors to the development of ischemic heart disease. Yet, platelets play a complex and dual role in cardiovascular disease. Upon activation, platelets release a variety of bioactive molecules, including adhesion molecules, growth factors, inflammatory mediators, and proteolytic enzymes, which can promote inflammation, microthrombi formation, (micro)vascular dysfunction, and enhance atherosclerotic plaque progression. However, growing evidence has demonstrated that platelets also exert cardioprotective functions during coronary ischemic events. As such, platelets secrete molecules that promote myocardial healing, reduce ischemia-reperfusion injury, modulate immune responses, and enhance angiogenesis. Moreover, platelets act as carriers of ischemic-conditioning signals that can protect the heart. This dual functionality underscores the complex nature of platelet activity in ischemic heart disease. Although antiplatelet therapies, such as aspirin and $P2Y_{12}$ receptor antagonists, are central to managing ischemic heart disease by reducing thrombotic complications and exerting direct cardioprotection, they may inadvertently disrupt these beneficial functions of platelets, potentially limiting their positive roles in myocardial protection and repair. Recognizing the dual nature of platelet activity in cardiovascular disease is essential for developing targeted therapies that reduce platelet-induced damage while maximizing their beneficial properties.

S 02 | A multitude of interactions: Unraveling Ion Channel Complexes

S 02-01

Ion channel complexes in the context of pain

Manuela Schmidt

University of Vienna, Department of Pharmaceutical Sciences, Vienna, Austria

Content

Question

I will discuss how recent advances in comprehensive proteome profiling can be exploited to decipher cellular protein networks, including those of ion channels, which are relevant for pain-related disorders.

Methods

[...]I will initially introduce key principles and cellular components implicated in pain processing. Thereafter I will provide a brief methodological overview of mass spectrometry-based pain proteomics and ion channel interactomics established in my laboratory.

Results

[...]I will highlight examples showing the successful integration of proteome/interactome and phenotypic data to construct pain-related signaling networks, which helped reveal disease signatures of pain. Overall, my presentation aims at showcasing how protein-based systems biology contributes to (i) uncovering mechanisms underlying pain, and (ii) determining disease signatures associated with pain phenotypes across species.

Conclusions

[...]Ultimately, this knowledge can be integrated in a systems biology framework to identify novel pain targets and develop effective treatments for pain-related conditions.

S 02-02

The sodium leak channel and friends:identifying protein partners of the NALCN complex.

Samuel Usher[1], Estelle Toulmé[5], Roberta Florea[2], Stanislau Yatskevich[3], Christine Jao[3], Janne Colding[1], Prajakta Joshi[4], Inna Zilberleyb[4], Thorsten Trimbuch[5], Bettina Brokowski[5], Alexander Hauser[1], Alexander Leitner[2], Christian Rosenmund[5], Marc Kschonsak[3], Stephan Pless[1]

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[5] Charité—Universitätsmedizin Berlin, Institute of Neurophysiology, Berlin, Germany

Content

The sodium leak channel NALCN is vital for the regulation of electrical activity in neurons and other excitable cells, and mutations in the channel or its auxiliary proteins lead to severe neurodevelopmental disorders. We developed an integrative computational approach to identify protein-protein interactions of this channel, and have shown that the neuronal SNARE complex proteins syntaxin and SNAP25, which enable synaptic transmission in the nervous system, inhibit the activity of the NALCN channel complex in both heterologous systems and primary neurons. The existence of this interaction suggests that the neurotransmitter release machinery can regulate electrical signalling directly, and therefore modulate the threshold for its own activity. We further find that reduction of NALCN currents is sufficient to promote cell survival in syntaxin-depleted cells. This suggests that disinhibited NALCN may be the culprit behind the puzzling phenomenon of rapid neuronal cell death in the absence of syntaxin. This interaction may offer opportunities for future drug development against genetic diseases linked to both NALCN- and SNARE protein-containing complexes.

S 02-03

AMPA receptor complexes - not a kind of its own

Uwe Schulte, Jochen Schwenk, Sami Boudkkazi, Aline Brechet, Akos Kulik, Gerd Zolles, Wolfgang Bildl, Alexander Haupt, **Bernd Fakler**

University of Freiburg, Institute for Physiology, Freiburg, Germany

Content

AMPA-type glutamate receptors (AMPARs) are central elements of excitatory neurotransmission in the mammalian brain. These ligand-gated ion channels conduct the depolarizing currents required for fast and precise synaptic transmission and dynamically adjust their number to endow synapses with the context-dependent plasticity required for memory formation. Comprehensive functional proteomics (i.e. multi-epitope affinity purification mass spectrometry) uncovered their *interactome*, an ensemble of roughly 40 protein building blocks of the native receptor channels, that not only revealed the large molecular diversity of AMPARs but also provided access to study the complex molecular physiology of these receptors.

I will present our latest advances in elucidating the specific roles of the identified AMPAR constituents for their functional/biophysical properties, their biogenesis and trafficking, as well as their positioning and synaptic stabilization in the mammalian brain. Specifically, I will demonstrate the role of identified secreted proteins and their associated networks and discuss their significance for memory formation.

S 02-04

A new auxiliary subunit associates with the extracellular domain of ASIC1a to control its surface expression.

Dominik Wiemuth[1], Sven Kuspiel[1], Maria Schilling[1], Felicitas Weiß[1], Alison Wiesehahn[1,2], Günther Schmalzing[2], Stefan Gründer[1]

[1] *RWTH Aachen University, Institute of Physiology, Aachen, Germany*
[2] *RWTH Aachen University, Institute of Clinical Pharmacology, Aachen, Germany*

Content

Ion channels are often regulated by complex networks of interaction partners. To unravel the interactome of acid-sensing ion channel 1a (ASIC1a) we performed affinity purification mass spectrometry and identified secreted protein 1 (SEP1), a protein of unknown function, as a new interaction partner of ASIC1a. SEP1 strongly increased ASIC1a current density when co-expressed in HEK293 cells. While SEP1 did not alter the biophysical properties of ASIC1, it strongly increased its surface expression. Moreover, SEP1 enhanced maturation of trimeric ASIC1 complexes but did not affect endocytosis or recycling of ASIC1a. In cortical neurons from SEP1$^{-/-}$ mice, ASIC currents were completely absent and ASIC1-dependent hippocampal long-term potentiation was strongly decreased in slices from SEP1$^{-/-}$ mice, highlighting the crucial role of SEP1 for the physiological function of ASIC1. SEP1 is secreted, and when applied from

the extracellular side, still enhanced ASIC1 current amplitudes. In addition, our data indicate that SEP1 is endocytosed and trafficked retrogradely to the ER in a rab6-mediated fashion, suggesting that it can increase ASIC1 forward trafficking, even when secreted from adjacent cells. Single-cell transcriptomic and proteomic data from mouse brain suggest that SEP1 is expressed in many neurons but also in astrocytes, in contrast to ASIC1a. Interestingly, SEP1 secreted from cultured astrocytes also rescued ASIC1 currents in neurons from SEP1$^{-/-}$ mice. Taken together, our results establish SEP1 as a novel auxiliary subunit of ASIC1a that acts as a chaperone. Moreover, we describe a novel mechanism for the regulation of ion channel surface expression by a secreted protein.

S 03 | Inflammatory processes contributing to pathological pain perception

S 03-01

The role of Interleukin 4 for shaping human nociceptor morphology and function

David Zimmermann, Clemens L. Schöpf, Georg Kern, Theodora Kalpachidou, Maximilian Zeidler, **Michaela Kress**

Medical University Innsbruck, Institute of Physiology, Innsbruck, Austria

Content

Question

Interleukin-4 (IL-4) is an anti-inflammatory type 2 cytokine generated and secreted by a variety of immune cells including macrophages or T-cells. It regulates immune cells but also has important roles in the nervous system where it promotes neuronal outgrowth and repair in rodents.

Methods

To study the importance of IL-4 in human models, we assessed expression of IL-4R and IL-13RA receptor subunits in nociceptors (iNocs) derived from human induced pluripotent stem cell (iPSC) and assessed IL-4 effects on morphological, transcriptional and electrophysiological properties as well as transcriptomic signatures of iNocs.

Results

iNocs expressed both components of the IL-4 receptor complex (IL-4ra and IL-13ra1) and signaling machinery (Jak1,2, STAT5, PKC isoforms, translation factor EiF4E) during differentiation. IL-4 induced de novo formation of boutons immunoreactive for vesicular glutamate transporter(vGLut1) after 24 h of treatment which was largely reduced upon pharmacological inhibition of translational and cellular signaling components. RNA-seq unveiled distinct transcriptomic changes characterized by 932 significantly up- and 1577 downregulated genes in Il-4 treated iNocs. GO-analysis revealed biological process ontologies for "synapse", "neuron projection development", "axonogenesis","regulation of membrane potential" and "calcium ion transmembrane transport".

Conclusions

The IL-4 induced massive changes in transcript levels of proteins for structuring of glutamatergic synapses partially reflect transcriptional changes from mouse nerve injury models suggesting that IL-4 is critically involved in

(re)connecting primary afferent neurons to their projections in the spinal dorsal horn indicative of a more general role of IL-4 in the control of developing neuronal networks.

S 03-02

The role of silent nociceptors in inflammatory pain

Stefan G. Lechner

University Medical Center Hamburg-Eppendorf, Department for Anesthesiology, Hamburg, Germany

Content

Question

Silent nociceptors are sensory afferents that are insensitive to noxious mechanical stimuli under normal conditions but become sensitized to such stimuli during inflammation. The exact role of silent nociceptors in the development and maintenance of inflammatory pain as well as the molecular mechanism underlying their 'unsilencing' are, however, still unknown.

Methods

Here, we combined behavioral assays with electrophysiological techniques, RNAseq, AAV-mediated gene transfer and mouse genetics to examine the contribution of silent afferents to the induction and maintenance of pain hypersensitivtiy in a mouse model of inflammatory knee joint pain.

Results

Using RNA-sequencing and quantitative RT-PCR we demonstrate that knee joint inflammation upregulates the expression of the transmembrane protein TMEM100 in silent nociceptors and electrophysiology revealed that over-expression of TMEM100 is required and sufficient to unsilence silent nociceptors in mice. Moreover, we show that mice lacking TMEM100 do not develop secondary mechanical hypersensitivity—i.e., pain hypersensitivity that spreads beyond the site of inflammation—during knee joint inflammation and that AAV-mediated overexpression of TMEM100 in articular afferents in the absence of inflammation is sufficient to induce mechanical hypersensitivity in remote skin regions without causing knee joint pain.

Conclusions

Taken together, our work identifies TMEM100 as a key regulator of silent nociceptor un-silencing and reveals a physiological role for this hitherto enigmatic afferent subclass in triggering spatially remote secondary mechanical hypersensitivity during inflammation.

S 03-03

A human skin lipopolysaccharide inflammation model

Michael J.M. Fischer

Medical University of Vienna, Center for Physiology and Pharmacology, Vienna, Austria

Content

Background: Inflammatory pain causes an increased response to noxious stimuli or pain due to normally innocuous stimuli. To better understand the mechanisms underlying hyperalgesia, it is essential to develop practical and relevant models of inflammation. In contrast to the large number of animal models, only a few studies have looked at human models that reflect bacterial inflammation.

Methods: To induce inflammation in human skin, lipopolysaccharide (LPS) was injected intradermally into the volar forearms of healthy volunteers. To study the time course of inflammation, LPS was injected at intervals of 50-1.5 hours before blood flow and sensitivity to noxious stimuli were assessed. Sensitivity to mechanical, thermal and chemical stimuli was tested at inflamed or non-inflamed control sites. Volunteers rated the perceived pain during the injection using a numerical rating scale.

Results: Injection of LPS resulted in localised inflammation, as indicated by increased skin blood flow and increased pain sensitivity to mechanical and acidic stimuli. Hyperaemia peaked at 4.5 hours, pain hypersensitivity at 4.5 - 6 hours. Both has largely resolved 50 hours after LPS injection.

Conclusions: This model of skin inflammation reliably induces hyperalgesia in human skin. The model can therefore be used to test substances that interfere with the development of inflammation and inflammatory pain.

S 04 | Regulation and dysregulation of mineral balance in health and disease

S 04-01

Calciprotein particles: sand in the physiological gears?

Jan-Luuk Hillebrands

University Medical Center Groningen, Pathology & Medical Biology, Groningen, Netherlands

Content

Cardiovascular diseases, including vascular calcifications (VC), play a significant role in the mortality and morbidity of patients with chronic kidney disease (CKD). CKD is characterized by declining renal function, which correlates with elevated phosphate levels. Phosphate has the ability to bind calcium and interact with serum proteins, initiating the formation of calciprotein monomers. Over time, these monomers can mature into primary calciprotein particles (CPP1) and crystalline secondary calciprotein particles (CPP2). Accelerated CPP2 maturation is associated with VC development and adverse outcomes in CKD. While vascular smooth muscle cells (VSMCs) are well-studied in calcification within the vascular media, research on endothelial cells (ECs) during CPP-induced medial VC remains limited. *In vivo*, ECs—rather than VSMCs—are the first cells to interact with circulating CPP2. Furthermore, EC dysfunction is a critical hallmark of cardiovascular disease (CVD) and an initiating factor in its pathogenesis. In this presentation, recent preclinical and translational data regarding the potential relationship between CPP2 and ECs will be discussed. Focus will be on NO metabolism, oxidative stress, EC activation, and EC-VSMC paracrine signaling. Notably, our findings underscore the importance of endothelial cells, which are often overlooked, in promoting CPP2-induced medial calcification in human CKD.

S 04-02

Dysregulation of FGF23, klotho and phosphate - sticks and stones breaking the heart?

Reinhold G. Erben

Ludwig Boltzmann Institute of Osteology, Vienna, Austria

Content

Fibroblast growth factor-23 (FGF23) is a bone-derived hormone, mainly produced by osteoblasts and osteocytes in response to increased extracellular phosphate and circulating vitamin D hormone. Endocrine FGF23 signaling requires co-expression of the ubiquitously expressed fibroblast growth factor receptor 1c (FGFR1c) and the co-receptor *α-Klotho* (Klotho). In tissues not expressing Klotho such as the heart, Klotho-independent FGF23 signaling has been reported at high concentrations of FGF23. Bony hypersecretion of FGF23 is a hallmark not only of renal phosphate-wasting diseases but also of chronic kidney disease (CKD). Although the mechanisms driving FGF23 secretion are controversial, there is solid evidence that FGF23 blood levels are an independent predictor of disease progression,

untoward cardiovascular outcomes, and overall mortality in CKD. An important question in this context is why? Possible explanatory scenarios involve i) elevated FGF23 concentrations may directly target the heart via a FGFR4-mediated Klotho-independent signaling cascade, ii) lower circulating concentrations of kidney-derived soluble Klotho may provide less cardio-protection, and iii) hyperphosphatemia alone or in combination with other factors may induce maladaptive cardiac changes. During recent years, it has become clear from different animal models that elevated FGF23 per se does not induce cardiac hypertrophy in animals with intact kidney function. The latter findings suggest that increased FGF23 signaling induces maladaptive changes in the heart only in the presence of additional disease-promoting factors such as hyperphosphatemia or other factors driving left ventricular hypertrophy. Emerging data suggest that the association between excessive FGF23 and untoward outcomes in patients with left ventricular hypertrophy may be explained by FGF23-mediated changes in cardiac energy metabolism.

S 04-03

FGF23: a pro-inflammatory wolf in a phosphaturic sheep skin

Daniela Egli

University of Zurich, Zurich, Switzerland

Content

Fibroblast growth factor 23 (FGF23) is a bone derived phosphaturic hormone which is critical to maintain phosphate homeostasis in health and disease. In acute kidney injury (AKI), FGF23 and phosphate levels rise very rapidly and partially independent of phosphate. Elevated FGF23 levels were associated with inflammation and morbidity and mortality in renal and non-renal inflammatory diseases and in the general population, however, the underlying mechanism of this association and its relation to phosphate is unknown. In the folic acid (FA) induced AKI mouse model we investigated the impact of normal and low dietary phosphate on the hormonal regulation of phosphate and inflammation. The LP diet lowered the rise in plasma FGF23 and prevented that of parathyroid hormone (PTH) and calcitriol as well as osseous but not splenic or thymic *Fgf23* mRNA expression. Absence of *Pth* prevented the rise in calcitriol and reduced the elevation of FGF23 in FA-AKI with the NP diet. The LP diet lowered renal inflammation by attenuating the rise in renal and plasma IL-6, alleviating the initial decline in renal α-Klotho, and reducing the formation of calciprotein particles. After 14 days of FA-AKI, the LP-FA group maintained less elevated plasma FGF23 levels and significantly improved survival by preventing the development of metabolic acidosis, hypocalcemia, hyperkalemia, and cardiac electrical disturbances. In conclusion dietary phosphate in combination with renal injury serves as a very strong trigger for plasma and osseus FGF23 expression as well as inflammation while extra-osseus FGF23 expression is not affected by dietary phosphate.

S 04-04

Vascular calcification: how tubes turn to pipes and does it matter at all?

Ioana Alesutan

Johannes Kepler University Linz, Inst. for Physiology and Pathophysiology, Linz, Austria

Content

Vascular calcifications occur as ectopic depositions of calcium and phosphate in the medial or intimal layer of the arteries. Medial vascular calcifications are associated with increased mortality and are observed during aging, diabetes mellitus and chronic kidney disease. The process of arterial calcification is complex and involves weakening of anti-calcific and propagation of pro-calcific mechanisms. Deranged phosphate homeostasis and insufficient mineral buffering with increased formation of calciprotein particles is considered a critical factor to accelerate ectopic calcification. A hallmark in the development of calcification is a phenotypical alteration of vascular smooth muscle cells, which alter the extracellular environment to favour calcium-phosphate depositions. The signalling pathways underlying this remodelling are incompletely understood, but involve inflammatory pathways, a putative contrast to physiological bone mineralization. Although our understanding of the links between mineral dysregulation, vascular inflammation, calcification and mortality is incomplete, deciphering these mechanisms may develop towards new therapeutic opportunities.

S 05 | Muscle Matters: Innovative Perspectives on Skeletal Muscle Health and Disease

S 05-01

Novel insights in ageing sarcopenia

Marco Sandri

University of Padova, Biomedical sciences, padova, Italy

Content

The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. The systems that are often activated both in short and prolonged stress conditions is autophagy lysosome and ubiquitin proteasome systems. Autophagy is required to clear the cell from dysfunctional organelles and altered proteins and is reported to be involved in muscle wasting during cancer growth and age-related sarcopenia. The regulation of protein breakdown as well as protein synthesis is under the control of transcription factors belonging to different signaling pathways. Here the last findings about novel genes and signaling pathways that control proteostasis in ageing sarcopenia will be presented.

S 05-02

Muscle disuse and unloading. Single fiber proteomics and novel spatial approaches

Marta Murgia[1,2]

[1] *University of Padova, Biomedical Sciences, Padova, Italy*
[2] *Max-Planck-Institute of Biochemistry, Proteomics and Signal Transduction, Martinsried, Germany*

Content

Question

[Skeletal muscle plasticity is a highly complex phenomenon, as this tissue is composed of multinucleated cellular units, muscle fibers, with different molecular and physiological properties. Do fast and low fibers undergo different changes when muscles atrophy as an effect of disuse and unloading?..]

Methods

[.We use mass spectrometry-based proteomics to measure dynamic changes occurring in skeletal muscle during both short and long-term plasticity in humans. Our workflows range from the analysis of lysates of muscle biopsies, to single muscle fibers and to a recently developed spatial approach, Deep Visual Proteomics (DVP).

Results

[I will show how we have applied these different proteomic approaches to analyze muscle biopsies of volunteers unergoing 10 days of bed rest. I will compare the effects of bed rest to those of spacefligh, showing the effects of the absence of gravity on the skeletal muscle of astronauts in a six months mission on the International Space Station ..]

Conclusions

[Our data show fiber type specific remodeling of the skeletal muscle proteome during disuse. I will discuss the perspectives and advantages of spatial proteomics for the analysis of skeletal muscle plasticity in a fiber type-resolved manner.

S 05-03

The mechanistic underpinnings of muscle plasticity in acute exercise and chronic training

Christoph Handschin

University of Basel, Biozentrum, Basel, Switzerland

Content

Skeletal muscle has an enormous capacity to adapt to different types of stimuli, most importantly changes in the relative levels of physical activity. Surprisingly, despite the undisputed health benefits of exercise, our understanding of the molecular mechanisms that control muscle plasticity in exercise training remains poor. For example, it is unclear how

the short-term perturbations evoked by an individual exercise bout ultimately lead to long-term chronic adaptations, how the molecular patterns of an untrained and a trained muscle at rest differ, and even how specification, e.g. in endurance compared to resistance training is brought about. In my presentation, I will summarize the current knowledge and present recent data providing more insights onto the fundamental process of muscle adaptation to contractile activity.

S 05-04

Super-relaxed myosins contribute to respiratory muscle hibernation in mechanically ventilated patients

Coen Ottenheijm

Amsterdam UMC, Physiology, Amsterdam, Netherlands

Content

Question

Patients receiving mechanical ventilation in the intensive care unit (ICU) frequently develop contractile weakness of the diaphragm. Consequently, they may experience difficulty weaning from mechanical ventilation, which increases mortality and poses a high economic burden. Because of a lack of knowledge regarding the molecular changes in the diaphragm, no treatment is currently available to improve diaphragm contractility.

Methods

We compared diaphragm biopsies from ventilated ICU patients (N = 54) to those of non-ICU patients undergoing thoracic surgery (N = 27). We integrated data from myofiber force measurements, x-ray diffraction experiments, and biochemical assays.

Results

We found that in myofibers isolated from the diaphragm of ventilated ICU patients, myosin is trapped in an energy-sparing, super-relaxed state, which impairs the binding of myosin to actin during diaphragm contraction. Studies on quadriceps biopsies of ICU patients and on the diaphragm of previously healthy mechanically ventilated rats suggested that the super-relaxed myosins are specific to the diaphragm and not a result of critical illness. Exposing slow- and fast-twitch myofibers isolated from the diaphragm biopsies to small-molecule compounds activating troponin restored contractile force in vitro.

Conclusions

These findings support the continued development of drugs that target sarcomere proteins to increase the calcium sensitivity of myofibers for the treatment of ICU-acquired diaphragm weakness.

S 06 | New Challenges in Physiological Teaching

S 06-01

Teaching through the backdoor: Smartwatch-based cardiovascular measurements in the physiology laboratory

Robert Patejdl[1]

[1] *Health and Medical University Erfurt, Physiology, Erfurt, Germany*
[2] *University of Rostock, Physiology, Rostock, Germany*

Content

Question

Smartwatches offer a low-threshold access to parameters of cardiovascular function outside the context of the laboratory. The question of this ongoing research project is if and how smartwatch-based measurements can be utilized to increase students' motivation for learning cardiovascular physiology.

Methods

Medical students are surveyed regarding their knowledge on and usage of smartwatch-based measurements of electrocardiogram (ECG), heart rate and blood pressure. Furthermore, they are a simultaneous recording of a single channel ECG by a smartwatch and a regular ECG-machine. Both traces are then overlayed and students are asked to discuss the relevance and possible limitations of smartwatch-based ECG-measurements.

Results

Due to the ongoing nature of this project, only preliminary results can be reported. Although a vast majority of students reported to have used cardiovascular measurement functions of their smartwatch devices at least once, there is only little knowledge about the underlying principles of measurement, their accuracy and their medical relevance. Nevertheless, the directs comparison between the conducted smartwatch recording of their ECG with the simultaneous ECG-machine recording greatly increased their interest in both the principle of measurement and the quality of signals in relation to classical ECG-interpretation.

Conclusions

The low threshold for recording students' own cardiovascular function parameters combined with the possibility to compare their results with those from "real" laboratory measurements offers an opportunity to increase motivation for active participation in physiology laboratory experiments. This may offer an improved starting point for the transfer of theoretical concepts and a critical appraisal of measurements in clinical routine.

06-02

Team-based Learning: Does a permanent team matter?

Michael J.M. Fischer

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Content

Background: Assigning students to stable teams is a fundamental design principle of team-based learning (TBL), a teaching format that favors active learning. It is assumed that a stable team composition promotes learning success, as stable teams were reported to support the emergence of collaborative problem solving. Students who are more familiar with each other would exchange more information and solve problems by jointly discussing the pros and cons of individual arguments, which would promote the acquisition of knowledge and understanding. However, these assumptions have not yet been investigated using an intervention design with subsequent measurement of learning success.

Methods: In an online course for 490 students, half of the students were assigned to a new team for each individual task to be solved during the lesson, while the other half worked in stable teams. The learning success was tested by a timely test of specialist knowledge.

Results: Data from 464 students (95%) were analyzable. As expected, the repetition of a subject area in the team-based learning format improved subject knowledge in the areas covered. However, whether students worked in a permanent or in a temporary TBL team had no influence on their test results.

Diskussion: Overall, the stable TBL team seems to be less important for learning success than previously assumed. Since medical practice often requires decision making through collaboration in temporary teams, training skills for teamwork in temporary teams is important. Whether to work with stable or temporary TBL teams should be decided individually for each TBL teaching setting.

S 06-03

Role of physiology in new non-(dental) medical degree programs

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Content

In recent years, newly established health science programmes have imposed several challenges for physiology, especially with “integrated curricula”. Here, the development of an integrated midwifery curriculum (iMID) in Hamburg

will be presented to discuss some representative challenges and to generalise to a broader description of needs in physiology.
The bachelor programme iMID was launched in 2020. As the midwifery programme has only been recently transformed from midwifery schools to a bachelor's degree at university level, there was (and still is) no consented catalogue of learning objectives in Germany. Also, basic needs of midwifery students and expectable performances were unknown. Transfer of concepts from other (medical) curricula was limited due to the specfic requirements of midwifery. In iMID, Physiology is taught in an “Integrated Strand (IS)” together with the departments of biochemistry, health care research, and midwifery with weekly topics, which are based on the “care sheet of midwifery”. Content that could not be integrated into the IS is provided in a separate section. Whilst the IS has clear advantages, e.g. for relevance, it also limits the ability to teach other aspects of basic human function.
From a personal perspective, the most relevant challenges for physiology were the undefined role of physiology in a curriculum without consented learning objectives, missing feedback from the work environment, and time (and money) required to develop integrated curricula with relevant contents and continuous adaptation. This underlines the need for a continuous exchange within the society of physiology and a commitment within the relevant professional groups.

S 06-04

Consideration of individual differences when comparing face-to-face lectures and asynchronous online lectures in physiology

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The author has objected to a publication of the abstract.

S 07 | New Trends in Studying Islet Cells in Health and Disease

S 07-01

Caloric Restriction Reverses T2DM: Lessons from a Mouse Model

Maša Skelin Klemen[1], Jan Kopecky[1], Eva Paradiž Leitgeb[1], Jasmina Kerčmar[1], Lidija Križančić Bombek[1], Viljem Pohorec[1], Ismael Valladolid-Acebes[2], Jurij Dolenšek[1,3], **Andraž Stožer**[1]

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Content

Diet-induced obesity (DIO) mouse models are valuable tools for studying the pathophysiology of type 2 diabetes mellitus (T2DM), despite some methodological drawbacks. Clinical studies by Taylor et al. strongly suggest that caloric restriction can lead to effective remission of T2DM in humans, although there is limited mechanistic explanation at the beta cell level. To address this gap, we developed a novel DIO mouse model that more closely reflects human T2DM to elucidate the functional and morphological changes following caloric restriction-induced remission of T2DM.

In our study, C57BL/6J mice were fed a western diet for 12 weeks staring at 12 weeks of age. This regimen resulted in a T2DM phenotype characterized by fasting hyperglycemia, impaired glucose clearance and increased insulin resistance. Remarkably, seven days of caloric restriction completely reversed the diabetic phenotype, normalizing body mass, glucose handling, and insulin sensitivity. To investigate the mechanistical explanation for both the DIO and remission following caloric restriction at the level of beta cell function, we performed functional multicellular confocal calcium imaging on acutely prepared pancreatic tissue slices. A left shift in the glucose dependence was detected in the DIO group. Short term caloric restriction completely reversed the above compensatory left shift in beta cells and their oscillatory activity at a given glucose concentration decreased to that of the control group.

Our findings provide a deeper understanding of the impact of caloric restriction on T2DM and demonstrate that our novel mouse model is an effective platform for studying this condition.

S 07-02

Alpha cell spotlight: Insights into their function and role in health and disease.

Julia Panzer, Alberto Pugliese

City of Hope, Los Angeles, USA

Content

Question

Increased glucagon release from alpha cells is the main defense against hypoglycemia, a mechanism lost in Type 1 Diabetes (T1D). We hypothesize that the loss of beta cell inhibitory signals impairs alpha cell response to hypoglycemia in T1D. Reactivating paracrine and autocrine signaling could restore glucagon secretion.

Methods

We conducted experiments using isolated islets and pancreatic tissue slices from both non-diabetic organ donors and donors with T1D to determine alpha cell responses to (a) changes in glycemia, (b) agonists, antagonists, and positive allosteric modulators of autocrine and paracrine signaling pathways, and (c) reference stimuli such as adrenaline and KCl depolarization by live cell imaging and synamic hormone secretion. We further performed *in vivo* studies using mouse models with defective glucose counterregulation to determine whether alpha cell responses to hypoglycemia could be restored.

Results

In T1D, alpha cells fail to respond to low glucose despite normal glucagon content and responses to KCl depolarization. Diminished Ca2+ responses to glucose reduction and glutamate receptor stimulation were observed. Reactivating glutamate receptors with cyclothiazide and aniracetam or individual paracrine signals (serotonin, GABA, somatostatin) restored glucagon secretion in T1D tissues.

Conclusions

We demonstrate that alpha cell responses are brief and not sustained compared to beta cells. They require active reset by inhibitory signals to function properly. In T1D, inefficient glucagon response is due to deficient glutamate receptor signaling and loss of paracrine inhibitory input. Restoring both autocrine and paracrine signaling rescues glucagon secretion, offering potential targets to reduce hypoglycemic episodes and improve T1D therapy.

S 07-03

Single-cell quantification of β-cell secretory capacity and transcriptome in health and diabetes

Vishal Salunkhe[3], Marta Gironella-Torrent[1,2], Neha Sinha[3], Sansan Hua[3], Anders Rosengren[3], **Joan Camunas-Soler**[1,2]

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Content

A major determinant of disease progression in type 2 diabetes (T2D) is deteriorating insulin secretion from the pancreatic β-cells. However, the molecular changes that underlie β-cell dysfunction in T2D are still elusive. Recent single-cell studies have revealed transcriptional heterogeneity among β-cells, highlighting the need to investigate the relationship between β-cell molecular heterogeneity and functional decline in T2D. A limitation to current approaches is the difficulty in establishing functional profiles for different subtype of β-cells.

Here we perform fluorescent labelling of individual β-cells according to their secretory capacity and combine it with single-cell RNA-sequencing in the same cell. Our approach is based in the use of fluorescent tracers of endocytic membrane retrieval, which we show can be used as a proxy for insulin granule fusion and secretion. We use this methodology to characterize β-cell states associated with higher secretory function in nondiabetic donors and to identify potential mediators of β-cell failure during progression to T2D.

By investigating islets from mouse models with increasing levels of β-cell dysfunction, as well as human islets from donors with and without T2D, we identified genes and pathways associated to decreased β-cell secretory capacity in both mouse and human β-cells. Some of these genes are known regulators of insulin exocytosis and mitochondrial function and are now being investigated for further mechanistic studies. Overall, our approach sheds light on the relationship between molecular heterogeneity and secretory capacity in β-cells and offer potential avenues for the development of new treatments targeting β-cell secretory failure.

S 08 | What‘s new in …..? Knowing Neurophysiology state-of-the-science

S 08-01

What is new in motion planning ?

Jochen Roeper

Goethe University Frankfurt, Neurophysiology, Frankfurt, Germany

Content

Generations of medical students have been introduced to the role of basal ganglia in motor control with reference to the Indirect/Direct Pathway model and its reciprocal control via dopamine. While the model still does a good job in predicting some clinical effects like hypokinesia of dopamine depletion in Parkinson Disease, its simple depiction as an antagonistic Go/NoGo system leaves important issues unadressed. This contribution will suggest how recent research studies based on the recording and manipulation of in vivo dynamics of basal ganglia signaling via genetically encoded sensors for relevant neurotransmitters (DA, ACh) and neural activity (GCaMP, electrical recording) from defined basal ganglia neurons inform our understanding of motor control.

S 08-02

What's new in taste?

Timo Kirschstein

University of Rostock, Department of Physiology, Rostock, Germany

Content

Taste is an archetypal chemical sensation, and flavours can govern food intake and emotional comfort. In addition, the perception of gustatory stimuli is relevantly affected by olfactory, somatosensory, and nociceptive stimuli in the oral cavity. Hence, it is instrumental to understand the molecular and cellular mechanisms of signal transduction in taste buds as well as the pathways of higher order information processing. Taste buds are complex systems with differentiated sensory cells capable of detecting sweet, salty, sour, bitter, umami, and probably also fat compounds. Novel findings have emerged on signal transduction and transformation on gustatory afferents. They have therefore challenged our previous notion of the polymodal taste cell. This talk will give an overview of recent progress in understanding taste bud receptor signalling and central gustatory pathways and suggest how this progress may be translated into medical education.

S 08-03

What‘s new in fear extinction? Neural circuits of a dopaminergic prediction error signal underlying fear extinction learning

Ximena I. Salinas-Hernandez

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

Content

Among the various innate mechanisms animals possess to protect themselves, the ability to learn from experience which stimuli in the environment predict danger is particularly valuable. However, in an ever-changing environment, it is equally important for adaptive behavior to extinguish defense responses when those stimuli no longer predict danger. Fear extinctionis an associative learning process during which a stimulus previously associated with an aversive unconditioned stimulus (US) comes to be recognized as safe after being repeatedly presented in the absence of the US. As a result, conditioned defense responses, such as freezing, are progressively reduced. Deficits in this form of safety learning are a hallmark of anxiety disorders and thus understanding the neural basis of fear extinction has clinical significance. According to associative learning models, learning is initiated by teaching signals, also known as prediction error (PE) signals, that compute the difference between expectations and outcomes. Decades of research have revealed that the activity of midbrain dopamine (DA) neurons plays a critical role in encoding PEs during reward learning. In contrast, the role DA plays in encoding PE signals during fear extinction have reminded largely unknown. In this talk, I will discuss data that uncovers the functional architecture of a dopaminergic extinction PE signal (EPE) mediating fear extinction learning.

S 08-04

Do strong synapses live longer? All-optical interrogation of the structure-function relationship at individual excitatory synapses in vivo

J. Simon Wiegert

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

Content

Question

Memory traces formed during learning have been hypothesized to be stored at synapses and therefore spines. During learning, experience-dependent activity triggers synaptic plasticity, modifying synaptic strength. Most excitatory inputs form synapses on dendritic spines and changes in synaptic strength result in structural modifications of spines. The Schaffer collateral synapse between areas CA3 and CA1 of the hippocampus is considered one of the prototypical small glutamatergic synapses in the central nervous system and the hippocampus is required for the formation and

consolidation of declarative, episodic-like memories. The formation of new memories and memory updating in a fluctuating environment implies dynamic restructuring of the network, which is in conflict with the necessity for memory stability. Thus, an open question is how synaptic stability is related to synaptic plasticity and how synaptic plasticity shapes synaptic connectivity.

Methods

We combine chronic two-photon calcium imaging of ipsilateral CA1 spines with repeated optogenetic activation of pre-synaptic CA3 pyramidal neurons in slices and in the awake mouse. Using this approach, we are able to induce local, synaptically evoked calcium responses at individual spines and to assess the stability of these functionally identified synapses for days to weeks.

Results

We show that synaptic plasticity influences the stability of synapses and therefore synaptic connectivity in the network.

Conclusions

All-optical synaptic physiology in slices and in awake mice revealed the structure-function relationship at individual Schaffer collateral synapses.

S 09 | Immune cell trafficking in the microcirculation: from the fetus to the adult

Supported by DFG funded Transregio TRR359 PILOT

S 09-01

Atypical chemokine receptors in control of immune cell trafficking

Antal Rot

William Harvey Research Institute, Queen Mary University of London, London, UK

Content

Atypical chemokine receptors (ACKRs) are a group of seven-transmembrane spanning serpentine receptors that are structurally homologous to classical G-protein-coupled receptors and bind cognate chemokines with high affinities, but fail to engage G-proteins, trigger their signalling or induce cell migration. However, ACKRs efficiently modify the availability and function of chemokines, albeit through dissimilar mechanism, by either targeting them for lysosomal degradation or transporting them. In addition, some ACKRs can signal via intracellular effectors alternative to G-proteins. Currently we recognise five different ACKRs, which ligate partially overlapping sets of chemokines, are expressed in distinct tissue microenvironments and play complex dissimilar roles in physiology and disease. We examine how the highly characteristic expression of two prototypical ACKRs, ACKR1 and ACKR4 in their distinct cellular microenvironments and their dissimilar functions impact on the chemokine-induced trafficking of immune cells during host defence and cancer progression.

S 09-02

Pre- and perinatal dynamics of tissue-resident macrophages

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The author has objected to a publication of the abstract.

S 09-03

Preimplantation factor (PIF) is a critical modulator of neutrophil functions during pregnancy

Roland Immler[1], Wiebke Nadolni[2], Johanna M. Franz[1], Annika Bertsch[1], Vasilios Morikis[3], Monika Pruenster[1], Lou M. Wackerbarth[1], Matteo Napoli[1], Ina Rohwedder[1], Anna Yevtushenko[1], Aleksnadra Kurova[1], Oliver Soehnlein[4], Markus Moser[5], Steffen Dietzel[1,6], Eytan Barnea[7], Thomas Vogl[8], Scott I. Simon[3], Claudia Klein[9], Susanna Zierler[2,10], Markus Sperandio[1]

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The author has objected to a publication of the abstract.

S 09-04

Intracellular signaling in mouse and human inflammatory disease models

Attila Mócsai

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Content

Inflammatory diseases such as autoimmune joint, skin and kidney diseases and autoinflammatory diseases such as gout place a major burden on the health care systems and the affected individuals worldwide. During the last several years, we have tried to identify novel intracellular signaling components in myeloid cells that are critical for in vivo animal and ex vivo human models of inflammatory diseases. Using genetically modified mouse strains, we have shown that myeloid Src-family kinases, Syk, PLCγ2 and CARD9 are all required for autoantibody-induced inflammatory reactions such as K/B×N serum-transfer arthritis, autoantibody-induced skin blistering or accelerated nephrotoxic glomerulonephritis models. Src-family kinases are also involved in autoinflammatory diseases such as MSU crsytal-induced gout model or the genetically determined motheaten phenotype. The genetic deficiency of those signaling molecules also prevented accumulation of myeloid cells at the site of inflammation, likely through their involvement in the generation of the inflammatory microenvironment. Using lineage-specific deletion approaches, we have shown that Syk, PLCγ2 and CARD9 are likely critical within the neutrophil compartment. Pharmacological studies in an ex vivo human bullous pemhigoid model suggest a role for the above signaling molecules in human autoimmune skin blistering, as well. Taken together, the Src-family–Syk–PLCγ2–CARD9 pathway plays a critical role in various autoimmune and autoinflammatory disease models, raising the possibility of the exploitation of this pathway as a potential therapeutic target.

S 10 | Calcium channelopathies: from molecular mechanisms to clinical diversity

S 10-01

***CACNA1D* variants in endocrine and neurological disease**

Gabriel Stölting

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Content

The voltage-gated L-type calcium channel $Ca_V1.3$ (encoded by the *CACNA1D* gene) mediates calcium influx and electrical signaling of various cells. It is expressed in several tissues, including the CNS, inner ear, heart, pancreas, and the adrenal gland. Over the past years, novel genetic techniques including whole exome and genome sequencing have led to the association of $Ca_V1.3$ variants to various diseases and informed us about its physiological role.

The loss of $Ca_V1.3$ function in humans leads to a syndrome with sinus bradycardia and deafness, recapitulating earlier findings from knock-out mice. Variants that instead facilitate channel activation and calcium influx, however, have rather been linked to endocrine and neurological disease. The actual disease presentation is variable and may include primary aldosteronism, seizures and neurological abnormalities (PASNA syndrome) but also autism and hyperinsulinemia.
With the increasing number of discovered $Ca_V1.3$ variants, it has become apparent that a more detailed functional differentiation of pathogenic gain-of-function variants is warranted. Besides these advances in identification and classification of $Ca_V1.3$ variants, several groups have also begun exploring pharmacological interventions in animal models and in patients. While some successes have been observed, particularly for endocrine disease, neurological symptoms seem to be harder to treat. In this talk, I will summarize the latest findings and highlight future directions in the exploration of $Ca_V1.3$ physiology and pathophysiology.

S 10-02

Neurodevelopmental disorders related to *CACNA1C* variants; beyond Timothy Syndrome

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The author has objected to a publication of the abstract.

S 10-03

Disease variants in the activation gate of T-type calcium channel CACNA1I linked to neurodevelopmental disorders and epilepsy

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Content

The voltage-gated calcium channels (VGCC) family consists of ten isoforms that can be divided in high voltage activated (HVA) and low-voltage-activated (LVA) channels. $Ca_V3.1$-$Ca_V3.3$ are the LVA channels, also known as T-type channels. These channels activate and inactivate at potentials close to the resting membrane potential of the neurons where they are expressed, making them perfectly equipped to regulate cellular excitability.

We identified the gene for $Ca_V3.3$ (CACNA1I) for the first time as a disease gene for neurodevelopmental disorder and epilepsy. Using a combination of structure modelling, site-directed mutagenesis, patch-clamp recording, and NEURON computer modelling, we identified four gain-of-function disease variants located in the channel activation-gate. These variants show left-shifted voltage-dependence of activation and inactivation, and slower inactivation and deactivation kinetics, resulting in increased calcium influx during rest and activity. Our results indicated that these changes lead to hyper-excitability and calcium toxicity, underlying the epilepsy and neurodevelopmental disorder in the patients. We are currently studying three new CACNA1I activation gate variants linked to epilepsy and/or developmental delay.
Our results link structure to function and show how changes of only one amino acid can have tremendous effects on channel gating. The exact gating property that is altered (e.g. voltage-dependence and kinetics of activation vs. inactivation), depends on the precise location and substitution of the amino acid. To understand the function and pathogenicity of T-type channels, it is important to study the complete channel structure, how different functional units are connected and ultimately all cooperate in opening the channel gate.

S 11 | Neuropeptide modulation of fear- and anxiety-related networks

S 11-01

NPY-mediated changes in the BLA that reduce anxiety

William F. Colmers

University of Alberta, Pharmacology, Edmonton, Alberta, Canada

Content

Anxiety is widespread in humans. One root cause is the maladaptive response to prolonged stress. Neuropeptide Y is an endogenous anti-stress molecule with key actions in the Basolateral amygdala (BLA). As a model to understanding mechanisms associated with the development of resilience, we study the sites and mechanisms of NPY actions in BLA. NPY reduces stress-like behaviors through actions in the BLA. Acute application of NPY hyperpolarizes BLA principal neurons (BLA-PNs) through suppression of the voltage-dependent H-current (*Ih* -a mixed Na^+-K^+ conductance) which tonically depolarizes neurons. Further studies show that inhibition of projections to the bed nucleus of the stria terminalis (BNST) are key for the anti-stress actions of NPY. We do not observe similar effects of NPY in female rats which could be due to sex differences in the activity of BLA neurons projecting to the BNST.
Repeated (rp) stress exposure, or rp corticotrophin-releasing hormone (rpCRH) produces persistent increases in anxiety behaviors and in excitability and dendritic complexity of BLA-PNs. Conversely, rpNPY applications reduce anxiety, and induce prolonged (>1-2 month) stress resistance, with hypotrophy of BLA-PN dendrites. The BLA expresses 3 different NPY receptors with divergent actions. [JU1] [JU2] NPY_1 receptors (Y_1R) suppress *Ih* and are acutely anxiolytic. Y_2 receptors inhibit GABA release onto BLA-PN dendritic $GABA_B$ receptors, facilitating synaptic plasticity. Y_5 receptors (Y_5R) induce hypoplasia in BLA-PNs resulting in persistent anxiolysis. Knockdown of Y_5R in BLA is anxiogenic both in females and males, suggesting a complex role for NPY in the modulation of BLA activity and behavioral responses.

S 11-02

"Modulation of fear-relevant circuits by Neuropeptide S in mice"

Kay Jüngling[1]

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The author has objected to a publication of the abstract.

S 11-03

Role of neuropeptide-expressing neurons in the integration of fear/anxiety and hunger

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Medical University Innsbruck, Institute of Pharmacology, Innsbruck, Austria

Content

Question

The bed nucleus of the stria terminalis (BNST) is a sexually diverse forebrain structure that acts as a critical integrator of essential needs. In particular, anxiety and sustained fear responses, but also metabolic processes crucially depend on BNST functioning. Several neuronal subpopulations with multiple neurotransmitters and co-localized neuropeptide modulators coordinate a complex intrinsic and extrinsic signaling. However, identification and functional contribution of individual neuronal ensembles and in particular the relevance of specialized neuropeptide systems is only beginning to emerge.

Methods

We investigate the questions whether specialized neuropeptide-expressing BNST subpopulations drive dedicated emotional-affective and metabolic behavioral states or if they rather coordinate feeding behavior with anxiety- and fear-related processing.

Results

By illustrating neurochemical, anatomical and behavioral characteristics of the tachykinin neurokinin B (NKB) and somatostatin / neuropeptide Y and expressing neurons, we identified non-overlapping neuropeptide-expressing BNST ensembles with specific and integrative functional properties.

Conclusions

Thus, our studies indicate that NKB-expressing neurons of the BNST play a substantial role in orchestrating emotional-affective with metabolic processes in a sex-specific manner, probably by interacting with defined amygdala and brainstem nuclei.

S 11-04

Fear memory under circadian strain: hippocampal circuit modulation by orexin

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Content

Question

Fear memory consolidation and retrieval are coupled to the circadian rhythm and disturbed by circadian strains such as sleep deprivation or shifts in the circadian rhythm. This is related to disruptions in hippocampal microcircuit functions and the interaction of the hippocampus with its related network structures. Wake-promoting neuromodulators, such as orexins originating in the hypothalamus may alleviate the detrimental effects of circadian strains by modulating intra- and extrahippocampal circuits.

Methods

Using RNAScope, high-resolution quantitative PCR and viral tracing techniques we determined the susceptibility to orexinergic modulation of a network comprising the medial prefrontal cortex (mPFC), the supramammillary nucleus (SUM) and the dorsal hippocampus (dHipp). We tested the activation of these network structures during fear memory formation under circadian strain induced by acute phase shift and under pharmacological orexinergic modulation using cFos immunohistochemistry. A special focus was set on fear memory engram cells within the dentate gyrus and putative hippocampal microcircuits regulating their involvement.

Results

Receptors for orexins are expressed in the a mPFC-SUM-dHipp network with a high level of regional differentiation, including mPFC-to-dHipp projection neurons in the SUM and distinct classes of interneurons in the hippocampus. The activity of the intra- and extrahippocampal network structures was modulated by acute circadian phase shift and further by intranasal administration of orexin. Within the dHipp, acute phase shifts reduced the number of dentate gyrus engram cells and orexin application regulated interneuron activation.

Conclusions

This project provides insights into the neurocognitive circuits regulating fer memory strength and their modulation by orexin.

S 12 | The physiology of SLC26 transporters and their clinical significance

S 12-01

Pathophysiological role of SLC26A4 (pendrin) in the inner ear

Silvia Dossena

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Content

Pendrin (*SLC26A4*) is a multifunctional exchanger for monovalent anions expressed on the apical membrane of distinct epithelial cells in the cochlea and endolymphatic sac and duct. The physiological functions of pendrin in the inner ear include the modification of the ion composition, pH, and volume of the endolymph. Pathogenic variants of the pendrin protein rising from genetic mutation cause non-syndromic autosomal recessive deafness B4 and Pendred syndrome. Studies in transgenic mice illuminated the understanding of pendrin physiology in the inner ear; however, no mechanistic approaches to prevent hearing loss in Pendred syndrome/DFNB4 have been developed.

Testing of the function of pathogenic pendrin variants revealed a partial or total impairment of ion transport activity. The main pathomechanism of disease was initially believed to be the retention of the misfolded pathogenic variants within subcellular compartments. Recently, however, we have shown that reduction of expression levels, rather than mis-localization, is the key feature of pathogenic pendrin protein variants. We also have shown that cellular expression levels of wild-type pendrin and its pathogenic variants are controlled by the ubiquitin-proteasome system (UPS) and clinically approved and investigational UPS inhibitors can rescue pendrin protein expression at the plasma membrane and ion transport function. We have found that pendrin is recruited to UPS by a zinc-finger and BTB domain-containing receptor-adaptor protein forming a complex with Cullin 3 and the ubiquitin-ligase Roc I. A specific sequence within the C-terminus of pendrin is crucial in determining its degradation and might therefore represent a novel therapeutic target in Pendred syndrome/DFNB4.

S 12-02

Intestinal acid/base and electrolyte transport by SLC26A9, A6 and A3 in health and disease

Ursula E. Seidler

Hannover Medical School, Department of Gastroenterology, Hannover, Germany

Content

The transport of bicarbonate across the enterocyte cell membrane regulates the intracellular as well as the luminal pH, and is an essential part of directional fluid movement in the gut. This presentation will give an overview on the transport functions of the three members of the SLC26 family which are expressed in the apical membranes of the gastrointestinal epithelial cells and have been implicated in gastrointestinal barrier maintenance and the regulation of

the pH-microclimate above the enterocytes, namely SLC26A9 in the stomach, SLC26a6 in the small intestine, and SLC26A3 in the colon.
The molecular mechanisms behind the wide range of transport modes of the SLC26 family members are currently being unraveled. In this presentation, I will describe the recent structure-function studies for SLC26A9 and SLC26A6 and relate these new findings to the physiological functions that have been elucidated for these transporters in the stomach and small intestine. SLC26A3 is a Cl^-/HCO_3^- exchanger highly expressed in the colon, and mutations in this gene result in congenital chloride diarrhea (CCD, CLD). These patients display an extremely high incidence of inflammatory bowel disease. The *slc26a3*$^{-/-}$ mouse closely recapitulates the human disease and has proved a valuable model for a detailed understanding of SLC26A3 transport regulation, the factors that may explain the high incidence of colonic inflammation associated with its loss of function, and potential treatment options.

S 12-03

Secretin is a homeostatic bicarbonate hormone

Peder Berg

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Content

Secretin activates renal bicarbonate excretion. The secretin receptor (SCTR) is functionally expressed on the basolateral side of the base-secreting beta intercalated cells (β-ICs) of the kidney collecting duct. Here, secretin stimulates CFTR and pendrin-dependent bicarbonate secretion. Hence, in mice lacking pendrin or CFTR, secretin-induced urine alkalization is absent. Intriguingly, plasma secretin levels increase during acute metabolic alkalosis. Further, mimicking metabolic alkalosis in isolated perfused small intestines causes an ~2-fold higher secretin release. In SCTR KO mice, secretin-induced urine alkalization is absent and secretin cannot activate the β-ICs in isolated perfused cortical collecting ducts. In response to acute oral base loading, SCTR KO mice have impaired renal bicarbonate excretion. Congruently, SCTR KO mice develop a more pronounced and prolonged metabolic alkalosis when exposed to acute oral or intra-peritoneal $NaHCO_3$ loading and experience a transient marked depression of respiration. During base loading for 1 to 8 days, SCTR KO mice have diminished urine alkalization and a blunted correction of the imposed metabolic alkalosis. After 24h base loading, SCTR WT and KO mice increase pendrin protein abundance similarly, however, WTs have an ~2-fold higher increase of pendrin function than KOs.
In conclusion, secretin release is increased during metabolic alkalosis, most likely from intestinal S-cells. During acute and prolonged metabolic alkalosis, secretin activates renal β-ICs to increase urine bicarbonate excretion and ameliorate metabolic alkalosis. During prolonged base loading, the SCTR is not needed to increase pendrin protein abundance but is important to increase pendrin function. We suggest that secretin is a homeostatic bicarbonate hormone.

S 12-04

Pendrin: An renal anion exchanger linking acid-base to blood pressure regulation

Dominique Eladari

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The author has objected to a publication of the abstract.

ORAL SESSIONS

OS 01 | Contractile function

OS 01-01

Impact of titin cleavage in vivo on cardiac tissue regeneration and function

Paulina Hartmann[1], Johanna K. Freundt[1], Andreas Unger[1], Lydia Wachsmuth[2], Cornelius Faber[2], Oliver J. Müller[3], Wolfgang A. Linke[1]

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Content

Question

Titin abnormalities play a crucial role in various heart disorders. However, the impact of titin stiffness loss on cardiac activity is incompletely understood. Using the titin cleavage (TC) mouse model, we aimed to investigate how cleavage of elastic titin in living hearts may affect cell regeneration, tissue remodeling, and heart function.

Methods

The TC mouse contains a tobacco etch virus protease (TEVp) recognition cassette in the titin springs. Titin was cleaved in vivo by cardiac specific overexpression of AAV9-TEVp; AAV9-eGFP served as a control. Immunohistochemical staining with Ki67, DAPI, and WGA was employed to determine the cycling cell count, picrosirius red to quantify fibrosis. Ventricular morphology and function were assessed through cardiac MRI.

Results

Time-resolved immunohistochemistry with Ki67 staining showed a mitotic index of 6.71±0.78% (mean±SEM) on day 6 post-TEVp-injection, followed by a decline to 2.1±0.53% by day 13. In eGFP-injected mice, less than 1% of cardiac cells were Ki67-positive. Ki67-positive cells appeared to be non-cardiomyocytes, probably fibroblasts. Fibrotic cardiac area was 1.44±0.52% in AAV9-GFP injected hearts (N=6), increasing to 18.12±2.19% two weeks after AAV9-TEVp injection (N=12). Cardiac MRI of TC mice at days 6 and 13 post-injection revealed a progressive decrease in left ventricular diameter and volume during both systole and diastole, in TEVp vs. GFP controls. Systolic interventricular septum thickness increased, whereas the outer heart diameter was unchanged. While cardiac output was reduced, ejection fraction remained unaltered.

Conclusions

Specific in-vivo titin cleavage reduces living heart function and causes concentric growth likely explained by fibrotic remodeling.

OS 01-02

Comparison of the cardiac mitochondrial proteome of Göttingen minipigs to that of lean Ossabaw minipigs before manifestation of the metabolic syndrome

Chantal Eickelmann[1], Nilgün Gedik[1], Helmut R. Lieder[1], Svenja Idel[2], Laxmikanth Kollipara[2], Albert Sickmann[2,3,4], Michael Sturek[5], Gerd Heusch[1], Petra Kleinbongard[1]

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Content

Question:

Ossabaw minipigs differ from Göttingen minipigs in their unique genetic predisposition to develop a full metabolic syndrome and their non-responsiveness to cardioprotective interventions, even before the development of the diseased phenotype. Previous DNA sequencing data revealed differences in a cluster of mitochondrial genes between the two pig strains. Thus, we here aimed to compare the cardiac mitochondrial proteome of adult, lean Ossabaw minipigs with that of Göttingen minipigs.

Methods:

Mitochondria were isolated from cardiac left ventricular tissue samples of both pig strains (n=4, each) by differential centrifugation and density-gradient ultracentrifugation. The mitochondrial proteome was analyzed by LC-MS/MS. Proteins were classified as of mitochondrial origin and intra-mitochondrial function according to MitoCarta3.0 and Beyond MitoCarta.

Results:

A total of 1050 proteins were identified in all samples, 710 were classified as mitochondrial proteins. Among them, 26 proteins were differentially expressed between both pig strains. Most of them were classified as related to mitochondrial metabolism (10 higher expressed/7 lower expressed in Ossabaw minipigs), six (higher expressed) as related to mitochondrial transcription and translation, and two as related to oxidative phosphorylation (one higher expressed/one lower expressed), one as related to protein import, protein sorting and homeostasis (higher expressed), and two as related to small molecule transport (lower expressed).

Conclusion:

The mitochondrial proteome is largely comparable between Ossabaw and Göttingen minipigs. However, the proteins that were differentially expressed are related to mitochondrial metabolism and mitochondrial transcription and translation. Both play a crucial role in the development of metabolic syndrome and ischemic heart disease.

OS 01-03

Cannabinoids attenuate myotonic responses of mouse muscles via activation of cannabinoid receptor 1

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Content

Question

Myotonia is defined by the inability of skeletal muscles to quickly relax after voluntary contraction. The underlying effect is an increased excitability of the muscle fibre membranes due to mutations in genes encoding muscular ion channels. Myotonia further occurs as part of multisystemic genetic diseases such as Myotonic dystrophy. Recently it was observed that the severity of the myotonic symptoms of myotonia patients was attenuated after consumption of cannabinoids. In the current study we analyzed whether cannabinoids can attenuate myotonia, experimentally induced in mouse soleus muscles *in vitro*.

Methods

To this end muscles were bathed in chloride-free solution and were electrically stimulated under isometric conditions. Upon 50-Hz stimulation the muscles responded with aftercontractions lasting up to 10 s.

Results

To quantify the effects of cannabinoids on myotonia we calculated the areas included by the response curves after stimulation stop. Under physiological conditions (137 mM NaCl) the areas yielded on average 183 ± 83 (arbitrary units) and rose to 3637 ± 534 after changing to chloride-free bathing solution. The addition of Δ^9-tetrahydrocannabinol (Δ9-THC, 10 µM) reduced this parameter to 1827 ± 224, i.e. by 50%. Since Δ^9-THC is an unspecific phytocannabinoid, we tested the effects of two selective cannabinoid receptor agonists. While ACPA (10 nM), an agonist of cannabinoid receptor 1 (CB1) caused a similar reduction of the myotonic responses as Δ^9-THC, JWH133, an agonist of cannabinoid receptor 2, was ineffective.

Conclusions

Cannabinoids can attenuate experimentally induced myotonia of mouse muscles, most likely via activation of CB1.

OS 01-04

A novel approach for accessible or retrospective echocardiographic diastolic function assessment in mice

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Content

Despite the demand for basic research in diastolic (dys-)function due to its clinical relevance in heart failure progression, echocardiographic mouse studies are held back by the level of expertise that is needed for performing the Apical-four-chamber-view (A4CV), mandatory for all common diastolic function protocols. This study aims to establish a full-scale diastolic function protocol exclusively based on parasternal-long-axis brightness-mode (PSLAX B-mode) cine loops, arguably the most common view in rodent echocardiography.

PSLAX B-mode images were processed using speckle-tracking analysis allowing continuous left ventricular volume (LVV) measurement creating a LVV/time curve. By deriving this curve, visualization of isovolumetric sections permitted assessment of the isovolumetric relaxation time (IVRT), a key parameter of diastolic function. To verify our novel approach it was tested against the gold standard (based on pulsed-wave Doppler in A4CV) in groups of mice (n=8 each) with normal heart function, mild heart failure (HFmrEF) and severe heart failure (HFrEF). The results were evaluated using Pearson´s correlation and Bland-Altman analysis, respectively.

In comparison to the gold standard the bias of the new approach in mice with normal heart function was 4.1% (r^2=0.5589, p-value=0.0330). In HFmrEF mice it was -6.4% (r^2=0.8991, p-value=0.0003) and in HFrEF mice -12.07% (r^2=0.7809, p-value=0.0036). With all groups taken together the bias averaged -4.8% (r^2=0.9312, p-value<0.0001).

Our approach, via deduction of IVRT, complements an existing echocardiographic protocol hence permitting accurate and accessible diastolic function assessment exclusively using PSLAX B-mode data. Most noteworthy, this approach is also applicable for re-analysing previously obtained data sets based on PSLAX B-mode approach.

OS 01-05

Transcriptional bursting could explain intercellular variability associated with hypertrophic cardiomyopathy induced by mutations in different sarcomeric proteins

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Content

Question

Mutation induced hypertrophic cardiomyopathy (HCM) is often associated with intercellular variability of transcriptional activity, mRNA expression, contractility, and morphological parameters. This variability is significantly higher in patient heart tissue than in donor heart tissue. Both the functional effect of the mutation and the variability from cardiomyocyte to cardiomyocyte could contribute to the observed phenotype of HCM. We hypothesize that in the case of a heterozygous mutation of a sarcomeric gene the variability can be explained by transcription of both the allele with the mutation and the allele without the mutation, occurring in independent stochastic bursts.

Methods

We developed a numerical model based on the Euler method which entails (i) the polyploidy of cardiomyocytes, (ii) the stochastic burst-like activation of both alleles, (iii) the synthesis of pre-mRNA, (iv) the splicing of pre-mRNA to mRNA and mRNA degradation, and (v) the synthesis and degradation of the protein. We applied the model to two different heterozygous myosin mutations (R723G, A200V) and one heterozygous troponin I mutation (R145W).

Results

By fitting the distribution of ploidy and the rate constants for activation and inactivation of transcription and for splicing of pre-mRNA to mRNA, we were able to closely reproduce the observed distributions of active transcription sites in the nuclei, of the absolute mRNA counts, of the mutant to wildtype mRNA ratio, and of the produced force at intermediate calcium concentrations.

Conclusions

We conclude that, for all three studied HCM mutations, the observed phenotypical variations could be associated with the mechanism of stochastic transcriptional bursting.

OS 01-06

In vivo titin cleavage shows graded effects on cardiac function, structure, and immune response

Christine M. Loescher[1], Johanna K. Freundt[1], Andreas Unger[1], Richard Holtmeier[2], Susanne Hille[3], Oliver J. Müller[3], Annika J. Klotz[1], Sophie van Linthout[4], Wolfgang A. Linke[1]

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Content

Question

Titin is essential for regulating myocardial passive stiffness and a reduction in functional titin results in heart failure. However, to what extent the heart can tolerate titin loss before functional consequences arise is unclear.

Methods

Using the titin cleavage mouse model, with a TEVp-cleavable cassette in its titin spring, we injected homozygous (Hom) and heterozygous (Het) mice with an AAV9-TEVp cardiac specific plasmid to induce titin cleavage. AAV9-eGFP served as control. Left ventricular tissue was used for gel electrophoresis, EM, and permeabilized fiber mechanics analysis. Monocytes were isolated from the spleens.

Results

In 14-day post-TEVp injection Hom tissue with ~60% titin cleavage, EM images and Z-disc disorder semi-automated analysis showed either complete sarcomere degradation, highly disordered Z-discs and loss of I-bands, or more typical sarcomeres, comparable to eGFP control. Het-TEVp treated tissue with ~30% titin cleavage showed wavy Z-discs but otherwise typical sarcomeres.

Hom-TEVp treated fibers were stiffer than eGFP control samples at 20% strain (53 ± 15 kPa, n=8, and 29 ± 14 kPa, n=14, respectively), likely due to massive fibrosis. Additionally, Hom-TEVp treated fibers produced less Ca^{2+}-activated maximum force (18 ± 5 kPa, n=6) compared with untreated Hom control fibers (70 ± 4 kPa, n=8).

In addition, Hom-TEVp treated mice showed increased proinflammatory monocytes from day 6 to 14, with no change in anti-inflammatory monocytes. The opposite was seen in Het-TEVp treated mice.

Conclusions

These findings highlight the critical role of titin in the heart, where severe loss disrupts structure, stiffens tissue, weakens contraction, and promotes inflammation.

OS 02 | Renal: Physiological and pathophysiological aspects

OS 02-01

Unveiling Nephron Segment-Specific Functions of NFAT5: Insights from Targeted Knockout Models

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The author has objected to a publication of the abstract.

OS 02-02

Transcriptomic analysis suggests an important role of the transmembrane serine protease 2 in regulation of epithelial permeability in the distal nephron

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Content

Regulation of the epithelial sodium channel (ENaC) in the distal nephron is crucial for sodium balance and blood pressure control. A unique feature of ENaC is its complex regulation by proteases. Recently, we identified transmembrane serine protease 2 (TMPRSS2) as a novel candidate protease involved in proteolytic ENaC activation (Sure et al. *J Biol Chem*. 2022 Jun;298(6):102004). Here, we performed RNA sequencing analysis to investigate the effects of TMPRSS2-knockout on the transcriptome of mouse cortical collecting duct ($mCCD_{cl1}$) cells. Among the serine proteases detected in control $mCCD_{cl1}$ cells, *Tmprss2* expression was highest. In TMPRSS2-ko cells, no compensatory upregulation of other serine proteases was observed. In line with this, TMPRSS2-ko cells demonstrated lower apical trypsin-like proteolytic activity as assessed by a fluorogenic substrate assay. Moreover, transepithelial current measurements revealed that TMPRSS2 deficiency impaired proteolytic ENaC activation and reduced ENaC activity. Interestingly, in TMPRSS2-ko cells expression of typical aldosterone target genes (*Sgk1*, *Zbtb16, Scnn1a*) was upregulated, possibly as a compensatory response. Furthermore, TMPRSS2-ko was associated with a significant transcriptional downregulation of Epcam/Claudin complex genes (*Epcam, Cldn3, Cldn7, Cldn23*) consistent with a lower transepithelial resistance of TMPRSS2-ko *versus* control cells. In summary, our data indicate that TMPRSS2 is involved in proteolytic ENaC activation in $mCCD_{cl1}$ cells and likely affects tight-junction permeability. Finally, our analysis of available human transcriptome data from the GTEx project revealed a strong positive correlation between

the expression of *TMPRSS2*, *EPCAM* and ENaC-encoding genes. The role of TMPRSS2 in distal nephron function *in vivo* remains to be determined.

OS 02-03

An atrial natriuretic peptide-mediated heart-adrenal axis prevents salt-responsive arterial hypertension

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Content

Cardiac atrial natriuretic peptide (ANP) regulates arterial blood pressure (ABP) and volume. Via its Guanylyl Cyclase-A (ANP) receptor, ANP lowers peripheral resistance and stimulates natriuresis. Based on studies with cultured zona glomerulosa (ZG) cells, physiology textbooks state that these actions involve the inhibition of adrenal aldosterone release. Whether ANP indeed mediates *endogenous* heart-adrenal communication is, in fact, unknown.

To dissect the role of endogenous ANP in aldosterone regulation, we generated a genetic mouse model with cell-restricted deletion of the GC-A receptor in ZG cells (*AsCre x GC-A$^{fl/fl}$* mice). In control mice, GC-A expression was remarkably higher in ZG cells as compared to other tissues or adrenal zones (immunoblot). In *AsCre x GC-A$^{fl/fl}$* mice, GC-A expression was abolished in adrenal cortices and fully preserved in other organs such as the kidney, brain, or heart. Already under normal dietary salt conditions (0.5% NaCl), the aldosterone plasma levels of such ZG GC-A KO mice were significantly elevated without impacting ABP. In control mice, 2-week administration of a high salt diet (HSD, 4% NaCl) suppressed plasma aldosterone levels and barely changed ABP. In their KO littermates, aldosterone levels stayed elevated, and ABP significantly increased. This was accompanied by mild cardiomyocyte hypertrophy without cardiac fibrosis (histology/morphometry). Cardiac contractile functions were preserved, but cardiac output was increased, indicating hypervolemia (invasive hemodynamic measurements).

In view of the contribution of inappropriate aldosterone release to therapy-resistant hypertension and its complications, unraveling the role of cardiac ANP in aldosterone regulation may have pathophysiological and therapeutic implications.

OS 02-04

Pharmaceutical activation of soluble guanylate cyclase reduces acute kidney injury progression to chronic kidney disease

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Content

Question

After an acute kidney injury (AKI), patients face high morbidity and mortality due to the frequent progression to chronic kidney disease (CKD). Restoring adequate blood supply can prevent renal tissue hypoxia and nephron loss. Soluble guanylate cyclase (sGC) is a promising target for promoting vasodilation and reducing inflammation. Our study investigates the hypothesis that pharmacological activation of sGC can attenuate AKI and prevent its progression to CKD.

Methods

Rats underwent unilateral ischemia-reperfusion injury (IRI) and were analyzed on days 3, 7, 14, and 84. The animals received a daily dose of the sGC activator Bay 60-2770 or a vehicle. Along with histological and immunohistochemical analyses, ex vivo experiments were conducted on isolated perfused renal microvessels. RNA sequencing was performed on HK-2 cells using two damage models.

Results

In vehicle-treated IRI rats, the kidneys developed inflammation and fibrosis, with increased expression of pro-inflammatory markers (Il-6, Tnf-α) and renal injury markers (Kim-1, Ngal). There was also a reduction in the diameter of medullary vessels and hypertrophic inward remodeling of cortical vessels in the late phase. Treatment with Bay 60-2770 attenuated these pro-inflammatory and pro-fibrotic responses, reduced kidney weight loss, and largely preserved renal vascular architecture. Additionally, ex vivo investigations of perfused renal microvessels demonstrated positive effects of sGC activation on angiotensin and acetylcholine responses. Bay 60-2770 also showed beneficial effects on human proximal tubular cells.

Conclusions

The results demonstrate the short- and long-term benefits of sGC activation in reducing AKI and lowering the risk of subsequent CKD.

OS 02-05

Effects of orally ingested microplastics on the structure and function of the kidney

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Content

Question

Plastics are chemically inert and durable materials with long-term deposition in the environment. Little is known about the functional consequences of microplastic particles (MPs) ingestion. Most MPs in the environment consist of fibers and irregular fragments. The size and shape of the MPs presumably determine their toxicity. Here we assessed the distribution of MPs in the organism and the consequences of MPs deposition in the kidney. We hypothesized that irregular particles, mimicking the real-world situation of MPs in the environment, are more deleterious compared with spherical particles.

Methods

Aspheric and spheric MPs of different polymers were synthetized, and fluorescence labeled (polystyrene, polypropylene, polyethylene terephthalate, 1-50 µm). The deposition of the particles was determined in mice after gavage (2.5 mg/d, 5 or 10 days, 30 mice (n=6 each)). 3-D histological assessment was performed by fluorescence confocal microscopy of tissue sections. The quantitative organ distribution was determined using MPs with embedded upconverting nanoparticles ($NaYF_4$:Yb,Tm).

Results

We found an enrichment of MPs in the kidney. MPs were present in renal blood vessels including glomerular capillaries. Large MPs (> 5 µm) entered some tubules of the renal cortex and were accompanied by an influx of immune cells. Experiments using the isolated perfused kidney showed the increased tubular uptake of labeled albumin in nephrons in which particles were located, suggesting a loss of the integrity of the filtration barrier.

Conclusions

In summary, ingested irregular MPs compromise the structural and functional integrity of the kidney and may trigger local inflammatory responses leading to kidney injury.

OS 02-06

Tracking Ca2+ Dynamics in Pancreatic Tissue Slices, from NOD Mice to Human Islets

Sandra Postic[2], Johannes U. Pfabe[1], Ya-Chi Huang[1,5], Joan Camunas-Soler[2], Marjan Slak Rupnik[1,3,4]

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Content

We tracked the progression of diabetes development in NOD mice, capturing all stages of islet destruction. We investigated inflammation-damaged islets and established a classification of islets based on the severity of islet destruction and observed changes in the functional response of **α-** and **β-**cells in different stages of disease severity. Finally, we discuss current approaches and challenges of the pancreatic tissue slice to study human cells and discuss conditions which can increase reproducibility.

The main method used here is high spatial and temporal imaging of Ca^{2+} oscillations in pancreatic tissue slices. We used hormone specific immunostaining to identify cell types in the slices previously imaged for Ca^{2+} oscillations.

We found that in NOD mice, beta cell glucose-induced synchronization diminishes in the early phases of islet inflammation. In the late islet destruction phase the synchronized short events recover in a subset of beta cells. Alpha cell glucose sensitivity persisted through the course of the disease, with occasional synchronized events in the late stages. To extend this methodology to human pancreatic slices, we tested various conditions to improve reproducibility. Data on healthy human donors indicate muscarinic receptor agonists, amino acids and elevated glucose are promising inducers of stable islet activity in tissue slices.

The pancreatic tissue slice is a promising approach to study T1D as it provides the intact architecture of the islet microenvironment. However, limitations to extend this methodology to human pancreatic slices still exist. This study combines high speed Ca^{2+} imaging and immunostaining to enhance reproducibility in the human pancreatic slice preparation.

OS 03 | Ion channels

OS 03-01

Regulation of selectivity filter gating in the model system of minimal viral potassium channels

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Content

In potassium (K^+) channels, the selectivity filter (SF) discriminates between different cation species and functions as a physiological gate (e.g. in hERG and K2P). SF gating is modulated by both K^+ occupancy and a complex interplay with the adjacent protein helices. Physiological signals act through this interaction network.

The minimal viral Kcv channels are a model system representing an “autonomous” close homologue of the mammalian K^+ channel pore, devoid of any regulatory protein domains. The are ideal to study the basic structure-function relations for SF gating.

With cell-free protein expression and electrophysiology on planar lipid bilayers, we characterized >40 channel mutants of Kcv_{NTS}. The position of the critical amino acids for regulating the SF are conserved between mammalian channels, bacterial channels and the viral channel. However, in agreement with the literature on mammalian and bacterial channels, the chemical nature of the involved interactions is in many cases not conserved. This suggest that these positions might be “evolutionary switches” to create different gating modes.

One example is an interaction network involving T45, near the S4 K^+ binding site in the SF, and several residues on TM2. Further mutational studies suggest a predominantly hydrophobic nature of this interaction. A similar network exists in the same position in KcsA and other channels, but with different amino acids involved.

In conclusion, Kcv channels can provide a reproducible and easily manipulated model system for studying the peculiar mixture of conserved and non-conserved mechanisms and interactions that regulate SF gating in K^+ channels.

OS 03-02

Dissecting the up and down state of TREK K_{2P} channels by computational and functional studies

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Content

TREK channels belong to the mechanosensitive subfamily of two-pore domain potassium (K2P) channels and are regulated by diverse physical and cellular stimuli, including stretch, phosphorylation, lipids, and pH. They are widely expressed in central and peripheral neurons and tissues, e.g. in brain, heart, smooth muscle and kidney, and are involved in diverse physiological functions such as sleep, anaesthesia, pain, or secretion.

Two crystallographic structures exist for TREK channels, a ‘down’ conformation which is thought to be the basal state, and the ‘up’ conformation, representing a high activity state which is achieved by mechanical stretch and which opens the selectivity filter via upwards movement of TM4. However, it is unknown if other stimuli act via such ‘up’ transition or if alternative activation mechanisms can apply.In this study, we combine computational and electrophysiological methods to systematically identify and explain highly active TREK mutants in the lower TM2/3/4 helices. We conduct an extensive scanning mutagenesis, predict and describe the conformational state of GOF mutants (i.e. by free energy calculation, conventional MD simulation, and network interaction analysis), and functionally determine their activation mechanism (i.e. by state-dependent inhibitor binding and competition experiments). Surprisingly, many activatory mutations preserved the ‘down’ state, which argues against the presence of a blocking lipid in this state. Importantly, we find that a big proportion of the highly active mutants adopt the ‘up’ state, indicating that it is stable in unstretched membranes, and we demonstrate that physiological stimuli as cellular lipids and pH induce this state to activate TREK channels.

OS 03-03

Regulation of TRPC3 by PIP_2 and DAG

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Content

The transient receptor potential canonical type 3 (TRPC3) channel plays a pivotal role in regulating neuronal excitability via its constitutive activity. Coordination of lipids within TRPC channels is essential for their Ca^{2+} signalling function. TRPC3 is regulated by DAG and positively modulated by PIP_2. By combining computational predictions with in vitro experimental testing, we identified the binding sites for DAG and PIP2. We could located the DAG binding site at channel protomer interface at the outer membrane leaflet. MD simulations showed rapid DAG accumulation within the L2, which is one of the two potential lipid binding site indicated by cryo-EM. Electrophysiological experiments using a photoswitchable DAG-probe revealed that initial DAG exposure generates a subsequently sensitised channel state that is associated with significantly faster activation kinetics, which we found to be specifically modified by mutations within L2. Experimentally we find that the stimulated and the constitutive TRPC3 activity require PIP_2. The coordination site of PIP_2 (L3) is a novel lipid binding site, which we could initially identify by extended MD simulations and located it at the intersection of pre-S1 and S1 helices. These in silico predictions were verified by side directed mutagenesis and patch clamp electrophysiological recordings, where we demonstrated that PIP_2 binding leads to local structural changes (PIP_2 sensing), while that channel activation involves a multistep mechanism that propagates from L3 to the pore domain via the VSLD, the TRP helix and a salt bridge between the TRP helix and S4-S5 linker.

OS 03-04

Structural Insights into Selectivity and Activation Mechanism of LRRC8 Channels

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Content

Volume-regulated anion channels (VRACs) are ubiquitously present in the plasma membrane of vertebrate cells and are key players in cell volume regulation. Upon cell swelling, VRACs mediate efflux of chloride and organic osmolytes. VRACs are involved in many physiological and pathological processes such as gliotransmission, hormone release, and innate immunity. VRACs are heteromers of the essential leucine-rich repeat-containing 8A (LRRC8A) and at least one other LRRC8B–E homolog. While several cryo-electron microscopy (cryo-EM) structures are available, molecular basis of VRAC permeability and gating remains elusive, and many activation mechanisms have been proposed. LRRC8 N-termini (NTs) were implicated in VRAC gating and ion selectivity, but remained unresolved in the previously published cryo-EM structures.

We report a 2.8-Å cryo-EM structure of human LRRC8A with resolved NTs. NTs fold inside the pore and constrict the permeation path, creating a second selectivity filter in addition to the previously described one formed by the first extracellular loop. They interact with pore-surrounding helices and the intracellular loops and support their compact arrangement. According to the molecular dynamics (MD) simulations, this tight interaction network is partially disrupted upon channel activation and single N-terminal helices may transiently unwind.

Our work suggests an unusual pore architecture with two anion-selective filters in series and a potential mechanism for VRAC activation by cell swelling. Furthermore, we hypothesize that rare NT unwinding events observed in the MD simulations may facilitate the passage of large organic substrates such as amino-acids and nucleotides, while the majority of channels dwell in a compact halide-selective conformation.

OS 03-05

Hearing loss mutations in human P2X2 receptor channels

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Content

Introduction: P2X receptors (P2XR) are cation channels and expressed in various cell types including neuronal and immune cells. In mammals, seven distinct subunits are described which can assemble into homo and heteromeric trimers. P2X2 receptors play important roles in cochlea adaption to elevated sound levels. Three mutations have been identified that cause inherited progressive hearing loss. These mutations localize to the transmembrane domain 1 (V60L), transmembrane domain 2 (G353R) and a b-sheet linking the ATP binding site to the pore (D273Y).
Methods: In this study we developed inducible HEK293 cells stably expressing the three human P2X2 mutations in homomeric P2X2 receptors as well as in heteromeric P2X2/3 receptors. We measured their localization in the plasma membrane by a red fluorescent protein attached to the C-terminus and the binding of a fluorescently labeled ATP derivative. The constructs were functionally characterized with the patch-clamp technique in the whole-cell configuration using a ligand application as well as a voltage step protocol.
Conclusions: 1. The mutations in the transmembrane domains V60L and G353 show robust localization in the plasma membrane and binding of fATP. 2. The mutation V60L has an increased affinity to fATP compared to the wildtype whereas the mutation G353 has similar binding. 3. Expression of mutated V60L hP2X2 channels has a dramatic effect on the cell viability leading to cell death after the second day of expression. This effect may support a role in the pathogenesis of hearing loss. 4. The mutated P2X2 subunits can assemble into P2X2/3 heteromeric channels.

OS 03-06

The pathophysiological effects of NMDA receptor activation in pancreatic β-cells can be mitigated by GluN2B antagonists

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Content

Question

Targeting NMDA receptors (NMDARs) in pancreatic β-cells might be a therapeutic avenue for treatment of type 2 diabetes. Hence, we aimed to characterize the sources of reactive oxygen species, stress pathways, and electrical

alterations upon NMDAR overstimulation in β-cells. Antagonists for the GluN2B receptor subunit were used to test the ability to restore the function of β-cells.

Methods

β-cells or islets from C57BL6/N mice and MIN6-cells were used. NOX activity assays, superoxide staining (mitoSOX), mitochondrial oxygen consumption rate (OCR), RT-PCR, Western blot, patch-clamp, and insulin secretion (ELISA) experiments were conducted. NMDA (5 mM) was applied for 48 h or acutely (500 µM NMDA/10 µM glycine).

Results

NMDAR activation increases mitochondrial superoxide production in β-cells, which is mitigated by the GluN2B antagonists WMS-1410 and Ro25-6981 (1 µM). In MIN6-cells NMDA elevates NOX activity, reduces ATP-linked OCR, and increases C/EBP homologous protein (*CHOP*) expression. GluN2B antagonists prevent the effect of NMDA on NOX activity and *CHOP* expression. The NMDA-induced decrease ATP-linked OCR is prevented by Ro25-6981. Patch-clamp experiments reveal that acute Ca^{2+} influx by NMDAR activation increases K^+-currents via $K_{Ca}3.1$ channels. This effect is sensitive to GluN2B antagonists and the $K_{Ca}3.1$ blocker senicapoc (1 µM). GluN2B antagonists also prevent NMDA-mediated alterations in insulin secretion.

Conclusions

Overstimulation of NMDARs in β-cells induces oxidative stress via NOX activation, mitochondrial dysfunction and ER stress. In addition, NMDAR activation increases K^+-currents through $K_{Ca}3.1$ channels. Targeting the GluN2B subunit partly restores β-cell function, highlighting the therapeutic potential of subtype-selective receptor inhibition for type 2 diabetes.

OS 04 | Neuroscience

OS 04-01

Reciprocal firing rate changes of dopamine substantia nigra neurons associated with self-paced movement initiation and termination

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Content

Dopamine substantia nigra (DA SN) neurons are important for voluntary movement and their degeneration in Parkinson Disease leads to motor impairments. We performed chronic multi-electrode recordings of pharmacologically identified DA SN neurons in freely-moving C57Bl/6N mice. In our data set of n=59 (N=16) DA SN neurons, a fraction of ~30% (n=17/59), predominantly found in the central SN (n=13/30), transiently increased their firing rates (+5.3±4.8Hz, mean±SD), while a similar fraction (n=18/59), predominantly found in the medial SN (n=11/22), transiently decreased their firing rates (-3.6±1.2Hz, mean±SD) in a time window (±500ms) around self-paced movements. Furthermore, we

carried out AA9-based retrograde axonal tracing in 7 adult DAT-Cre mice (3 male, 4 female, aged 8–20 weeks) to selectively express the inhibitory DREADD hM4D in either dorso-lateral striatum (DLS) or dorso-medial striatum (DMS) projecting DA SN neurons. A reduction of >40% of their mean firing rate within 30min after systemic injection of the agonist DCZ (100µg/kg) was used a criteria for identification of projection-defined DA SN neurons. We found that half of DLS-projecting neurons (n=4/8, N=3) and a similar proportion of DMS-projecting neurons (n=8/15, N=4) in the medial SN transiently increased their firing rate prior to movement initiation (DLS:+162,27±81,81% mean±SD, DMS:+104,67±108,67% mean±SD), while only a minority transiently decreased their firing rate shortly after movement initiation (DMS:1/15, DLS:1/8). Therefore, we are currently exploring the contribution of DA neurons in the medial SN projecting to the ventral striatum.

OS 04-02

Intradermal injection-based pain models

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Content

Question

The available knowledge how nociceptive neurons sense noxious stimuli is mainly based on in vitro data and animal models. This also applies to pain hypersensitivity commonly seen in inflammatory conditions. Most of these mechanisms have not been validated in humans.

Methods

We developed pain models that are based on intradermal injection on the volar forearm of healthy volunteers. A defined painful stimulus (chemical or thermal) was co-applied with specific ion channel inhibitors. This allowed for probing various targets for a potential involvement in the perception of the respective stimuli. Volunteers periodically rated the perceived pain during the injection using a numerical rating scale. Such stimuli were also investigated in local inflammation, which can be induced by intradermal injections of proinflammatory agents.

Results

Of the targets studied by the antagonists, only transient receptor potential cation channel subfamily V member 1 (TRPV1) contributed to acid-induced pain and heat-induced pain. Induction of inflammation increased pain ratings upon acidic injections and to mechanical stimuli. Both stimuli had a maximum sensitisation after about 5 hours for a clinically relevant inflammatory model.

Conclusions

The established pain models enable to apply noxious stimuli to human skin and at the same time evaluate multiple targets possibly important for the detection thereof. Human inflammation could be reliably modeled, and to determine the optimal time for intervention. This allows to investigate whether the relevance of the involved receptors in naive conditions changes by inflammation.

OS 04-03

The network-wide impact of pallidal deep brain stimulation in generalized dystonia

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Content

Question

The therapy of deep brain stimulation (DBS) of the globus pallidus internus (GPi) is an effective treatment of generalized or cervical dystonia but with previously unknown mechanisms. It is thought that DBS leads to an increase in GPi activity, thereby counteracting the hyperkinesia of dystonia. Our working hypothesis, therefore, involves increased inhibition of motor thalamic neurons as a result of increased GPi activity after pallidal DBS.

Methods

To confirm our working hypothesis, we investigated the inhibitory synaptic transmission and network activities of motor thalamic neurons with patch clamp in vitro in an animal model of paroxysmal generalized dystonia treated with pallidal DBS. We implanted bipolar stimulation electrodes bilaterally into the dt^{sz} mutant hamster's globus pallidus internus and the STELLA stimulation system in the hamster's flank for continuous long-term DBS (130 Hz, 50 µA) over 11 days.

Results

Our results indicated unexpected effects of pallidal DBS on the motor thalamic neurons by upregulating the excitatory tone rather than direct inhibitory projections. That may be an effect of raised excitability in cortical motor neurons, which indicated prolonged bursts with an increased number of spikes compared to the sham group. We also confirmed an enhanced neuronal network activity within the cerebellar layers after pallidal DBS that was recorded with high-density microelectrode arrays.

Conclusions

Our measurements on the synaptic and network activity of motor thalamic neurons and expanded to the motor cortex and the cerebellum pointed to global network effect of DBS rather than a local impact on the stimulation side.

OS 04-04

Serotonin acts pre- and postsynaptically to differentially modulate glutamatergic and GABAergic synaptic transmission in the anterior cingulate cortex

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The author has objected to a publication of the abstract.

OS 04-05

Enhanced *in vivo* firing of substantia nigra dopamine neurons projecting to the dorsal striatum in the *Df(16)A*$^{+/-}$ mouse model of schizophrenia

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Content

Elevation of dopamine (DA) levels in the striatum has been the hallmark of schizophrenia (SCZ) psychopathology. However, its unknown, whether this respective DA system is also preferentially affected in any model system. We studied a genetically modified mouse model (*Df(16)A*$^{+/-}$) of the human 22q11.2 microdeletion, known as the highest genetic risk factor for SCZ. Initially, we characterized putative DA midbrain neurons by chronic *in vivo* extracellular recordings in awake, freely moving *Df(16)A*$^{+/-}$mice and littermate controls. We detected ~50% persistently increased firing frequencies for medial substantia nigra (mSN) DA neurons in the *Df(16)A*$^{+/-}$mice in comparison to controls (male: WT FR:5.15Hz,n=84,N=6; *Df(16)A*$^{+/-}$ FR:7.84Hz,n=130,N=7; 1.5–fold; female: WT FR:5.05Hz,n=95,N=6; *Df(16)A*$^{+/-}$ FR:8.06Hz,n=95,N=5; 1.5–fold). A similar degree of hyperactivity was also observed in DA neurons recorded in the lateral SN of *Df(16)A*$^{+/-}$mice (WT FR:6.51Hz,n=49,N=7; *Df(16)A*$^{+/-}$ FR:9.75Hz,n=52,N=7; 1.4-fold), but no differences of DA neurons in the VTA. For a projection-specific characterization of DA SN subpopulations, we performed a chemogenic DREADD approach in *DAT-Cre*$^{+/-}$*XDf(16)A*$^{+/-}$double transgenic mice. Identified dorso-medial striatum (DMS)-projecting DA SN neurons displayed a 50% higher firing rate in *DAT-Cre*$^{+/-}$*XDf(16)A*$^{+/-}$ mice compared to controls (DMS: *DAT-Cre*$^{+/-}$ FR:4.94Hz,n=26,N=6; *DAT-Cre*$^{+/-}$*XDf(16)A*$^{+/-}$ FR:7.54Hz,n=49,N=7; 1.5–fold). In contrast, DLS-projecting DA SN neurons displayed only a 25% higher firing rate (DLS: *DAT-Cre*$^{+/-}$ FR:8.02 Hz,n=42,N=7; *DAT-Cre*$^{+/-}$*XDf(16)A*$^{+/-}$ FR:10,13Hz,n=39,N=7; 1.2–fold). Our findings provide robust evidence for a preferentially enhanced dopaminergic firing activity in DMS-projecting DA SNc neurons. Thus, the *Df(16)A*$^{+/-}$ mice might be suitable to study the molecular and cellular mechanisms for regional DA dysfunction in SCZ.

OS 04-06

Modulation of cold pain by inhibition of TRPM8, TRPA1, NaV1.7 and NaV1.8 in humans

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Content

Question

Do TRPM8, TRPA1, NaV1.7, and NaV1.8 ion channels mediate cold-induced pain in humans?

Methods

We developed a human cold pain model using intradermal injections of progressively colder fluid to elicit cold pain. The model was used in a randomized, double-blinded placebo-controlled, crossover trial with 36 participants, testing individual and combined effects of antagonists of TRPM8, TRPA1, NaV1.7 and NaV1.8. Pain was quantitatively assessed using a numerical rating scale every 5 seconds during injections.

Results

Cold injections resulted in higher pain ratings than room temperature injections ($p < 0.001$), co-injection of the local anesthetic lidocaine largely reduced cold pain ($p < 0.001$), validating the model. Based on the pre-specified analysis, there was no evidence that inhibition of TRPM8, TRPA1, NaV1.7, and NaV1.8 or their simultaneous inhibition eliminates the cold pain in the final 30 s period of a prolonged cold stimulus. Nevertheless, 95% confidence intervals are compatible with some involvement of the tested ion channels in cold pain perception. Exploratory analyses indicated shifts in cold pain thresholds to lower temperatures with receptor antagonists, most pronounced for TRPA1 inhibition.

Conclusions

Single or combined inhibition of the investigated ion channels show only a minority contribution to cold-induced pain in humans, indicating an additional principal cold sensor. Nevertheless, the results suggest a partial contribution of TRPM8, TRPA1, NaV1.7, and NaV1.8 to cold pain, particular in setting the threshold for cold pain.

OS 05 | Cardiac intervention and arrhythmia

OS 05-01

IGF-1/AKT kinase-mediated phosphorylation of HCN4 channels increases their cell surface expression

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Content

The hyperpolarization-activated cyclic nucleotide gated (HCN) channels mediate the cation current I_f, which plays a major role in cardiac pacemaker activity of the sinoatrial node. I_f is modulated by cyclic nucleotides, phosphoinositides, accessory proteins of the HCN channel complex, or post-translational modification of the HCN proteins. A previous phospho-proteomic analysis revealed four AKT kinase-dependent phosphorylation sites in HCN4, the predominant HCN isoform in the mammalian heart.

Here, we investigated the impact of AKT kinase-mediated phosphorylation on HCN4 channel physiology using electrophysiological and cell biological approaches. The extracellular application of insulin-like growth factor 1 (IGF-1), which canonically activates AKT kinase, resulted in a rapid more than 2-fold increase in HCN4 current amplitudes recorded in the heterologous expression system of Xenopus laevis oocytes. Whereas voltage-dependent gating remained unaffected, the amount of channel protein on the cell surface increased by a factor of three. These effects could be mimicked by the direct AKT kinase activator SC-79. Mutation of the four previously predicted N-terminal phosphorylation sites to phosphorylation-deficient amino acids rendered HCN4 insensitive to the application of either IGF-1 or SC-79, while phospho-mimicking mutants anticipated the described IGF-1 effects on current amplitudes. Extending the findings from the heterologous expression system to native tissue, an IGF-1 induced increase in pacemaker action potential frequency could be observed by optical voltage mapping of a mouse sinoatrial node preparation.

In summary, this study indicates a role for IGF-1/AKT kinase-induced phosphorylation in HCN4 channel surface trafficking, which may impact on cardiac rhythmogenesis.

OS 05-02

Paroxysmal atrial fibrillation under prolonged JDP2 overexpression

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Content

It is assumed that cardiac remodeling in paroxysmal atrial fibrillation (AF) contributes to disease progression and that there is a point of no return. In a mouse model with inducible cardiac specific JDP2 overexpression we tested, if paroxysmal AF develops into persistent AF.

After 5 weeks of JDP2 overexpression, which led to paroxysmal AF, JDP2 overexpression was interrupted for 5 weeks. In this group, all changes in ecg recordings declined and HW/BW was not different to WTs. In another group, JDP2 was overexpressed for the entire 10 weeks. In this group, PQ time prolongation, widening of the QRS complexes and paroxysmal AF were still present. Furthermore, HW/BW was increased. The initial 5 weeks of JDP2 overexpression reduced atrial mRNA expression of the calcium-handling proteins NCX, Cav1.2 and RyR2, and connexin40. mRNAs of the hypertrophic marker gene ANP, pro-fibrotic collagen, pro-inflammatory MCP1, and markers of immune cell infiltration (CD68, CD20) increased. All these mRNA expression changes declined 5 weeks after cancellation of JDP2 overexpression. In mice with prolonged JDP2 overexpression reductions in mRNA expression of Ca handling proteins and connexin 40, and enhancement of ANP-mRNA still were visible. However, all pro-inflammatory markers went back to normal.

In conclusion, paroxysmal AF and changes in gene expression were reversible upon cessation of JDP2 overexpression. AF occurred only when JDP2 was overexpressed, but prolonged expression did not lead to a worsening of the phenotype. Therefore, changes occurring during paroxysmal AF were not sufficient to provoke aggravation and progression to persistent AF in JDP2 mice.

OS 05-03

Tachyarrhythmic pacing in long-term culture of right atrial appendage pectinate muscles from patients in sinus rhythm induces atrial fibrillation-like remodeling

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Content

Question

Atrial fibrillation (AF) affects millions worldwide, contributing to elevated hospitalization rates and mortality. AF sustains itself through adverse atrial remodeling. However, the precise causes of AF-induced remodeling remain inadquately elucidated. Here, we explore, whether and how long-term arrhythmic tachypacing induces remodeling characterisitcs typical of AF.

Methods

We cultured beating right-atrial appendage pectinate muscles (trabeculae) obtained from patients in sinus rhythm for two weeks. During the second week, trabeculae were paced either regularly at 1Hz or with a 30% beat-to-beat standard deviation at 5Hz. Contractile parameters were assessed daily. After one week of 1Hz or 5Hz pacing, we isolated myocytes, measured transmembrane currents and quantified transcription levels of ion channel subunits and Ca^{2+} handling proteins.

Results

Arrhythmic pacing at 5 Hz reduced the transient outward current I_{to} at V_{Pip} = 60 mV by 51% in comparison to 1Hz pacing (n=19/8 and 16/6, p<0.005) in isolated cardiomyocytes. The transcription levels of the L-type Ca^{2+} channel pore-forming subunit was reduced by 24% (n = 6/6 each, p < 0.05), and of SERCA by 40% (n =7/7 each, p<0.01). The effective refractory period, assessed by contraction, was shorter after 5Hz pacing (363±28.2ms) than after 1Hz pacing (484±40ms, n=8/8 each, p<0.05). Contractile force after 5Hz pacing reached only ~33% of the force in 1Hz paced tissue (1050±181µN, n= 14/8, vs 363±68.4µN, n=12/8, p< 0.01)

Conclusions

Tachy-arrhythmic electrical pacing of healthy human atrial myocardium over a period of one week is sufficient to induce remodeling characteristics commonly found in the atrial myocardium of AF patients.

OS 05-04

Optogenetic current clamp in the intact heart to quantify cardiac excitability and explain cardiac arrhythmia initiation and protection

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Content

Altered cardiac excitability is an important factor promoting cardiac arrhythmia and can be quantified by the input resistance (R_m), calculated by the change in membrane potential in response to subthreshold current injections, and the pacing threshold (PT), defined as the minimum inward current sufficient to trigger an action potential. To determine these parameters in the intact heart, we developed a novel optogenetic current clamp method, which allows for cycle-dependent measurement of R_m and PT.

Precisely timed depolarization of confined myocardial tissue was performed by epicardial illumination of Langendorff-perfused mouse hearts expressing the light-gated ion channel channelrhodopsin-2 in all cardiomyocytes. The resulting subthreshold membrane potential changes were measured by microelectrodes and the injected current was computed by a channelrhodopsin-2 gating model.

R_m was smallest at diastole (63.3±13.1 MΩ, N=10) and significantly larger during plateau and repolarization phase. Pharmacological block of the inwardly rectifying potassium current (I_{K1}) by $BaCl_2$ (10 µM) as well as depolarization induced I_{K1} block by optogenetic depolarization of the resting membrane potential significantly decreased PT and increased diastolic R_m indicating a critical role of I_{K1} in generating the low R_m at diastole (N=5-8). Simulations in a cardiomyocyte model (openCARP) showed the importance of I_{K1} inward rectification for explaining the experimental data and preventing pathologically high excitability, and were used to classify anti-arrhythmic mechanisms of multi-channel blockers.

Thus, combining precise optogenetic current injection and microelectrode recording enables quantification of cardiac excitability in the intact heart and can be used to identify arrhythmia mechanisms and therapeutic strategies.

OS 05-05

Vago-splenic signal transduction in the release of cardioprotective factors by remote ischemic conditioning and auricular tragus stimulation in humans

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Content

Questions: The spleen serves as an important relay organ which releases cardioprotective factor(s) upon vagal activation during remote ischemic conditioning (RIC) in rats and pigs. The translation of these findings to humans was attempted.

Methods: RIC or electrical auricular tragus stimulation (ATS) were performed in 10 healthy young volunteers, 10 volunteers with splenectomy and 20 matched controls. Venous blood samples were taken before and after RIC/ATS or placebo, and a plasma-dialysate (12-14 kDa cut-off, dialysis ration 1:10 against saline buffer) was infused into isolated perfused rat hearts subjected to global 30 min ischemia and 120 min reperfusion. In a subgroup of 6 healthy volunteers, intravenous atropine was used to address the role of vagal activation.

Results: Neither RIC nor ATS altered heart rate and heart rate variability in the study cohorts. With the plasma-dialysate prepared before RIC or ATS, respectively, infarct size (% ventricular mass) in the recipient rat heart was 36±6% with RIC and 31±5% with ATS, and decreased with the plasma-dialysate from healthy volunteers after RIC to 20±4& and to 19±4% with ATS. Infarct size was still reduced with plasma dialysate 4 days after ATS and 9 days after RIC. Such infarct size reduction was abrogated by intravenous atropine. Infarct size reduction by RIC or ATS was also abrogated in 10 volunteers with splenectomy, but not in their 20 matched controls.

Conclusions: In humans, vagal innervation and the spleen as a relay organ are decisive for the cardioprotective signal transduction of RIC and ATS.

OS 05-06

Neuro-glial interaction in the heart

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The author has objected to a publication of the abstract.

OS 06 | Best Abstract Competition

OS 06-01

Downstream activation of K_{Ca} channels as amplifiers of TRPV4-mediated pulmonary edema formation

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The author has objected to a publication of the abstract.

OS 06-02

Differential K_v4 channel functions among projection-defined dopamine neurons of the ventral tegmental area

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Content

K_v4 channels are well established regulators of pacemaking frequency in dopamine (DA) midbrain neurons. Although previous studies have characterized K_v4 channel properties in substantia nigra (SN) and some ventral tegmental area (VTA) DA neurons, the contribution of K_v4 in axonal projection-defined VTA DA subpopulations is still unknown.

To investigate K_v4s biophysical properties in projection-defined VTA DA subpopulations, we combined axonal retrograde tracing with nucleated outside-out patch-clamp recordings. In DA neurons projecting to the medial shell of nucleus accumbens (mNAc) the K_v4 steady-state activation curves were shifted to significantly more depolarized potentials compared to those in lateral shell (lNAc)-projecting DA neurons ($V_{50\text{-}A}$: mNAc:-1.4±6.0mV; lNAc:-11.7±3.5mV). Additionally, the K_v4 steady-state inactivation curves were more negative ($V_{50\text{-}I}$: mNAc:-88.6±2.4mV; lNAc:-72.2±2.3mV). These distinct K_v4 gating properties suggested functional differences regarding K_v4-mediated pacemaker control. To test this, we recorded the spontaneous activity of retrogradely traced VTA DA neurons before and after wash-in of the specific K_v4-inhibitor AmmTx3 (1µM). Consistent with the biophysical differences, K_v4 inhibition increased the mean firing frequency in lNAc DA neurons by ~60%, while it did not affect discharge rates in mNAc DA neurons.

These results demonstrate a differential K_v4 channel function for projection-defined VTA DA subpopulations. Like in SN DA neurons, K_v4 controls pacemaker frequency in lNAc DA cells. In contrast, in mNAc DA neurons K_v4 appears to operate only in the subthreshold voltage range. Using this new biophysical K_v4 data, we are currently refining our subtype-specific computational VTA DA models to predict the functional implications of K_v4 diversity for *in vivo*-like states.

OS 06-03

Identification of new gain-of-function variants located in the erythropoietin gene locus resulting in hereditary polycythemia

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The author has objected to a publication of the abstract.

OS 06-04

The Calcium Sensing Receptor in the Thick Ascending Limb regulates paracellular calcium permeability

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Content

Question

The calcium-sensing receptor (CaSR) plays a critical role in the maintenance of calcium homeostasis. In the kidney, it is predominantly expressed in the basolateral membrane of the thick ascending limb (TAL). The TAL is responsible for the reabsorption of approximately 25% of the filtered calcium. The basolateral CaSR allows the direct response to

elevated basolateral Ca^{2+} concentrations, however, this local regulatory response remains to be delineated in detail. We studied the effect of hypercalcemia on TAL transport in a nephron-specific CaSR knockout mouse model.

Methods

Electrophysiological transport properties were assessed in freshly isolated TAL segments of mice with kidney-specific (Ksp-Cre) CaSR deletion (cKO) and Cre-negative littermates (WT). Hypercalcemia was induced with Vitamin D analog (Dihydrotachysterol, DHT) diet for 3 days and balance studies performed in metabolic cages.

Results

CaSR antibody staining confirmed the loss of CaSR in TAL of cKO. Experimental hypercalcemia increased urinary Ca^{2+} excretion in WT but not in cKO. Hypercalcemia did not change transepithelial voltage. The transepithelial resistance was increased in TAL of WT but not in cKO mice. This indicated changes mainly in paracellular properties. Accordingly, in contrast to cKO, WT TAL showed a selective decrease in paracellular sodium and calcium permeability.

Conclusions

Tissue-specific deletion of CASR in TAL unmasked local modulation of TAL transport. While the voltage gradient generated by transcellular transport remained unaltered, paracellular transport properties were modulated to allow higher urinary excretion of Ca^{2+} under hypercalcemic conditions.

OS 06-05

Secretin: a novel modulator of GFR and diuresis

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Content

Secretin is one of the key gastro-intestinal hormones and known to stimulate pancreatic HCO_3^- secretion. Its actions extend far beyond this, also comprising regulation of renal functions. By serendipity we discovered that bolus application of secretin in anaesthetized mice triggered a very pronounced, transient decrease of urine production. Here, we provide a comprehensive description of the underlying mechanism. Key results comprise the following: 1. Secretin application (0.2 µg/25 g) interrupted urine production transiently which returned to pre-control values after ~ 20 minutes. This effect was absent in the secretin receptor KO mouse. 2. No systemic blood pressure effects were measured at this dose. 3. secretin receptor KO mice had significantly elevated urine production and GFR. 4. Isolated tubule perfusion experiments in TALs and collecting ducts revealed no secretin-dependent effects on electrolyte or water transport. 5. Intravital two-photon microscopy showed a marked secretin-induced reduction of early proximal

tubular flow with close temporal overlay with secretin´s antiduretic effect. 6. In isolated angiotensin II pre-constricted vas afference and vas efference, secretin caused a markedly vasodilation of vas efference while not affecting vas afference. These results describe a novel physiological effect of secretin. The effect is explained by its vasodilation of vas efference, thus reducing the effective filtration pressure and hence diuresis. This unexpected finding suggests an intimate functional relationship between the gut and the kidney. It may serve to sustain sufficient volume provision in the digestive period during which volume needs to be re-directed into the intestine for digestive purposes.

OS 06-06

Muscle Ankyrin Repeat Protein 1 (MARP1) alters sarcomere structures in mammalian skeletal muscle via titin association

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Content

Question

The muscle ankyrin repeat protein (MARP) family is associated with titin in striated muscle and plays a role in force production, and muscle remodelling. Especially MARP1, is upregulated from trace levels after eccentric overload exercise, linking titin to actin to increase titin-based passive force at muscle lengths stretched above normal. To evaluate the ultrastructural consequences in sarcomeres to MARP1 upregulation, we used small-angle X-ray diffraction on mammalian skinned fiber bundles before and after recombinant MARP1 incubation.

Methods

Small-angle X-ray diffraction allows for measurements of sarcomere structures in functioning fibers at near-physiological conditions. Permeabilized fiber bundles were prepared from human anterior tibialis biopsies placed in a muscle mechanics rig within the X-ray apparatus. X-ray images were taken at various sarcomere lengths before and after incubation.

Results

MARP1 treatment in muscle fibers reduced the distance between myosin heads and thin filaments and increased the fiber's force production potential, often described as length-dependent activation (LDA). MARP1-mediated titin-thin filament interactions increase titin-based force after stretch, which is known to affect the properties of LDA. Therefore, one possible role of MARP1 in disease is potentially enhancing force production, perhaps as a compensation mechanism for force-deficient myopathic fibers.

Conclusions

To our knowledge, this is the first demonstration of length-dependent priming in passive fibers of human skeletal muscle, suggesting changes to Ca2+ sensitivity during contraction. Stretch of MARP1 incubated fibers further “primed” thick filament vs. control, which allows them to produce greater forces.

OS 07 | Renal: Calcium-/Phosphat Homeostasis

OS 07-01

The effect of intestinal depletion of Claudin-4 on paracellular phosphate absorption

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Content

Question

Intestinal phosphate absorption is in part mediated by the paracellular pathway. Claudin-4 is a main anion selective tight junction protein expressed along the whole murine intestinal tract. It has generally been described as barrier-forming, but its relation to the intestinal paracellular absorption of phosphate is not defined. Here, we generated intestine specific *Cldn4*-deficient mice and analyzed their baseline phenotype with regards to phosphate homeostasis.

Methods

Intestinal *Cldn4*-knockout (KO) mice and wildtype littermates received a standard phosphate diet. Faeces, urine, blood, intestinal segments and kidneys were collected. Measurements included urinary and plasma concentrations of phosphate and calcium, plasma levels of phosphate-regulating hormones and evaluation of trans- and paracellular phosphate transport across jejunum and ileum.

Results

While the urinary excretion of phosphate and calcium were similar between genotypes, *Cldn4*-KO mice showed increased paracellular phosphate transport in ileum but unaltered fluxes in jejunum. In the ileum, the active transcellular transport component tended to be lower in KOs, suggesting a compensatory response to the enhanced passive flux. *Cldn4* KOs also showed reduced plasma PTH levels compared to wildtypes.

Conclusions

Our data suggest a role of Claudin-4 in intestinal paracellular phosphate fluxes. Normal urinary phosphate excretion suggests compensatory mechanisms to maintain phosphate balance. A detailed analysis of other organs and factors involved in phosphate balance are required and on-going. Dietary challenges may further uncover the role of Claudin-4 in intestinal phosphate and mineral handling.

OS 07-02

The sodium phosphate cotransporter NaPi-IIb is expressed and upregulated in kidneys of patients with chronic kidneys disease.

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Content

Question

Renal phosphate reabsorption is mediated by NaPi-IIa, NaPi-IIc & Pit2 in the luminal brush border membrane of the proximal tubule. These proteins have been shown to have reduced expression in animal models of kidney disease. We demonstrated that NaPi-IIb is also expressed in the loop of Henle. Its mRNA expression is enhanced in rodent kidney injury models. However, expression of NaPi-IIb has not been characterized in humans and whether NaPi-transporters are regulated in human chronic kidney disease (CKD) is unknown.

Methods

We examined localization of NaPi-IIa/IIb/IIc in kidneys from healthy individuals and patients with diabetic nephropathy, Anti-GBM nephritis, and hypertensive nephropathy by immunofluorescence. Moreover, we analyzed transcriptome databases (ERCB, Neptune) from healthy individuals and CKD patients.

Results

In control kidneys NaPi-IIb staining was restricted to thin and thick limbs of the loop of Henle. NaPi-IIa/IIc were found in proximal tubule cells. In patient biopsies, NaPi-IIb was found in collecting duct and proximal tubule cells. The staining of NaPi-IIa/IIc was reduced in all CKD patients. Functional experiments in human kidneys are on-going.

The analysis of the ERCB and Neptune databases showed reduction of *SLC34A1* and *SLC34A3* mRNA and elevation of *SLC34A2* mRNA expression with loss of kidney function.

Conclusions

NaPi-IIb is highly upregulated in CKD patient kidneys and its expression spreads to proximal tubules and collecting ducts. The expression of NaPi-IIa and NaPi-IIc is reduced in CKD. These findings may have implications for the development and maintenance of hyperphosphatemia in CKD patients and may limit specific NaPi-IIa/NaPi-IIc-inhibitor use.

OS 07-03

Association of mineral stress with mortality in the geriatric population

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The author has objected to a publication of the abstract.

OS 07-04

Assessing the clinical relevance of *SCL34A1* and *SCL34A3* variants in kidney stone disease through biochemical parameters and in vitro functional studies

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Content

Phosphate homeostasis is mainly regulated by the action of *SLC34A1* and *SLC34A3* in the kidneys. Both are responsible for phosphate reabsorption and their function is mainly regulated by phosphate intake, parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), electrolyte levels, and acid-base balance. Biallelic variants in *SLC34A1* and *SLC34A3* causes perturbances in phosphate and calcium metabolism. Phosphate wasting stimulates calcitriol production increasing intestinal calcium absorption and resulting in symptomatic hypercalcemia with hypercalciuria. Eventually it leads to kidney stone disease.

The Swiss Kidney Stone Cohort (SKSC) is a multicenter longitudinal observational study including adult kidney stone formers and non-kidney stone formers. Whole exome-sequencing identified monoallelic *SLC34A1* and *SLC34A3* variants, that may be pathogenic increasing the predisposition to develop nephrocalcinosis and kidney stone disease. Selected functional and biochemical parameters (TmP/GFR, phosphate, PTH, FGF23, calcitriol in plasma and calcium to creatinine ratio in urine) from variants' carriers have been compared with the other SKSC participants and distinct differences were found for some variants. However, the wide range of phenotypic symptoms, is not directly indicative for the transporter functionality. Therefore, *in vitro* analysis is being conducted. Transport activity of the variants is

studied by heterologous expression in Xenopus laevis oocytes, while trafficking and subcellular localization will be defined through transient transfection in mammalian renal cells and confocal microscopy. Variants in *SLC34A1*, *SLC34A3*, are common in patients with renal phosphate wasting and kidney stones. Linking clinical data with protein function is clinically important and allows to further define the physiological role of these transporters.

OS 07-05

Assessing human renal phosphate transporters and their dependency on age and sex

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Content

Plasma phosphate (Pi) levels are tightly regulated mainly by the kidneys to mitigate risks such as cardiovascular and renal disease. Renal Pi reabsorption is mediated by Na+-dependent Pi cotransporters from the SLC34 family, NaPi-IIa and NaPi-IIc, with the SLC20 family likely playing a minor role. Most functional information on renal Pi reabsorption is derived from animal models, with only genetic studies in humans. Studying cotransporters' contribution to Pi transport and its age and sex dependency is critical to gain a better understanding of phosphate homeostasis in health and disease. We investigated renal Pi transport in adult and neonate human kidney.

Time-course experiments demonstrated the feasibility of measuring Pi transport in brush border membrane vesicles (BBMVs) prepared from frozen human kidneys from healthy donors. Further, concentration-response experiments with two pharmacological inhibitors, one for NaPi-IIa and a pan-inhibitor for SLC34 transporters, were conducted.

Pi uptake into human renal BBMV was Na+-dependent exhibiting the characteristic overshoot, due to fast Pi accumulation. Our data revealed near-linear Pi uptake up to 30 seconds across all experimental groups. Adults showed a significant Pi uptake inhibition using 100 mM of the two inhibitors while Na+-dependent leucine transport remained unaffected. In adults, the pan-inhibitor blocked only partially and differences are seen between both inhibitors.

Currently, protein and gene expression of SLC34 and SLC20 transporters in human renal cortices is being investigated. Pi uptake experiments in neonate renal BBMV with the two inhibitors will be conducted. Eventually, all experiments will be conducted with mouse kidneys to evaluate species differences.

OS 07-06

DUSP1 mediates anti-calcific effects of fisetin in vascular smooth muscle cells

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The author has objected to a publication of the abstract.

OS 08 | Vascular physiology and signalling

OS 08-01

Nuclear eNOS interacts with and S-nitrosates ADAR1 to modulate type I interferon signalling and endothelial function.

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Content

Aim: Nitric oxide (NO) generated by the endothelial NO synthase (eNOS) elicits its effects by interacting with heme-containing proteins or by protein S-nitrosation. eNOS is localized in different cell compartments i.e. at the cell membrane and in the Golgi apparatus as well as in the nucleus. However, the relevance of nuclear eNOS/NO signalling for vascular homeostasis remains poorly understood.

Methods and Results: Nuclear translocation of eNOS was stimulated with vascular endothelial growth factor. Co-immunoprecipitation coupled with proteomics revealed that nuclear eNOS interacted with double-stranded RNA-specific adenosine deaminase (ADAR1), which was also S-nitrosated. The knockdown of eNOS in endothelial cells resulted in an increase in double-stranded RNA (dsRNA) and altered ADAR1-mediated adenosine-to-inosine editing. The accumulation of dsRNA in cells lacking eNOS led to the activation of the type I interferon (IFN) signalling pathway and a marked downregulation of cell cycle-related genes. ADAR1 knockdown elicited similar effects. As a result, growth factor-stimulated cell proliferation was abrogated and basal as well as tumour necrosis factor- or H_2O_2-induced cell

death were increased. Similarly, endothelial dysfunction in mice and in patients with atherosclerosis was accompanied by the accumulation of dsRNA and the activation of the type I IFN signalling pathway. Preserving NO bioavailability *in vivo* fully prevented these effects.

Conclusions: Our findings uncover a novel mechanism linking nuclear eNOS-generated NO to ADAR1. Reduced NO bioavailability is directly linked to the activation of a type I IFN response in the endothelium, which contributes to atherogenesis.

OS 08-02

Endothelial cytochrome P450 reductase-derived cholesterol limits angiogenesis

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Content

The cytochrome P450 reductase (POR)/CYP51-monoxygenase is a redox system important for sterol synthesis. Cholesterol is determined by uptake and de novo synthesis. High circulating cholesterol is linked to cardiovascular diseases but the role of endogenous cholesterol synthesis for endothelial function, in contrast, is unknown. To inhibit cholesterol synthesis in endothelial cells, POR and CYP51 CRISPR knockout was performed in human aortic endothelial cells (HAEC) and in human umbilical vein endothelial cells (HUVEC). Furthermore, an endothelial-specific POR knockout mouse (ecPOR$^{-/-}$) was generated. Knockout of POR and CYP51 in HAEC led to an accumulation of cholesterol precursor lanosterol, whereas desmosterol was reduced. Functionally, knockout of POR was linked to an increased in angiogenic sprouting in HUVEC and aortic segments in ecPOR$^{-/-}$ mice. Importantly, increased angiogenesis was also observed in retina of ecPOR$^{-/-}$ mice. Cellular cholesterol levels are sensed by the SREBP2 (sterol regulatory element-binding proteins) system, and indeed, SREBP2 activation (cleavage and translocation to the nucleus) was increased after deletion of POR in cultured cells as well as *in vivo* (*en face* of aorta). Overexpression of the active, cleaved nuclear SREBP2 in cells increased angiogenesis similar to the knockout of POR and CYP51. RNAseq of POR$^{-/-}$ HAEC showed significant upregulation of cholesterol related genes LRP1, VLDLR and ABCG1, as well as pro-angiogenic genes such as VEGFA and ADM2. Remarkably, the complete set of genes from the cholesterol synthesis pathway was significantly downregulated. Altogether, inhibition of the endothelial POR/CYP51-axis impairs endogenous cholesterol production which correlates with the transcription of genes that promote angiogenesis.

OS 08-03

Pericyte-actions of Atrial Natriuretic Peptide foster reparative postischemic angiogenesis

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Content

Tissue repair after ischemia requires an angiogenic response. New capillaries are generated by proliferating endothelial cells (ECs) and pericytes in response to proangiogenic factors released by hypoxic cells. Unravelling this intercellular communication can help to find novel targets for proangiogenic therapies. The natriuretic peptides ANP and BNP are among the first “myokines” induced in cardiomyocytes and skeletal muscle satellite cells in response to hypoxia. Their shared guanylyl cyclase A (GC-A) receptor is expressed by microvascular ECs and pericytes and might mediate local effects.

To study whether the NP/GC-A/cGMP pathway stimulates angiogenesis, we (co)cultured EC and pericytes. ANP concentration-dependently stimulated the proliferation of both, ECs and pericytes. Moreover, in the presence of pericytes, ANP enhanced the proliferation of GC-A-deficient endothelia, suggesting the release of proangiogenic pericyte factors. To elucidate the relevance *in vivo*, we studied mice with pericyte-restricted GC-A deletion. Two weeks after experimental myocardial infarction, control mice had increased endothelial and pericyte densities in the border of the infarct zone, demonstrating an angiogenic response. Notably, this response was markedly blunted in pericyte GC-A KO mice, which was associated with augmented scarring. Corroborating this observation, vascular regeneration in response to critical hind limb ischemia was also severely impaired in such KO mice.

Our studies indicate that ANP and BNP, produced by satellite cells within skeletal muscle or by ventricular myocytes in response to hypoxia, exert local paracrine actions on neighboring pericytes, thereby stimulating a proangiogenic pericyte-to-endothelial communication. Our current studies are directed to elucidate the intra/extracellular mechanisms mediating these cell-cell crosstalks.

OS 08-04

Role of lipoma-preferred partner in the phenotype control of vascular smooth muscle cells

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Content

Question

Hypertension remains one of the main causes of death worldwide contributing to ischaemic heart disease, stroke, and renal pathologies. Chronically elevated blood pressure increases arterial wall stress, and lipoma preferred partner (LPP), a Lin11–Isl1–Mec3 (LIM)-domain protein, is involved in sensing and/or transducing this mechanical cue. LPP is primarily expressed by vascular smooth muscle cells (VSMCs) and seems to maintain the quiescent contractile phenotype of these cells in the face of hypertension, whereby its exact mechanism of action remains indistinct.

Methods and **Results**

In 2D and 3D models, primary aortic LPP-deficient (LPP-KO) VSMCs display higher rates of proliferation and motility but lower contractility compared to control cells. When exposed to cyclic stretch, LPP-KO VSMCs seem to adopt a proinflammatory expression profile. Own analyses of publicly available single-cell RNA sequencing data reveal that expression of LPP in patients with aortic aneurysms is significantly lower as compared to control subjects. Moreover, expression of LPP is significantly lower in human VSMCs with an activated synthetic phenotype as compared to cells with a quiescent contractile phenotype. When made hypertensive, LPP-deficient mice present with strong vascular pathologies, including aortic dissections as well as ruptures, that result in a high premature mortality amongst male but not female animals. Moreover, functional analyses of isolated perfused third order mesenteric artery segments of these mice show a significantly reduced myogenic response.

Conclusions

Overall, our data indicate an important role of LPP in VSMC phenotype control as well as in the adaptation of the vascular wall to increased wall stress.

OS 08-05

Pharmacological Gq protein inhibition prevents and reverses pulmonary hypertension

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Content

Gq proteins are key regulators of pulmonary vascular tone and critically involved in the main hallmarks of pulmonary hypertension (PH), i.e. excessive vasoconstriction and pulmonary vascular remodeling. Therefore, we investigated if the specific pan-Gq inhibitor FR900359 (FR) could be of therapeutic value for PH.

Pulmonary arterial tone was examined by several *ex vivo* measurements (wire-myography, precision-cut lung slices, isolated perfused lung) in mice, pigs and humans. Cell growth and migration were investigated in murine pulmonary artery smooth muscle cells (mPASMC). The effect of FR on pulmonary hemodynamics *in vivo* was determined by pressure catheter measurements in mouse and we also assessed prevention and reversal of PH by FR in a hypoxia-induced murine PH model.

We found a strong vasodilatory effect of FR on pulmonary arteries in wire-myograph measurements in pigs and mice that was similar or superior to relaxation by equal concentrations of currently used triple therapy. A strong vasorelaxation by FR we also detected in lung slices of humans and in the IPL model of mouse. FR reduced cell growth and migration of mPASMCs under PH conditions. Furthermore, FR acutely diminished right ventricular systolic pressure (RVSP) by about 13% at 20 min after *in vivo* application and chronic application of FR prevented and reversed RVSP increase, vessel remodeling and RV hypertrophy without obvious side effects in PH mice.

Thus, we show that the pharmacological Gq inhibitor FR targets main hallmarks of PH and could therefore be a promising therapeutic option for PH in the future.

OS 08-06

Targeting S1P-associated immune activation to lower blood pressure in Angiotensin II-associated hypertension.

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Content

Hypertension is the most common preventable risk factor for cardiovascular disease and the leading contributor to all-cause mortality. Although evidence of immune system involvement in hypertension development and progression emerged, no immune-targeting anti-hypertensive treatment is available. We previously showed that higher plasma levels of the bioactive phospholipid sphingosine-1-phosphate (S1P) associate with increments in blood pressure (BP) and correlate with biomarkers of inflammation in humans. Hypertension-associated plasma S1P elevation links to the S1P generating enzyme sphingosine kinase 2 (SphK2) and a higher frequency of circulating T-cells. The current study investigates the potential efficacy of therapeutic SphK2 inhibition to lowering elevated BP by attenuating hypertension-associated immune system activation. Upon established experimental hypertension (at four weeks after initiation of Angiotensin II (AngII) infusion), mice were subcutaneously injected with an SphK2 inhibitor (SphK2i) or vehicle for two weeks. SphK2i treatment resulted in BP normalization irrespective of plasma S1P levels or vascular responses. SphK2i-mediated BP lowering was associated with a reduction in circulating $CD4^+$ T-cell counts. Interestingly, SphK2i therapy did not elicit the same BP lowering effects in mice lacking T-cell receptor alpha, which have significantly lower circulating $CD4^+$ T-cells at baseline. Our data indicates that SphK2i-mediated BP normalization during AngII-induced hypertension is mainly mediated through immune-based mechanisms. Taken together, our work identified first evidence for SphK2i as promising target in hypertension therapy.

OS 09 | Blood & oxygen

OS 09-01

Roxadustat affects inflammatory response in human leukocytes

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Content

Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitors are used to treat anemia associated with chronic kidney disease. However, they have been shown to increase infection rates in treated patients. The effects of HIF stabilization on circulating leukocytes have been neglected thus far. This study investigates the effects of the prolyl hydroxylase inhibitor Roxadustat on human peripheral blood mononuclear cells (PBMCs).

Protein and gene expression of the HIF pathway were analyzed to confirm the activity of Roxadustat in PBMCs. In addition, its effect on cell viability was evaluated. Metabolic flux analyses were performed to determine the time-dependent oxygen consumption rate and extracellular acidification rate of living cells, which correlates with their metabolic function. To induce total HIF stimulation, we combined lipopolysaccharide-induced inflammation with Roxadustat treatment and measured protein and gene expression. Finally, single-cell RNA sequencing of PBMCs revealed the downstream molecular effectors targeted by Roxadustat-induced HIF stimulation.

The concentration of Roxadustat used did not cause cell death or increase longevity in PBMCs. Roxadustat resulted in the accumulation of HIF-1α protein, expression of HIF target genes, and a shift towards anaerobic energy production by glycolysis. Lipopolysaccharide treatment further enhanced the inflammatory response of PBMCs.

Prolyl hydroxylase inhibitors strongly activate the HIF-induced metabolic shift towards glycolysis in circulating leukocytes, indicating an activated state of the leukocytes. Therefore, it is important to consider the potential effects on leukocyte function to ensure the safety and usability of these drugs.

OS 09-02

Secreted modular calcium binding protein 1 is required for TGF-b-mediated resolution of inflammation

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Content

Macrophages play an essential role in inflammation, and their repolarization to pro-resolving phenotype is a prerequisite for its resolution. Transforming growth factor (TGF)-β plays a major role in driving the latter process. Given

that secreted modular calcium binding protein (SMOC1) modulates TGF-β signalling, the aim of this study was to determine the impact of SMOC1 on inflammation and its resolution. A single dose of streptozotocin (STZ) was used to induce pancreatic inflammation in wild-type and SMOC1$^{+/-}$ littermates. Wild-type mice demonstrated a mild inflammatory response but most SMOC1$^{+/-}$ died within 4 days. In the absence of SMOC1 there was a massive pancreatic infiltration of monocytes (Ly6C^{+}) as well as T cells (CD4^{+} and CD8^{+}) and a concomitant accelerated loss of β and δ cells. Providing recombinant SMOC1 via minipumps rescued the phenotype and promoted islet survival. In isolated TGF-β repolarized macrophages, SMOC1 deletion accelerated phagocytosis. Actively phahocytosing cells were then subjected to RNA-sequencing and proteomics. While SMOC1-expressing macrophages showed an overrepresentation of genes related to endocytosis and phagosomal degradation, genes related to inflammatory pathways (TNF-α, IL-17 and NF-kβ) dominated in SMOC1-deficient cells. Moreover, macrophages from SMOC1$^{+/-}$ mice lacked several key proteins involved in lysosomal maturation as well as function, and failed to form functional phagolysosomes. In conclusion, SMOC1 is essential for the resolution of inflammation by promoting the lysosomal degradation of phagocytosed material. The failure to clear cell debris aggravates the inflammatory response resulting in T cell recruitment and islet cell death.

OS 09-03

The organic cation transporter 3 (OCT3) mediates the transport of histamine in basophilic granulocytes

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Content

The organic cation transporter 3 (OCT3) is a polyspecific membrane transporter, which transports organic cations and is inhibited by corticosterone (Cort.). Histamine, an important mediator secreted by granulocytes during allergy, is an endogenous OCT3 substrate. Since a specific histamine transporter has not yet been identified, we investigated whether OCT3 is involved in histamine release by basophilc granulocytes. To do this, a human basophilic cell line (KU812), bone marrow cells (BMC) and sera from wild type mice (WT) and mice with genetic deletion of OCT3 (OCT3$^{-/-}$) were used. Histamine release from KU812 and BMC was induced by anti-IgE after incubation with IL-3/IgE. BMC were subjected to RNAseq and metabolomic analysis. WT and OCT3$^{-/-}$ sera were compared by metabolomic analysis. Histamine release in KU812 cell line was significantly reduced by Cort. co-incubation (-33±19 %, mean±SEM, n=5). Interestingly, serum histamine concentration in female mice were found to be higher than in male animals. Histamine release by BMC from female WT mice were lower than that from male animals (85±5, vs 69±2 ng histamine/mg protein, mean±SEM, everywhere n=6). Stimulation of histamine release in BMC increased expression of metabolites belonging to the histidine metabolic pathway, the precursor of histamine. RNAseq analysis of BMC from WT and OCT3$^{-/-}$ mice

showed no significant differences in gene regulation of other known histamine transporter and other important genes for histamine metabolism. Taken together, these results suggest that OCT3 mediates a part of histamine release by basophils and may be the long-sought histamine transporter in granulocytes. Supported by DFG (CI107/14-1).

OS 09-05

Targeted inhibition of hypoxia-inducible factors in a model of age-related macular degeneration protects from cell death and metabolic dysregulation

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Content

Age-related macular degeneration (AMD) is a progressive degenerative disease of photoreceptors located in the macula of the retina leading to loss of central, high acuity vision. In industrial countries, AMD represents the leading cause of vision loss in the elderly population. Dry AMD, the predominant subtype of late-stage AMD, lacks effective treatment options. Therefore, novel treatment options are needed that prevent irreversible damage to photoreceptors. Major hallmarks of dry AMD are hypoxia and oxidative stress in retinal pigment epithelium (RPE), a monolayer of pigmented cells located adjacent to the photoreceptors. To mimic the pathophysiology of dry AMD, we established a novel combination model by exposing RPE cells to oxidative stress under hypoxic conditions.

This combination potentiated oxidative damage and induced cell death by ferroptosis, an iron-dependent cell death mode by modulating iron and redox homeostasis. Moreover, we observed metabolic dysregulation in this combination model, characteristic of AMD. Hypoxia leads to an accumulation of hypoxia-inducible factors (HIFs), notably HIF-1 and HIF-2, which are dimeric transcription factors pivotal in cellular adaptation to hypoxia. Silencing HIF-α subunits elucidated distinct roles: HIF-1 exacerbated oxidative damage and ferroptosis by modulating iron homeostasis, while HIF-2 protected RPE cells from cell death. Furthermore, HIF-1α knockdown ameliorated metabolic perturbations. Based on these findings, we identified small molecule inhibitors that protected RPE cells from damage induced by the combined induction of oxidative stress and hypoxia.

Taken together, targeting HIF-1 emerges as a promising avenue for novel dry AMD treatment approaches, offering potential to mitigate disease progression and preserve vision.

OS 09-06

Androglobin, a chimeric mammalian globin, is associated with ciliogenesis and sonic hedgehog signaling

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The author has objected to a publication of the abstract.

POSTER SESSION A

A 01 | Cardiac calcium and disease models

A 01-01

Functional and downstream signaling effects of protein kinase C (PKC) activation on rabbit and human myocardium in vitro

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Content

Question

Effects of PKC upregulation on myocardial contractility in heart failure remain unclear. Here, we investigate the functional and downstream signaling effects of PKC activation in rabbit and human myocardial slices

Methods

Contraction forces (CF) of left ventricular human (n=5) and rabbit (n=15) myocardial slices cultured under permanent electrical stimulation with/out 24h PMA treatment (human: 10-50nM after 9-15d; rabbit: 50nM at 2d) were measured. Rabbit slices (n=5) were exposed to transcription (2mM Actinomycin D) and translation (20mg/ml cycloheximide) inhibitors (2h), followed by PMA treatment (24h). PKC-specific phosphorylations of PKD at ser744-748, troponin I (TPNI) at S23/24 and T143 were analyzed via Western blotting after 30min, 90min, 150min and 24h PMA treatment. Ca^{2+} sensitivity in rabbit slices (n=4-5; control vs PMA(24h)) was assessed. Maximum contraction force (CF_{max}) was tested by addition of 100nM isoprenaline.

Results

PMA reduced CF of human (-80%) and rabbit (-33%) cardiac slices, while no changes were observed in controls ($p<0.01$). PMA enhanced phosphorylations of ser744-748 of PKD (2.2-fold) and T-143 of TPNI (4.4-fold) 24h after

treatment. TPNI-T-143 phosphorylation was already increased 30min (1.5-fold) and 90min (2.4-fold) after PMA addition. Actinomycin D and cycloheximide did not alter PMA-induced effects on CF and TPNI-T-143 phosphorylation. PMA reduced Ca^{2+} sensitivity in rabbit slices by 0.16 pCa units ($p<0.05$), but not $F_{c,max}$.

Conclusions

PKC activation hampers contractility of rabbit and human myocardium, presumably via phosphorylation of TPNI-T-143, thereby, reducing Ca^{2+} sensitivity. This occurs also in the presence of transcription and translation inhibitors, suggesting a direct PKC-TPNI interaction not requiring gene expression.

A 01-02

Acute effects of tunicamycin on cardiac calcium regulation and signaling pathways associated with endoplasmic/sarcoplasmic reticulum stress

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Content

Question

In cardiomyocytes, the endoplasmic/sarcoplasmic reticulum (ER/SR) is a key organelle for both calcium regulation and protein homeostasis. Disturbances of protein modification and folding lead to ER/SR stress, but whether this also affects SR calcium regulation remains elusive. Tunicamycin is a known inducer of ER/SR stress. We tested the hypothesis that acute exposure of cardiomyocytes to tunicamycin disturbs SR calcium regulation and activates signaling pathways associated with ER/SR stress.

Methods

Ventricular myocytes were isolated from adult rat hearts. Confocal calcium imaging was performed in electrically-stimulated myocytes loaded with Fluo-4/AM. Expression and phosphorylation of proteins was determined by immunoblotting in left ventricular (LV) tissue homogenates from Langendorff-perfused rat hearts. Myocytes or hearts were treated with 10µM tunicamycin for 10-15 minutes.

Results

Ventricular myocytes exhibited greatly disturbed calcium handling within minutes after tunicamycin exposure consisting of elevated diastolic calcium and pro-arrhythmic calcium release including calcium waves and subcellular calcium alternans. In LV tissue from Langendorff-perfused hearts treated with tunicamycin for 15 minutes, however, we did not observe any significant changes in expression or phosphorylation of major calcium-regulating proteins, including Cav1.2, RyR2, SERCA2 (N=8 for tunicamycin vs N=8 DMSO controls). Likewise, we did not find any changes in expression or phosphorylation of proteins involved in ER/SR stress, unfolded protein response or mTORC1 signaling: BiP/GRP78, eIF2a, Akt, ribosomal S6.

Conclusion

Tunicamycin acutely disturbs SR calcium regulation in ventricular myocytes, but this appears to occur in the absence of altered phosphorylation of major calcium-regulating proteins or activation of some signaling pathways associated with ER/SR stress.

A 01-03

Cardiac glia among the autonomic innervation: characterization of myelinating and non-myelinating Schwann cells in the heart

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Content

Question

Cardiac activities are under the control of the autonomic nervous system. Evidence suggests that glia associated with autonomic innervation participate in the regulation of cardiac functions. Schwann cells are known for their critical role in supporting axons in the peripheral nervous system. Therefore, we investigated the presence of myelinating and non-myelinating Schwann cells in human and murine hearts.

Methods

Human left ventricular heart samples were obtained from patients receiving a heart transplantation. Adult C57BL/6 mice of both sexes were used. We investigated ventricular cross sections from humans and mice hearts, as well as murine epicardium and whole hearts by staining with FluoroMyelin and immunohistochemistry for the glial marker S100B and the sympathetic nerve marker tyrosine hydroxylase (TH).

Results

While few FluoroMyelin-positive, epicardiac nerves were detected in human left ventricular epicardium, most of the sympathetic nerve fibers were myelin-negative. No Fluoromyelin-staining was found within the working myocardium. On the other hand, non-myelinating Schwann cells were detected abundantly throughout all layers of the heart and in epicardial nerve fibers. In the murine heart, no FluoroMyelin-positive nerve fibers were found, despite the presence of non-myelinating Schwann cells and nerve fibers within the epicardium.

Conclusions

Our data suggest that myelinating Schwann cells are present in larger nerve fibers in the epicardium of human hearts, but not in murine hearts, where all Schwann cells are non-myelinating. This indicates that species-differences should

be considered when studying cardiac glia. As glia have an emerging role in the autonomic control of the heart, further studies are needed.

A 01-04

Cardiac function of the living mouse after specific, graded cleavage of cardiac titin

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Content

Question

To detect the effects of titin stiffness loss on living heart function, we used a mouse model that allows specific cleavage of the titin springs in vivo.

Methods

In this titin cleavage (TC) mouse, a tobacco etch virus protease (TEVp) recognition site is cloned into elastic titin. Titin cleavage was performed by overexpressing (under a cTnT-promoter) AAV9-TEVp plasmid in the heart; AAV9-eGFP served as a control.

Results

Two weeks after AAV9-TEVp injection, 48.3±3.4% (N=5) of titin were cleaved in homozygous TC-mice. Cardiac MRI analyses of mice injected with AAV9-TEVp revealed significantly reduced left ventricular internal diameters in diastole and systole, cardiac output, and systolic and diastolic volume, compared to AAV9-eGFP (N=3) injected mice, which began to manifest 6 days post-injection and were aggravated 13 days post-injection. Systolic septum diameter was significantly increased 6 days post-AAV9-TEVp-injection, but the outer diameter of the heart remained unaltered. Cardiac function was reasonably well compensated until more than ~30% of titin became cleaved. Protein analyses of heart tissue demonstrated progressive ubiquitination of cleaved titin and activation of autophagy between 6 and 13 days post-injection. Histological staining illustrated a 6-fold increase in fibrosis two weeks post-injection. Microscopic analyses of cardiomyocytes revealed degenerated areas and accumulation of aggregates in titin-cleaved mice; sarcomeres with missing I-bands, thinned diameter, and wavy Z-discs were observed.

Conclusions

These findings demonstrate the important role of titin stiffness in cardiac contractility. With cleavage of more than 40% of elastic titin, the heart grows concentrically and cannot maintain its function under baseline conditions.

A 01-05

CHARGE-BALANCING OPTIMIZES ELECTROCHEMICAL COMPATIBILITY OF BIPHASIC TISSUE STIMULATION

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Content

Aim: The benefit of continuous electrical stimulation of excitable cells and tissues in long-term culture becomes more and more evident. However, electrical field stimulation is inevitably associated with electrode reactions, implying ill-defined redox reactions of all medium constituents. With the aim to optimize the continuous stimulation of long-term cultured myocardial tissues, we developed a convenient method for the quantification of the electrochemical reactivity of biphasic current-controlled impulses, and explored technical implementations of charge balancing.
Methods: Electrochemical compatibility was tested by exposing a phenol red solution (20mg/l in PBS) to biphasic impulses (3ms charge and discharge, 50mA) at a rate of 4Hz via graphite electrodes. Charges of positive or negative polarities were modulated by variation of the discharge duration, or balanced by capacitive electrode coupling or by feedback regulation of electrode polarisation. Redox reactions of phenol red were measured photometrically.
Results: Impulses with technically equivalent (balanced) positive and negative charges maintained phenol red absorbance at 74% after 24h. Dysbalance of charges (up to 2%) aggravated phenol red degradation to a residual fraction of 13%. The consequence of dysbalance was completely prevented by a serially integrated capacitor. Prolongation of decharge duration to 9ms (17mA current) improved phenol red stability to 85%. Automatic adaptation of decharge duration aiming at a pre-pulse electrode potential of 150mV further enhanced phenol red stability to 90%.
Conclusion: Phenol red sensitively detects electrochemical compatibility of stimulation impulses. Optimum implementation of charge balancing may greatly preserve chronically stimulated cells *in vitro*.

A 01-06

Optogenetic control of G_i signaling using long wavelength-sensitive opsin enables induction of atrial fibrillation in mice

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Content

Atrial fibrillation (AF) is a prevalent cardiac arrhythmia primarily caused by irregular electrical activity due to a reentrant mechanism. The latter is promoted by heterogeneous refractoriness which could be due to vagal G_i signaling activating G protein-coupled inwardly rectifying potassium channels (GIRK).

To investigate the spatial and temporal requirement of GIRK activation for AF by optogenetic G_i stimulation, we generated a novel flox mouse line for αMHC-Cre-dependent heart-specific expression of the G_i-coupled, human long-wavelength-sensitive Cone opsin (hLWO). Localized illumination of Langendorff perfused hearts from hLWO mice was performed with a macroscope and action potential duration (APD) was measured with a sharp microelectrode impaled in the right atrium. Illumination of the right atrium with red light (625 nm, 44 µW/mm^2) lead to a sustained decrease in APD of 44.9% similar to 40.3% APD reduction by the M_2-R agonist Carbachol (10 µM). Using different pulse durations and light intensities, we determined the half maximal light dose (4.9 µW/mm^2) and optimal pulse repetition for sustained APD decrease with only minor deactivation. To induce AF, we reduced coupling with the gap junction blocker Carbenoxolone (30 µM) and applied electrical burst stimulation. In control conditions, AF was not observed, however Carbachol (10 µM) or illumination of both atria (44 µW/mm^2) enabled AF induction, which terminated shortly after stop of illumination.

Thus, we enabled optogenetic control of G_i signaling in the atrium by hLWO, which will be useful to investigate the timing, location and sensitivity of G_i-GIRK dependent AF induction in the heart.

A 01-07

Investigating Electrophysiological Features of Right Ventricular Tissue Slices from Patients with Tetralogy of Fallot

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Content

Background: Patients withTetralogy of Fallot (ToF) are at increased risk for life-threatening arrhythmias, even after successful surgical repair. One potential cause may be persistent structural remodelling of the right ventricular myocardium even in regions unaffected by post-surgery scars. Here, increased fibrosis and altered cellular coupling via connexin-43 (Cx43) may interfere with electrical conduction and increase arrhythmia susceptibility.

Methods: We use live tissue slices of right ventricular myocardium, excised as part of clinically necessary practice from patients with ToF during repair or re-operation. We investigate conduction velocity, arrhythmia susceptibility, and action potential shape by visualising spatially resolved transmembrane voltage dynamics using optical mapping during electrical pacing at different stimulation frequencies and arrhythmia induction protocols. Immunohistochemistry and confocal microscopy on adjacent slices from the same samples provides three-dimensional structural information on the extent of fibrosis, presence of various cardiac cell types (cardiomyocytes, fibroblasts, endothelial cells, immune cells), Cx43 distribution, and Cx43-mediated cellular coupling.

Results and conclusion: Our pilot data confirm the plausibility of living human myocardial slices as a model to investigate the role of fibrosis in arrhythmogenesis in patients with ToF.

A 01-08

Automated patch-clamp assessment of cardiac chamber-specific electrophysiology in induced pluripotent stem cell derived cardiomyocytes

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Content

Atrial fibrillation (AF) is the most common cardiac arrhythmia reported in the clinics. Current AF therapeutics lack efficacy, and mechanistic strategies to examine ion channel remodelling in AF are limited by a lack of atrial specificity in expression systems or low throughput methods. Much progress has been made in the development of automated patch-clamp (APC) systems that allow for high throughput electrophysiological measurements. APC could therefore be a useful tool for increasing the throughput of electrical investigations into AF mechanisms.

We describe the application of the high throughput APC device (SyncroPatch 384) to deeply characterise key ionic currents and action potentials (AP) in human atrial- and ventricular specific iPSC-derived cardiomyocytes (iPSC-CM). Typical subtype-specific electrophysiological characteristics we observed including a shorter AP, smaller peak sodium current (I_{Na}), smaller L-type calcium current ($I_{Ca,L}$), and smaller inward rectifier potassium current (I_{K1}) in atrial cells compared to ventricular. In addition, activation of the atrial-specific acetylcholine-activated inward rectifier potassium current ($I_{K,ACh}$) was exclusively observed in atrial but not in ventricular iPSC-CM following application of the M-receptor agonist carbachol.

The successful application of a high throughput APC-system for the recording and characterization of atrial and ventricular APs and ionic currents in highly scalable iPSC-CM models implies that APC represents a very useful tool for future studies of AF mechanisms and may substantially impact AF-related drug development programs.

A 01-09

TISSUE INTEGRATION OF STEM CELL-DERIVED CARDIAC PROGENITOR CELLS: *IN VITRO* STUDIES IN LONG-TERM CULTIVATED SLICES OF HUMAN FAILING MYOCARDIUM

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Content

Background: Cardiac regeneration may be achieved by the integration of stem cell-derived cardiomyocytes with human failing myocardium. Human cardiac progenitor cells (CPCs) have the potential to differentiate into ventricular myocytes, and their engraftment in the human failing myocardium may enhance myocardial contractility and improve myocardial plasticity.

Methods: CPCs were derived from embryonic stem cells with a cardiac directed differentiation protocol. Human myocardium was acquired from explanted hearts of transplant recipients, and was cultured as living thin slices under biomimetic stimulation and continuous force measurements. CPCs were seeded on the surface of myocardial slices and were co-cultured for 5 weeks. Cardiac function assessments were performed daily. Morphological maturation and integration were characterized by no-stain labeling (eGFP and second-harmonic generation) and immunohistology.

Results: After 5 weeks of co-culture, we observed that CPCs autonomously migrated and uniformly distributed into deep layers of the myocardium, and expressed morphological characteristics of cardiomyocytes (positive for α-actinin and connexin 43) during co-culture. In comparison to plain myocardial slices, functional changes included an increased force development (+75.6%±22.4%), changes in force temporal kinetics, a more negative force-frequency relation (at beating rates >120bpm), increased maximum capture rate (+42.9%±20.5%), enhanced frequency-dependent acceleration of relaxation (at beating rates >80bpm), weakened post-pause potentiation (-16.1%±4.5%), but no change of refractory period in CPC-cocultured human myocardial slices.

Conclusion: CPCs differentiate into more mature cardiomyocytes and self-integrate with human failing myocardium during long-term co-culture. Integration promotes contractility and affects excitation-contraction coupling, possibly indicating cross-talk among CPCs and the various myocardial cell populations.

A 01-10

Contractile properties of human induced pluripotent stem cell-derived cardiomyocytes can be altered by cryopreservation

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Content

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a favorable *in vitro* model to study cardiac diseases. Therefore, well-characterized and standardized batches of hiPSC-CMs are required. However, large heterogeneity between differentiations is often observed. To reduce experimental variability, cryopreservation of differentiated hiPSC-CMs could help, but might also affect gene expression or functional parameters. Therefore, we asked: Do cryopreservation media CS10 and KSR influence hiPSC-CMs characteristics?

hiPSC-CMs were used freshly or after at least three month of cryopreservation before checking recovery rate and sarcomeric protein expression by immunofluorescence analysis. Furthermore, transcriptional activity of sarcomeric genes was analyzed by RNA-FISH and the influence on contraction parameters by video-based edge detection.

Recovery rate of hiPSC-CMs after thawing was on average 46% for KSR and 38% for CS10. In freshly cultivated hiPSC-CMs, cardiomyocyte fraction was 81%, which remained similar in KSR-frozen cells (84%) but was substantially reduced to 61% in CS10-frozen cells. Freezing revealed no crucial alterations of transcriptional activity of *MYH6*, *MYH7*, *MYBPC3* and *TNNI3*. Only minor changes were detectable at protein level. By analyzing twitch parameters at day 10 of cultivation, we found no changes for KSR-frozen vs. fresh CMs. Yet, in CS10-frozen CMs half-relaxation time was significantly reduced and remained low upon longer cultivation (d35).We conclude that freezing can affect contractile function of hiPSC-CMs in medium-dependent cryopreservation. This alteration is not caused by changes in myosin isoform, *MYBPC3* or *TNNI3* expression. Therefore, application of fresh and frozen hiPSC-CMs for functional studies requires appropriate controls to correct for potential cryopreservation-induced alterations

A 01-11

Impact of Replating on Stem Cell-Derived Cardiomyocyte Function and Myosin Heavy Chain Expression

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Content

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are a valuable tool for cardiac research with possible implications for therapeutic approaches. β-myosin heavy chain (MyHC) is an important marker of adult human ventricular CMs and a main determinant of cardiac contractile properties. hPSC-CMs can be directed towards predominant β-MyHC expression e.g. by long-term culture on a stiff matrix. We investigated the impact of disrupting the cell-extracellular matrix (ECM) connection by enzymatically detaching and replating hPSC-CMs after long-term culture, which is commonly performed for various applications. Following replating, crossbridge cycling kinetics of myofibrils measured in a micromechanical setup were accelerated compared to myofibrils from non-replated CMs. Accordingly, a significant increase of α-MyHC, which has a higher ATPase activity than β-MyHC, and of the corresponding *MYH6* mRNA expression was observed in replated hPSC-CMs. Functional and *MYH*/MyHC expression changes were reversed within the second week after replating. As determined by RNA-Seq-based gene enrichment analyses, the shift in *MYH*/MyHC expression was associated with alterations in mechanosensitive signaling pathways, particularly those involving integrin- and downstream focal adhesion kinase (FAK) signaling. In line, FAK inhibition led to decreased β-MyHC expression on a stiff matrix, providing evidence for a critical role of FAK in the regulation of MyHC isoform expression. These findings underline the relevance of ECM alterations for the phenotype of hPSC-CMs. Replating-induced changes in MyHC expression and its effects on cellular function are important to consider for downstream applications when culturing hPSC-CMs, as they may influence experimental outcomes.

A 02 | Ion channels (voltage-gated)

A 02-01

Electrochemical coupling at the plasma membrane by mammalian voltage sensitive phosphatase

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The author has objected to a publication of the abstract.

A 02-02

Single-channel conductance of mouse pacemaker channels mHCN1 to mHCN4 is tiny and specific

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Content

HCN channels are tetramers that evoke rhythmic electrical activity in specialized neurons and cardiac cells. They are activated by hyperpolarizing voltage, and the activation can be boostered by the second messenger cAMP. For HCN2 channels, the unitary conductance γ of ~1.5 pS has been determined before and the cAMP effect has been demonstrated. In contrast, the elementary functional properties of the isoforms HCN1, HCN3 and HCN4 are missing. We expressed all four mouse HCN isoforms in *Xenopus* oocytes and performed single-channel experiments at optimized low-noise conditions.

We show that γ is tiny for all 4 mHCN isoforms and specific: γ_{mHCN2}=1.52 pS confirmed previous results. γ_{mHCN1} of the most rapidly activating mHCN1 channel was observed as 0.84 pS, i.e. only about half as big as γ_{mHCN2}. The slowly activating mHCN3 and mHCN4 isoforms generated an even smaller unitary conductance of γ_{mHCN3}=0.55 pS and γ_{mHCN4}=0.51 pS, respectively. For comparison, we determined also for the human isoform of hHCN4 the unitary conductance γ_{hHCN4}=0.56 pS which is indistinguishable from the value of mHCN4 and mHCN3. All single-channel currents showed the characteristic slow activation by hyperpolarizing voltage. To further validate these exceptionally small unitary currents, we demonstrated for all of them that activation is accelerated by the agonist cAMP, which contrasts previous results for mHCN3. In conclusion, the specificity of the single-channel conductance of the four members of the HCN channel family opens a new window to better understand activation and permeation of these relevant channels and, also, paves the way to develop channel-specific drugs.

A 02-03

Modification of human Kv4 channels by KChIP1 splice variants

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Content

Voltage-dependent potassium (Kv) channels of the Kv4 subfamily mediate a rapidly inactivating somatodendritic A-type current, which controls dendritic excitation and synaptic plasticity. Kv4 channels in the brain are thought to form ternary complexes with two types of auxiliary beta-subunits, DPPs and KChIPs, which come in numerous splice variants and differentially modulate channel surface expression and gating. Notably, all DPP and most KChIP splice variants accelerate the recovery of Kv4 channels from inactivation. Only KChIP2e and KChIP1b seem to represent exceptions of this rule. In particular, KChIP1b has been reported to induce a second, extremely slow recovery component in Kv4.2 channels, however, this effect has neither been tested for other Kv4 channel subtypes nor in a ternary configuration (Kv4 + DPP + KChIP1). Utilizing two-electrode voltage-clamp in the *Xenopus* oocyte expression system we performed a comprehensive and detailed analysis of the modulatory effects of KChIP1a and KChIP1b on all Kv4 channel subtypes (Kv4.1, Kv4.2, Kv4.3S and Kv4.3L) in both binary (Kv4 + KChIP1) and ternary (Kv4 + DPP6 + KChIP1) channel configurations. We find biphasic recovery kinetics with an extremely slow component for both KChIP1a and KChIP1b in all Kv4 channel subtypes, which persist in the presence of DPP6. With longer (40 ms - 10 s) and less depolarized inactivating pulses (between +70 and -70 mV) the relative amplitude of the slow component of recovery from inactivation increases from 40 up to 100%, indicating a highly dynamic time- and voltage-dependent control of A-type current availability to be further elucidated mechanistically.

A 02-04

Gating-modifying *CACNA1D* (Cav1.3)missense variants lead to diverse clinical phenotypes

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Content

Question

Voltage-gated Cav1.3 Ca^{2+} channels (*CACNA1D*) mediate several physiological processes. Human homozygous *CACNA1D* loss-of-function (LOF) causes bradycardia and deafness (SANDD syndrome) whereas *de novo* (heterozygous) missense variants induce a neurodevelopmental disorder (NDD). Herein, we functionally characterize

variants causing SANDD (A376V) or NDD (V1447L and C1436X) to investigate whether distinct gating changes account for the symptomatic variability.

Methods

Whole-cell voltage-clamp recordings were performed in tsA201-cells transfected with full-length wild-type (WT), A376V, V1447L or C1436X (truncated channel lacking the C-terminus) human Cav1.3 α_1-subunit co-transfected with β_3, $\alpha_2\delta_1$ and EGFP.

Results

Cells expressing the V1447L variant (alone/co-expressed with WT) revealed a hyperpolarising shift in the half-maximal activation/inactivation voltage by ~-11 mV, in line with other NDD-associated variants. A376V also showed similar complex gating changes, including increased current density, activation/inactivation at more negative potentials (by ~-7 mV), and faster inactivation kinetics. Lastly, C1436X alone resulted in a non-conducting channel. However, C1436X co-expressed with WT induced a hyperpolarising shift of the voltage-dependent activation by ~-7 mV and faster inactivation kinetics.

Conclusions

We demonstrate the pathogenicity of the V1447L variant, and reveal a dominant effect on WT channels when co-expressed. Surprisingly, A376V is the first complex gating-modifier implicated in a clinical LOF phenotype. Finally, C1436X co-expressed with WT channels (as in the patient) induces typical NDD-associated gating changes. Compared to other NDD variants, V1447L and C1436X cause rather weak functional changes, in line with the milder clinical phenotypes (autism-spectrum-disorder with or without epilepsy, respectively). Increasing the number of studied variants/patients will help to establish genotype-phenotype correlations.

A 02-05

The biphasic effect of hydrogen peroxide on the voltage gated K^+-channel Kv1.5 expressed in *Xenopus*oocytes

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Content

Voltage-gated K^+-channels (Kv-channels) expressed in pulmonary arterial smooth muscle cells are known to be involved in the initiation of hypoxic pulmonary vasoconstriction (HPV). There is evidence that a hypoxia-induced release of hydrogen peroxide (H_2O_2) induces Kv-channel inhibition and subsequently triggers HPV. However, conflicting results exist with regard to whether H_2O_2 inhibits or activates Kv-channels. Therefore, we hypothesized that H_2O_2 might affect the activity of Kv-channels in a dose-dependent manner.

In order to test this hypothesis, cRNAs for the murine Kv1.5 α-subunit and two isoforms of the auxiliary Kvβ-subunits (Kvβ1.1, Kvβ1.4) were generated via *in vitro* transcription before being injected into *Xenopus laevis* oocytes for heterologous expression. 24 hours post injection, transmembrane currents of either homomeric (Kv1.5) or heteromeric

(Kv1.5/Kvβ1.1 or Kv1.5/Kvβ1.4) channels were assessed by Two-Electrode-Voltage Clamp. H_2O_2 was freshly added to the perfusate before starting each recording.
As result, low H_2O_2 concentrations significantly inhibited not only homomeric, but also heteromeric channels at a holding potential of +50 mV. Interestingly, this inhibition was also observed at -20 mV, which is in the range of the physiological membrane potential in PASMCs. However, the currents inhibition was observed from lower concentrations only in heteromeric channels, indicating that the Kvβ-subunits might sensitize the channels to H_2O_2. In contrast, higher H_2O_2 concentrations induced a significant activation of the different Kv-channels.
These results suggest that the activity of murine Kv-channels is dose-dependently affected by H_2O_2 in *Xenopus* oocytes, thereby delivering an explanation for the conflicting results described in the literature.

A 02-06

Functional characterization of *KCND2* missense variants affecting the Kv4.2 pore domain

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Content

Voltage-gated potassium (Kv) channels are composed of four alpha-subunits, each with six transmembrane segments, surrounding a central permeation pathway. The so-called pore loop, located between transmembrane segments 5 and 6, harbours specialized domains which control K^+ selectivity and conductivity in the tetramer. Kv channel alpha-subunits are encoded by many different genes, and in numerous cases Kv channel dysfunction has been shown to be associated with genetic diseases. Kv4.2 is highly expressed in the brain and mediates a rapidly inactivating somatodendritic A-type current controlling dendritic excitation and synaptic plasticity. Heterozygous patients with *KCND2* variants causing a single amino acid substitution in the Kv4.2 selectivity filter or pore helix clinically present mainly with developmental delays, sometimes combined with epilepsy. We have investigated whether and how *KCND2* missense variants affecting the pore loop modulate Kv4.2 channel function. For this purpose the corresponding *KCND2* variants were functionally characterized under two-electrode voltage-clamp in RNA-injected *Xenopus* oocytes and with patch-clamp experiments in transfected CHO cells. Selectivity filter variants do not express detectable currents. With equal amounts of wild-type and mutant cRNA the mean current amplitudes are less than 1/16 (6.25%) compared to Kv4.2 wild-type alone, indicative of a dominant negative effect, which allows only wild-type homomers to conduct. A pore helix variant, on the other side, does mediate A-type currents but with incomplete inactivation. The data suggest that both loss and gain-of-function variants of *KCND2* can critically influence brain function and cognitive development.

A 02-07

Influence of Kv8 subunits on Kv7 channels: Molecular determinants and functional implications

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The author has objected to a publication of the abstract.

A 02-08

Functional characterization of *KCND1* variants identifies new disease gene

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Content

We identified a cohort of individuals with hemizygous variants of *KCND1*, including two *de novo* missense variants, three maternally inherited protein-truncating variants and 12 maternally inherited missense variants. Affected subjects present with a variable neurodevelopmental disorder. *KCND1* encodes the alpha-subunit of Kv4.1 voltage-gated potassium channels, known to be involved in the control of low frequency repetitive firing. Variant-associated amino acid exchanges affect the cytoplasmatic N- or C-Terminus, except for two exchanges occurring in transmembrane segments 1 and 4, of Kv4.1. Functional assessment in the absence and presence of auxiliary beta-subunits KChIP2b and DPP6s was performed under two-electrode voltage-clamp in RNA-injected *Xenopus* oocytes. Analyzed parameters involved peak current amplitude, current decay kinetics, recovery from inactivation as well as the voltage dependences of activation and steady-state inactivation. Variant-specific alterations of biophysical channel properties were diverse and varied in magnitude. Significant differences compared to Kv4.1 wild-type in various channel configurations (Kv4.1 alone, Kv4.1 + KChIP2b, Kv4.1 + DPP6s and Kv4.1 + KChIP2b + DPP6s), and the absence of typical beta-subunit co-expression effects were quantified. Based on the resultant numbers a scoring system was established to define the PS3 criterion for the American College of Medical Genetics and Genomics (ACMG) classification. Combining genetic and biophysical data analysis we show that Kv4.1 channel dysfunction is involved in the pathogenesis of an X-linked neurodevelopmental disorder associated with a variable neuropsychiatric clinical phenotype, thereby identifying *KCND1* as a novel disease gene.

A 02-09

Light-induced action potentials and stem cell-derived sensory neurons: an automated patch clamp investigation

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Content

Induced pluripotent stem cells (hiPSCs) serve as a vital tool in biomedical research because of their relative abundance and human origin. What is more, light-activated channels can be expressed in cell lines to control excitability through light.

In this study we investigated voltage-gated sodium channels and their electrophysiological characteristics recorded from in iPSC-derived sensory neurons and cell lines. Using automated patch clamp (APC) techniques in voltage-clamp mode we characterized current amplitude and V_{half} of activation. In current-clamp mode we could measure resting membrane potential and action potential (AP) firing patterns.

We also explored blue light-activated voltage-gated channels, specifically the ChR2-$Na_V1.5$ channel, a fusion of the light-sensitive ion channel – channelrhodopsin-2 (ChR2) from the green alga *Chlamydomonas reinhardtii*, with the cardiac-specific isoform Na^+ channels – $Na_V1.5$ expressed in HEK cells. This channel exhibits the ability to modulate Na^+ channel kinetics and evoke photosensitive inward currents. Our investigation reveals a direct correlation between blue-light intensity and channel activity, with higher intensities yielding larger peak amplitudes under constant light exposure durations. Similarly, prolonging light exposure at constant intensity enhances current amplitude, indicating a cumulative effect on channel activation. Notably, shorter exposures induce more APs than longer ones, implying an optimal exposure range for neuronal excitability modulation.Our findings provide valuable insights into the electrophysiological and biophysical properties of hiPSC-derived sensory neurons and light-sensitive ion channels. Using APC devices enhances experimental efficiency and accuracy, further advancing our understanding of neuronal function and potential applications in regenerative medicine and drug discovery.

A 02-10

Student performance in a practical Physiology course after a preparatory asynchronous online teaching module: effect of an optional pre-course online test offering bonus scores

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Content

Question

How can students be motivated to learn? In the setting of our practical Physiology course, student compliance with mandatory pre-course reading assignments has been highly divergent calling for effective interventions.

Methods

As a timely learning incentive, students were offered bonus points through an optional pre-course online test to be added to their final course scores. To test the efficacy of this intervention, half of the yearly cohort of about 500 medical students enrolled in a mandatory Physiology course were provided the test before the start of their in-house practicum. The rest served as control. The preparatory student work load was structured by an online course with interactive self-learning modules within a learning management system (LMS). The hypothesis was that students in the intervention group intensify their pre-course learning activities increasing their performance. The students' preparation efficiency and effectiveness were quantified by LMS metrics and scores at a post-course written examination in addition to structured written and oral feedback by course tutors and students.

Results

Comparison of the student preparatory work efforts and learning outcomes revealed no significant differences between the intervention and control group. Remarkably, the outstanding performers fulfilled their mandatory learning assignments to 100 % without needing to be further incentivized, while students showing lower compliance could not be motivated by the prospect of added bonus points.

Conclusions

In the setting of our study, a positive effect of a pre-course bonus test on student preparatory efforts and performance could not be detected. More effective interventions are needed.

A 03 | Neuroscience (cellular)

A 03-01

Phospho-mimetic mutant of the AMPAR-associated protein Porcupine reduces Wnt5a secretion and neuronal outgrowth

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Content

AMPA-type glutamate receptors (AMPARs) are key factors in excitatory neurotransmission. They form multiprotein complexes consisting of four pore-forming subunits and a variety of auxiliary and associated proteins that modulate receptor function. One of these proteins is the membrane-bound O-acyltransferase porcupine (PORCN). Besides its moonlighting function in supporting AMPAR assembly in the endoplasmic reticulum, PORCN has a canonical enzymatic function in Wnt palmitoleoylation. This modification is essential for activation of the Wnt signaling pathway, which plays a critical role in human development including neurite and synapse development.
Here, we investigated the phosphorylation of PORCN as a possible regulatory mechanism for its function by overexpressing phospho-mimetic or phospho-deficient PORCN variants in HeLa cells and dissociated hippocampal neuron cultures. Overexpression of a phospho-mimetic PORCN mutant resulted in decreased Wnt5a secretion in HeLa cells compared to the phospho-deficient PORCN variant. In line with this finding, manual reconstruction of hippocampal neurons and Sholl analysis of their dendrite trees revealed a significant reduction in the length of apical and basal dendrites in neurons over-expressing a phospho-mimetic PORCN mutant.
In summary, this study suggests that phosphorylation of PORCN acts as a potential regulatory mechanism for its function, leading to decreased Wnt5a secretion and eventually to physiologically relevant changes in the growth of neurons.

This work was supported by the German Research Foundation (DFG, ER799/1-1).

A 03-02

Environmental Enrichment shapes lateral inhibition in the hippocampus

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The author has objected to a publication of the abstract.

A 03-03

Morphotype-specific calcium signaling in human microglia

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Content

Ca^{2+} signaling is crucial in many physiological and pathological functions of rodent microglia including monitoring of the brain state or surrounding neuronal activity, and sensing danger or damage in their microenvironment. Moreover, microglial Ca^{2+} dyshomeostasis is a disease hallmark in many animal models of neurological disorders.

While significant insights have been gained from mouse models, the Ca^{2+} signal properties of human microglia remain largely unexplored. In this study, we employ a newly developed toolbox, including slice culturing in human cerebrospinal fluid, microRNA-9-mediated viral labeling of microglia and a novel ratiometric Ca^{2+} indicator, to analyze microglial Ca^{2+} signaling in human organotypic cortical slices.

We found that the Ca^{2+} signaling patterns differ significantly across microglial morphotypes. Ramified microglia exhibited low basal Ca^{2+} levels with ongoing Ca^{2+} transients primarily localized in the processes, marked by large amplitudes and short durations. In contrast, the ameboid microglia showed significantly higher basal Ca^{2+} levels with widespread Ca^{2+} signals invading the cell somata. Moreover, the fraction of cells with ongoing Ca^{2+} signaling, the fraction and amplitude of process Ca^{2+} signals, and the duration of somatic Ca2+ signals decreased when moving along the microglia activation pathway, i.e., from ramified via hypertrophic to ameboid microglia. Conversely, the size of active compartments, the fraction and amplitude of somatic Ca^{2+} signals, and the duration of process Ca^{2+} signals increased with microglial activation.

Together, our data revealed the marked compartmentalization of Ca^{2+} transients in human microglia, with basic properties of these Ca^{2+} transients differing dramatically across the compartments as well as morphotypes.

A 03-04

Relaxin' cortical circuits: understanding the effect of relaxin in the mouse cortex

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Content

Question

Relaxin is a heterodimeric peptide whose biological effect is mediated via the family of G-protein coupled receptor Relaxin Family Peptide Receptor 1 (RXFP1). It was initially described as facilitator of parturition in reproductive endocrinology in the late 20s of the last century. However, the actions of relaxin are not limited to reproductive organs. Although studies suggest a wide distribution of RXFP1 in different brain areas, little is known about its biological actions in the brain.

Single-cell sequencing data show robust mRNA levels of relaxin-1 in a subgroup of GABAergic interneurons, namely somatostatin-expressing interneurons (SOM-INs), suggesting that relaxin-1 might amodulate neuronal activity.

Methods

We prepared acute coronal brain slices and recorded from infragranular pyramidal cells (PCs) and from 2 types of GABAergic interneurons namely parvalbumin-expressing interneurons (PV-INs) and SOM-INs. Experiments were performed on wild-type mice and on 2 transgenic mouse lines: 1) the GIN mouse line where a subset of SOM-INs expresses enhance green fluorescent protein, 2) a PV-Cre mouse line where PV-INs express robust tdTomato fluorescence following Cre-mediated recombination.

Results

H2-relaxin caused a sustained and reversible inward current in subtypes of PCs, PV-INs, and SOM-INs. In agreement with that, we found that H2-relaxin induced a depolarization of the resting membrane potential and an increase in the action potential discharge frequency in subtypes of each cell type. In addition, initial evidence suggests that H2 relaxin reduces c-fos expression in the cortex.

Conclusions

Altogether, these data suggest that H2-relaxin acts as a powerful neuromodulator of cortical circuits.

A 03-05

SOM kind of neuromodulator: Cell-type specific effects of somatostatin in the cortex

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The author has objected to a publication of the abstract.

A 03-06

Intrinsic rebound burst excitability is unique among dopamine midbrain neurons for those projecting to the dorso-lateral striatum

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Content

Projection-defined subpopulations of midbrain dopamine (DA) neurons exhibit significant variability in their rebound properties. Our *in vitro* patch-clamp approach revealed that only dorsolateral striatum projecting DA SN neurons (DLS-DA) displayed intrinsic rebound bursting (>10Hz, 2-3 APs, mean rebound delay [RD]:86ms, 3-fold frequency gain). In contrast, DA neurons projecting to the dorsomedial striatum (DMS-DA) and lateral shell of the nucleus accumbens (lNAc-DA) returned to pacemaking (frequency gain <2) after variable delays. We found that inhibition of Ca_v3-channel eliminated rebound bursting and prolonged rebound timing (mean rebound frequency [RF]: 4.11Hz, RD:192ms). In contrast, DMS- and lNAc-DA were affected to a smaller degree (DMS: RF:2.51Hz, RD:461ms; lNAc: RF:1.81Hz, RD:653ms). Moreover, inhibition of SK-channels amplified the differences in rebound gain between DLS-DA and the other two projections, while exhibiting a lower effect on rebound timing (DLS: RF:26.43Hz, RD:151ms; DMS: RF:5.91Hz, RD:443ms; lNAc: RF:3.68Hz, RD:685ms). However, K_v4 channel inhibition removed differences in rebound properties (DLS: RF:4.02Hz, RD:34ms; DMS: RF:6.05Hz, RD:48ms; lNAc: RF:5.19Hz, RD:25ms). In addition, we performed computational modeling using realistic morphologies of SN DA neurons combined with our *in vitro* findings. We tested the integration of synaptic signals in a balanced *in vivo* state. Transient synaptic $GABA_B$-sIPSC induced similar membrane hyperpolarization in all three projection-defined models of DA neurons. However, a substantial increase in rebound firing following the termination of synaptic $GABA_B$ inhibition was only observed in DLS-DA. To test these model predictions in the intact brain, we are currently carrying out *in vivo* patch-clamp recordings in defined DA SN neurons.

A 03-07

Autophagosomal dysfunction elicits neurodegenerative tauopathy in biallelic SPRED2 loss-of-function mouse models

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Content

SPRED-proteins are potent inhibitors of the Ras-MAPK-pathway and thus have regulatory effects on processes like cell proliferation, cell homeostasis, and important steps of autophagy. Biallelic SPRED2-KO-mice show clear alterations in autophagy related processes. The protein Tau is phosphorylated by MAPKs and then dissociates from microtubules and forms multimeric aggregates, a common marker of neurodegenerative tauopathies. Not only Tau-phosphorylation is linked to tauopathies, but also impaired Tau clearance mediated by autophagy. We hypothesize that missing inhibition of MAPK by SPRED2 leads to an increase in Tau-phosphorylation, dissociation from microtubules, and accumulation within the cell. Furthermore, dissociation of pTau from microtubules destabilizes them and impairs vesicle transport.

MRI and histology showed enlarged brain ventricles and cortex atrophy in SPRED2-KO-brains. Western blots and IHC demonstrated hyperphosphorylated Tau-aggregation in SPRED2-KO-brains, a common finding in various neurodegenerative diseases. We detected SPRED2 interaction with SQSTM1/p62, an adapter guiding ubiquitinated proteins to LC3-mediated autophagy. GST-pull-downs showed that full-length-SPRED2 and the SPRED2-EVH1-domain interact with p62. Western blot analyses revealed decreased expression of p62 in SPRED2-KO-brains, indicating that SPRED2 is required for p62 recruitment and autophagy induction. We detected reduced autophagic flux in SPRED2-KO-brains. Furthermore, a Dynein/Kinesin-ATPase-Assay with vesicle preparations from WT and SPRED2-KO-mice revealed that lack of SPRED2 impairs Dynein-, but not Kinesin-activity.

Our data indicate that autophagosomal dysfunction in SPRED2-KO-mice is connected to a tauopathy. Now that first people with mutations in the SPRED2 gene have been identified, the knowledge gained from this project is very important for a possible diagnosis or therapy of these rare diseases.

A 03-08

Projection-specific uni-directional organisation of dendritic dopamine transmission in the midbrain

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Content

Most midbrain dopamine (DA) neurons are inhibited by somato-dendritic D2-autoreceptors (D2R) (Lacey et al., 1987). The presence of genuine synaptic D2R signalling between pre- and postsynaptic DA neurons and spontaneous D2R-mediated IPSCs in midbrain DA neurons were demonstrated in midbrain slices in vitro (Beckstead et al., 2004, Gantz et al., 2013). Our previous studies reported different D2R and GIRK2 expression levels in midbrain DA neurons projecting to distinct target areas (Lammel et al., 2008), but the degree of synaptic and extrasynaptic D2R signalling across these DA subpopulations is currently unknown.

Therefore, we recorded electrically evoked, D2R-mediated, sulpiride-sensitive, slow inhibitory postsynaptic currents (eIPSCs) in retrogradely identified DA neurons in vitro. We observed significant differences in peak D2R-sIPSC amplitudes between DA neurons projecting lateral shell of nucleus accumbens (lNAcc), dorsomedial striatum (DMS) and dorsolateral striatum (DLS). Mean eIPSC amplitudes of lNAcc-projecting DA neurons displayed much larger synaptic currents compared to DA neurons projecting to the dorsal striatum (lNAcc: 27.1±2.5pA, n=21, N=9; DMS: 16.3±1.4pA, n=23, N=8; DLS: 12.5±1.3pA, n=18, N=7).

We optogenetically stimulated presynaptic DA neurons projecting to DLS while recording optically-evoked, sulpiride-sensitive D2-IPSC (oIPSC) in DA neurons projecting to lNAcc ($lNAcc_{DLS}$:14.9±3.7pA, n=13,N=4). When reversing pre- and postsynaptic DA neurons, little to no oIPSCs were recorded (DLS_{lNAcc}:3.6±1.4pA, n=9, N=3).

We currently are using rabies-tracing (cTRIO; Beier et al., 2015), modified to explore local connectivity relationships (Beier, 2022) to mark specific DA-DA connections for electrophysiological recordings. Furthermore, implementing opto-switchable botulinum-toxin, we are currently trying to manipulate local DA release in the midbrain.

A 03-09

Establishment of a human induced pluripotent stem cell-based *in vitro* model for the investigation of sex-specific differences in migraine pathophysiology

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Content

Migraine is a very common neurovascular disorder yet its underlying mechanisms are still not fully understood. Due to its complexity, a variety of genetic backgrounds including a loss of function mutation in the TWIK-related spinal cord potassium channel (TRESK), has been linked to migraine pathophysiology. While childhood prevalence is similar in both sexes, migraine incidences increase in women with rising age affecting females three times more often than males. There is evidence that this disparity is mediated by sex hormones, which were shown to affect the excitability and sensitization of rodent trigeminal nociceptors by modulating the transient receptor potential vanilloid 1 (TRPV1) receptor. This receptor was further shown to be influenced by the knockout of TRESK, leading to the activation of TRPV1 and subsequent higher levels of calcitonin gene-related peptide (CGRP), which is a central mediator of migraine pathophysiology. For the investigation of the interplay of sex-hormones, TRPV1 and TRESK signaling in migraine pathology, we are establishing a cohort of migraine patient-derived induced pluripotent stem cells (iPSCs) as a basis for a human cellular *in vitro* model. Currently, migraine patient-derived fibroblast cultures and healthy controls are used for the generation of iPSCs and whole genome sequencing analysis. Additionally, iPSCs were successfully differentiated into functional nociceptive neurons, validated by electrophysiological recordings using whole cell patch-clamp and immunocytochemical analysis. Using this model, we intend to investigate the molecular mechanisms underlying the pathophysiology of migraine, focusing in particular on the role of sex hormones and migraine-associated ion channels.

A 03-10

Action potential-evoked Ca^{2+} transients in basal dendrites of rat olfactory bulb granule cells

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Content

Within the olfactory bulb, granule cells (GCs) mediate recurrent reciprocal and lateral inhibitory synaptic interactions between principal mitral and tufted cells (MTCs) via their apical dendrite that extends toward the external plexiform layer. Nevertheless, GCs also possess a short brush of basal dendrites within the granule cell layer. Basal dendrites are known to receive inputs from both MTC axonal collaterals and olfactory cortical areas, while their properties with respect to signal integration are unknown so far.

As a first step, Ca^{2+} transients evoked by backpropagating action potentials elicited in the soma (sAP) were detected using 2-photon imaging and Ca^{2+} sensitive dye (OGB-1 100µM) in GC whole-cell recordings in juvenile rat brain slices. Transients in the basal dendrites had an average amplitude $(\Delta F/F)_{sAP}$ of 20±12 % (mean±SD, n= 58 locations in 13 cells), similar to transient amplitudes in the proximal apical dendrite of the same cells (24±12%, n=11 cells). Basal spine $(\Delta F/F)_{sAP}$ was similar to that in the adjacent dendrite. In contrast to the known increase in apical dendrite transients with distance, there was no significant dependency of $(\Delta F/F)_{sAP}$ on distance in the basal dendrites. A first set of synaptic responses $(\Delta F/F)_{syn}$ in basal spines (n = 8 in 3 cells) evoked by proximal electrical stimulation was

smaller in amplitude compared to $(\Delta F/F)_{sAP}$, and thus substantially different from $(\Delta F/F)_{syn}$ in reciprocal spines. These observations imply that the known functional differences between basal and apical GC dendrites are also reflected in their respective Ca^{2+} dynamics.

A 03-11

Membrane potential UP/DOWN-states enhance synaptic transmission in the human neocortex

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The author has objected to a publication of the abstract.

A 03-12

Exploring cell-to-network plasticity of parvalbumin interneurons in the rodent and human cortex

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Content

Question

Prevalent pathologies of the brain, including schizophrenia and Alzheimer's disease, can be characterized by deficiencies of parvalbumin interneurons (PVIs) on the cellular and fast oscillations on the network level. We previously demonstrated that physiological plasticity processes of PVIs transfer from cellular to network phenomena in a process we term "cell-to-network plasticity". We therefore argue that pathological states of PVIs and fast oscillations may emerge from non-functional plasticity and can thus be treated by reinstating physiological plasticity processes. This warrants a closer examination of PVI cell-to-network processes in the human brain and according experimental tools.

Methods

In acute brain slices from rodents and humans, we perform patch-clamp, local field potentital and muulti-electrode array recordings under consideration of parvalbumin interneuron phyisology.

Results

Fast network oscillations in the low gamma (25 - 40 Hertz) and ripple (120 - 300 Hertz) frequency range can be induced and observed with extracellular recordings. The emergence of oscillations, however, is guided by interneuron-specific confounders, such as the extracellular composition and the presence of neuropeptides. Cell-type specific staining of PVIs allow the investigation of cellular PVI properties.

Conclusions

We present a multi-level platform to investigate PVI cell-to-network plasticity in the human brain under consideration of previous knowledge obtained from rodents. Effects of neuromodulation explored here set the stage for a deeper analysis of human brain tissue in the future and may form future therapies.

A 03-13

A novel auxiliary subunit fine-tunes TARPed AMPA receptors

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The author has objected to a publication of the abstract.

A 03-14

Characterization of a Novel Enhancer of AMPA Receptor Fidelity

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The author has objected to a publication of the abstract.

A 04 | Metabolism

A 04-01

Investigating the cellular crosstalk between the endocrine and exocrine pancreas

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Content

The pancreas as a biglandular organ exerts both exocrine and endocrine functions. Chronic diseases of one compartment can have major effects on the other part. However, it still remains elusive if the crosstalk between islets of Langerhans and acinar cells is also driven by paracrine signalling leading to spatial differences in cellular function. We here employed the in situ pancreas tissue slice technique that allows the analysis of endocrine and exocrine tissue in a preserved native environment, enabling the investigation of potential functional differences in acinar cells dependent on their proximity to islets of Langerhans. We assessed $[Ca^{2+}]_i$ kinetics of acinar as well as endocrine cells upon stimulation with acetylcholine, caerulein and glucose in two different mouse strains (C57BL6/J, NSG). Preliminary data shows that the response of acinar cells to acetylcholine and caerulein does not follow a clear spatial pattern related to their distance to islet cells. However, distinct differences were observed between the two different mouse strains with regard to their responsiveness to the different stimuli, with acinar cells from C57BL6/J mice responding stronger to acetylcholine while caerulein induced a higher oscillatory frequency specifically in NSG mice. Additionally, enzyme secretion was not altered between the two mouse strains, suggesting potential additional signalling pathways controlling acinar cell exocytosis. Further studies including tissue morphometry as well as the analysis of endocrine and exocrine cell function during the development of chronic diseases like diabetes or pancreatitis will be necessesary to evaluate the importance of the endocrine to exocrine cell crosstalk.

A 04-02

Calory restricted mice show primarily IP3R related calcium release supported by elevated cAMP levels

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Introduction

Calory restriction (CR) improves glucose homeostasis and delays beta cell aging by reducing insulin release and turnover. Furthermore, CR beta cells have increased cAMP levels, and elevated mitochondrial mass and ATP generation, indicating higher energy efficiency.

We hypothesize that elevated cAMP levels ($[cAMP]_c$) observed in CR beta cells modulate calcium release from the ER, impacting beta cell function. Thus, we investigated cytosolic calcium oscillations of CR beta cells using advanced imaging and analysis techniques.

Methods

We put FVB-mice on either ad-libitum (AL) or 20% CR for 8 weeks. Afterward, we prepared acute pancreas tissue slices and performed calcium imaging with high spatio-temporal resolution using a low-affinity calcium indicator dye. We used sub-stimulatory glucose conditions as a baseline and stimulated islets with high glucose or acetylcholine (ACh). Epinephrine (Epi) was used to lower [cAMP], inhibiting islet activity. Finally, we analyzed the imaging data, extracting traces and distilling calcium events.

Results

In CR beta cells we observed significant shortening of events in the fast and slow time domain, however, with unchanged frequency of the events, indicating different calcium loads in the ER. Cross-correlations of the cells tend to be lower in CR beta cells. Additionally, CR cells were resistant to ACh-dependent changes in event duration, frequency, and activity depletion. Lowering $[cAMP]_c$ by epinephrine significantly decreased oscillation frequency in both dietary regimes.

Conclusions

Our results support elevated $[cAMP]_c$ in CR beta cells primarily influencing G_q/PLC/IP_3R-related calcium release from the ER, contributing to previously described beneficial effects on glucose homeostasis, energetics, and turnover.

A 04-03

Assessing Acute Pancreatitis Severity: Extent of Necrosis as a Novel Approach in Studies Employing Live Cell Imaging

Polona Kovačič[1], Maša Skelin Klemen[1], Eva Paradiž Leitgeb[1], Andraž Stožer[1], Jurij Dolenšek[1,2]

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Content

Question

Acute pancreatitis (AP) is sudden inflammation of the pancreatic exocrine region, causing tissue destruction through autodigestion. The interplay between exocrine and endocrine functions is crucial; acinar cell damage impacts endocrine cell function and vice versa. Traditional histological assessment methods hinder live-cell imaging, necessitating alternative approaches for evaluating tissue damage and cellular activity. In this study we address the possibilities of assessing live tissue damage and cellular activity without traditional histological methods.

Methods

We induced AP in adult male NMRI mice through repeated cerulein injections. Pancreatic tissue slices were prepared and stained with a calcium indicator dye to monitor beta cell calcium dynamics via confocal microscopy Additionally, slices were stained with a commercial fluorescence LiveDead assay to concurrently assess tissue damage. A separate cohort underwent AP induction for traditional histological assessment of tissue damage.

Results

We studied the dynamics of intracellular calcium ion concentration in beta cells in acutely prepared pancreatic tissue sections, particularly during the stable plateau phase post-glucose stimulation. AP increased the active time of oscillations at various glucose concentrations. Histological assessment showed pancreatic edema, necrosis, vacuolization, and inflammatory infiltration, with no significant regional differences in damage, and intact islets of Langerhans. The LiveDead assay correlated well with traditional histological methods in assessing pancreatic necrosis.

Conclusions

We employed a commercial LiveDead assay for simultaneous tissue damage and calcium imaging, providing quick imaging and damage assessment without tissue fixation. This approach reduces animal use and offers insights for AP pathophysiology and therapeutic development.

A 04-04

Hyperlipidemia *per se* does not induce β-cell lipotoxicity in mouse and human islets *in vivo*

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Content

Lipotoxicity is widely believed as a leading factor for T2D pathogenesis. Despite of numerous *in vitro* studies, the *in vivo* relevance of β-cell lipotoxicity is under long debate. Here, we address this question by combining metabolic phenotyping, high-resolution lipidomics and longitudinal *in vivo* imaging. Immunocompetent aIB6 mice and immunodeficient NSG mice developed similar degree of hyperlipidemia when fed with a high-fat diet (HFD) but had different metabolic phenotypes. HFD aIB6 mice developed obesity, insulin resistance and β-cell functional insufficiency. In contrast, NSG mice maintained a largely normal glucose homeostasis. Plasma lipidomics reveals comparable changes in all lipid classes and most of the lipid species between mouse strains and between HFD-responders and nonresponders. Importantly, these lipids correlate strongly with insulin resistance and β-cell (dys)function in aIB6 mice but not in NSG mice. Intravital imaging shows impaired glucose-induced calcium influx in mouse β-cells already after one-week in HFD aIB6 mice. In contrast, β-cells maintained normal calcium dynamics in HFD NSG mice. Furthermore, no difference in β-cell mitochondria mass and turnover was detected in human islets during HFD in transplanted NSG mice. Taking together, our data shows β-cells maintained normal insulin release, calcium dynamics and mitochondria turnover in NSG mice during HFD. We propose high-fat feeding in immunodeficient NSG mice a good *in vivo* model to explore β-cell lipotoxicity in a relatively “pure” hyperlipidemia environment on top of which other β-cell toxic suspects could be investigated. In conclusion, we show strong evidences that hyperlipidemia *per se* does not cause β-cell lipotoxicity *in vivo*.

A 04-05

Navigating Technical Considerations and the Interpretation of Functional Connectivity Analysis in Pancreatic Islets

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Content

In the islets of Langerhans beta cells operate in synchrony to facilitate proper insulin secretion and metabolic balance. The beta cell collectives are highly heterogeneous interconnected structures, and their dynamics is characterized by intricate patterns of activity that are known to be altered in disease. In recent years, interdisciplinary endeavours combining high-resolution calcium imaging with network analysis have been performed with the aim to understand how the multicellular activity is established within this complex syncytium. However, variations in experimental preparations, microscopic techniques, derivation of functional connectivity patterns, and signal processing methods among different research groups pose challenges for comparing findings and integrating them into a cohesive understanding. Therefore, in our study we provide a systematic analysis on how different approaches impact network representation of islet activity. Our findings indicate that method choice for constructing functional connectivity maps is not critical, but caution is advised when integrating data across islets. Network analysis conclusions are influenced by time series pre-processing, oscillatory component types, and experimental variations. The latter does not pertain to variations among mouse strains but rather to the differing types of signals commonly encountered in tissue slices versus isolated islets. Our study will hopefully help to address interpretational challenges in future studies, reconciling potentially conflicting viewpoints, advancing the understanding of collective beta cell function, and inspire more researchers to employ connectivity analysis to quantify complex multicellular dynamics in health and disease.

A 04-06

FT50 EXTRACT MITIGATES METABOLIC SYNDROME AND ENHANCES GLUCOSE TOLERANCE IN WESTERN DIET-FED MICE

Nika Polšak[1], Maša Skelin Klemen[1], Jasmina Jakopiček[1], Polona Kovačič[1], Jurij Dolenšek[2], Lidija Stopinšek[3], Andraž Stožer[1]

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The author has objected to a publication of the abstract.

A 04-07

The transcription factor WT1 inhibits white adipose tissue browning via activation of the WNT signaling pathway

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Content

Question

Cold exposure and β3-adrenergic agonists can induce the appearance of heat producing “beige” adipocytes in white adipose tissue (WAT). This phenomenon, which is known as “browning” has received attention for its potential therapeutic use in metabolic disease. Our previous findings indicate that the transcription factor WT1 represses browning of visceral WAT in mice. Here, we set out to investigate the molecular mechanisms along which WT1 inhibits WAT browning.

Methods

The stromal vascular fraction (SVF) containing the fat progenitor cells was isolated from epididymal WAT of mice. *Wt1* expression in cultured SVF cells was silenced by RNA interference and the transcriptome was analyzed by deep RNA sequencing.

Results

In total, 1489 transcripts were differentially expressed between SVF cells transfected either with *Wt1* siRNA or non-targeting siRNA. GO term analysis identified “inflammatory response”, “cytokine production” and “cell migration” as the prevalent biological processes targeted by WT1. Among the 150 mRNAs with the strongest increase in SVF cells upon *Wt1* knockdown was *Ppargc1a*, which encodes a transcriptional co-activator of uncoupling protein 1 (UCP1). *Wt1* silencing significantly lowered mRNA levels of *Wnt4* and other *Wnt* family members that activate the canonical β-catenin pathway. Conversely, overexpression of WT1 increased transcripts of *Wnt4* in SVF cells. Immunoprecipitation

with anti-WT1 antibody enriched the promotor region of the *Wnt4* gene. Overexpression of WNT4 significantly reduced mRNA levels of thermogenic genes including *Ucp1* and *Cidea* in differentiating SVF cells.

Conclusions

WT1 inhibits browning of visceral WAT, at least partially, by activating the WNT signaling pathway in progenitor cells.

A 04-08

Comparison of Glucose-Stimulated Calcium Responses in Non-Diabetic and Diabetic Human Pancreatic Islets

Viljem Pohorec[1], Jurij Dolenšek[1,2], James Lyon[3], Aliya Spigelman[3,4], Nancy Smith[3,4], Amanda Gomes[3,4], Andraž Stožer[1], Patrick MacDonald[3,4]

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Content

Question

Pancreatic islet calcium signaling dynamics and insulin release are traditionally studied using animal models, which often fail to capture species-specific variations crucial for understanding type 2 diabetes (T2D). This study aims to elucidate these variations by comparing glucose-stimulated calcium dynamics in human pancreatic tissue slices and insulin secretion from isolated islets from non-diabetic and diabetic donors.

Methods

Human pancreata were obtained from three deceased organ donors: two non-diabetic and one diabetic. Tissue slices (130-140 micrometers) from the pancreatic tail were analyzed using confocal microscopy to observe glucose-stimulated calcium dynamics in response to increasing glucose levels. Additionally, isolated islets were studied for secretory function after enzymatic digestion.

Results

Tissue slices were perifused with glucose concentrations of 3, 6, 9, and 12 mM, and epinephrine was used to deactivate beta cells. Some cells were active at 3 mM glucose, while others responded to higher levels with fast calcium oscillations. Non-diabetic donor islets showed a glucose-dependent increase in active time and oscillation frequency, whereas diabetic donor islets had no clear glucose dependence, with only a slight increase in activity between 3- and 12-mM glucose. Insulin secretion was notably reduced in T2D donor islets compared to non-diabetic donor islets.

Conclusions

Our study shows that non-diabetic islets exhibit a clear glucose-dependent increase in the frequency of fast calcium oscillation, while diabetic islets have attenuated glucose responsiveness, suggesting a mechanism for beta-cell

dysfunction in T2D. These findings enhance our understanding of the pathophysiological changes in pancreatic islets in diabetes.

A 04-09

Impact of Dietary Protein Variation on Human Metabolome: Insights from NMR-based Analysis

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Content

Question

How do different levels of dietary protein affect metabolomic profiles, and what can these changes reveal about nutritional impacts?

Methods

Our study employed nuclear magnetic resonance (NMR) spectroscopy to analyze urine samples from 38 healthy participants assigned to three dietary interventions: low-protein (lp), high-protein from natural foods (hp), and high-protein supplemented with protein shakes (hps). Over a seven-week period, participants first adhered to a standard Western European diet during a four-week baseline phase, followed by a three-week intervention phase. Metabolomic profiles were analyzed to detect differences in small molecule metabolites related to dietary changes, focusing on changes induced by varying protein levels and sources.

Results

Metabolomic analysis revealed distinct differences in the profiles of participants across the three groups, highlighting significant shifts in metabolites such as inosine, formate, alanine, urea, trimethylamine, and citrate. These metabolites showed pronounced variability, reflecting the impact of different protein intake levels and sources. The study effectively mapped the shifts in metabolic pathways influenced by the variations in dietary protein. Specifically, increased protein from shakes led to unique alterations not observed with natural protein sources.

Conclusions

The findings illustrate that alterations in dietary protein intake significantly affect metabolomic profiles, underscoring the potential of NMR-based metabolomics in nutritional research. By detailing the specific metabolites affected, our study contributes to a deeper understanding of the metabolic implications of dietary protein, which can aid in developing personalized dietary recommendations based on individual metabolomic responses.

A 04-10

Reversal of diet-induced beta cell dysfunction of type 2 diabetes with caloric restriction

Jan Kopecky, Viljem Pohorec, Maša Skelin klemen, Lidija Križančić Bombek, Jasmina Kerčmar, Eva Paradiž Leitgeb, **Jurij Dolenšek**, Andraž Stožer

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Content

Diet-induced obesity (DIO) mouse models are a pivotal in studying pathophysiology of type 2 diabetes mellitus (T2D). Currently used models have some inherent methodological drawbacks, such as beta cell plasticity and composition of the diet used to induce T2DM. Clinical studies hint at T2D remission with caloric restriction in humans, however limited mechanistical explanation is available at the level of beta cell function. We therefore constructed a novel mouse model of DIO that more closely reflects T2D in humans. Male and female C57BL/6J mice were fed a western diet (WD) for 12 weeks staring from 12 weeks of age, after which they exhibited a T2D phenotype in the form of fasting hyperglycemia, impaired glucose clearance and increased insulin resistance. 7 days of caloric restriction (35 % of the caloric intake of the control group) completely reversed the diabetic phenotype, with normalization of body mass, normalization of glucose handling and insulin sensitivity. To provide a mechanistical explanation for both the DIO and remission following caloric restriction at the level of beta cell function and glucose sensitivity, we performed functional multicellular confocal calcium imaging on acute pancreatic tissue slices. A left shift in the glucose dependence was detected in the DIO group, which together with hyperglycemia could account for hyperinsulinemia observed *in vivo*. Short term caloric restriction completely reversed the compensatory left shift in beta cells and decreased their oscillatory activity. Our findings further elucidate the impact of caloric restriction on T2D and our model provides a novel platform for studying T2D.

A 04-11

Investigating the impact of GLP-1 receptor agonists on beta cell calcium dynamics

Eva Paradiž Leitgeb[1], Jasmina Kerčmar[1], Lidija Križančić Bombek[1], Viljem Pohorec[1], Marjan Slak Rupnik[1,2,3], Marko Gosak[1,3], Jurij Dolenšek[1,3], Andraž Stožer[1]

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Question

The precise regulation of beta cell stimulus-secretion coupling is crucial for maintaining nutrient homeostasis. Glucose serves as the main regulator, while incretin hormones like GLP-1 enhance beta cell function by modulating cytosolic Ca^{2+} dynamics. However, how incretins affect various phases of beta cell activity—from recruitment and activation to functional connectivity and deactivation—remains inadequately described

Methods

We investigated the effect of Ex-4, a GLP-1 receptor agonist, on beta cell calcium dynamics in healthy mice using acute pancreas tissue slices and multicellular confocal Ca^{2+} imaging. Beta cell function was assessed both through conventional physiological measures and novel network-based analyses.

Results

Ex-4 activated approximately 25% of beta cells in otherwise substimulatory glucose. Co-stimulation with Ex-4 and 10 mM glucose accelerated beta cell activation, halving the time to half-maximal activation and increasing the active time and regularity of $[Ca^{2+}]_{IC}$ oscillations, especially early in the response. Adding Ex-4 to already active cells did not significantly enhance activity. Network analyses showed increased connectivity during activation and activity with Ex-4, with unchanged hub cell roles. Ex-4 had a biphasic effect on deactivation, prolonging delays at pharmacological concentrations and shortening them at higher concentrations.

Conclusions

Co-stimulation with Ex-4 and glucose enhances $[Ca^{2+}]_{IC}$ during beta cell activation and activity, suggesting that incretin effects are largely due to enhanced [Ca2+]IC signals. GLP-1 receptor signaling supports cellular activity early in the beta cell response, and previous incretin stimulation does not significantly prolong deactivation, supporting their low risk of hypoglycemia.

A 04-12

The role of sEH deletion on hepatic lipid metabolism and lipid droplet dynamics and its effect on cardiovascular function under a fat and carbohydrate rich diet

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Content

Therapeutic approaches to lower lipid accumulation in steatohepatitis by increasing lipolysis in the liver can result in toxic effects caused by free fatty acids and subsequent systemic inflammatory responses. Lipophagy, the autophagic removal of excessive Lipids would be an alternative approach to lower the hepatic lipid load. Given that soluble epoxide hydrolase (she) is associated with lipid droplets and its deficiency is associated with a decrease in cholesterol synthesis, as well as the downregulation of key lipid-metabolizing enzymes under normal diet in hepatocytes the following study investigated the effects of sEH deletion on lipid metabolism and cardiovascular function. We could show that sEH-deficient mice displayed improved glucose handling, insulin sensitivity, and reduced blood pressure when fed a high fat and high carbohydrate diet in comparison to the respective control animals. Furthermore, the study revealed that sEH deletion led to alterations in hepatic cholesterol and triglyceride levels, with decreased triglyceride content and altered lipid droplet size. This effect could be linked to changes in lipid metabolism related proteins and pathways, including perilipin 2 and lysosome-associated proteins. Furthermore, an increased number of lysosomes as well as mitochondria could be observed in cultured hepatocytes derived from sEH deficient animals. The study suggests that deletion of sEH may affect lipid droplet content by modulating lysosomal and/or mitochondrial degradation. These results suggest that sEH is a potential target for the treatment of metabolic diseases and protects animals from hyperglycemia, insulin insensitivity, hypertension and endothelial dysfunction associated with overeating and abnormal lipid accumulation in the liver.

A 05 | Renal Pathophysiology

A 05-01

Do different expression patterns of prolyl-4-hydroxylases or different behavior of fibroblast subpopulations explain the lack of erythropoietin induction in medullary fibroblasts?

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Content

Renal erythropoietin (EPO)-producing cells are PDGFR-β$^+$ interstitial fibroblasts, primarily localized at the corticomedullary border. During increased EPO demand, cortical fibroblasts are predominantly recruited, despite the potential EPO production capacity of medullary fibroblasts. Therefore, we wanted to analyse whether different expression patterns of prolyl-4-hydroxylases (PHD) 2 and 3 involved in EPO regulation could account for these findings. Alternatively, whether subpopulations of PDGFR-β$^+$ fibroblasts like SMMHC$^+$ contractile pericytes behave differently in EPO production.

To this aim, PHD2 and 3 expression patterns were analysed using RNAscope. Moreover, the influence of both PHD isoforms on EPO expression in different fibroblast subpopulations was investigated using mice with inducible PDGFR-β and SMMHC cell-specific PHD2, PHD3, and PHD2/PHD3 deletions.

RNAscope analysis revealed that some interstitial fibroblasts express only PHD2, whereas others express both PHD isoforms. While PHD3 deletion alone did not affect renal EPO expression levels, codeletion of both PHD isoforms doubled EPO levels (6000 EPO cells/section) detected due to PHD2 deletion alone (2600 EPO cells/section). However, EPO induction occurred without any zonal pattern evenly across cortex, outer medulla, and partly inner medulla in both models. PHD2/PHD3 deletion only increased the density of EPO$^+$ cells. SMMHC$^+$ contractile pericytes showed similar results in terms of EPO expression.

Taken together our results show that although PHD2$^+$ and PHD2/PHD3$^+$ fibroblast subpopulations exist, they cannot be solely responsible for the typical physiological EPO expression pattern. Further, the EPO expression pattern cannot be explained by contractile pericytes behaving differently from other interstitial PDGFR-β$^+$ fibroblasts in terms of PHD expression or EPO inducibility.

A 05-02

Do differences in the zonal expression or stabilization of the hypoxia-inducible transcription factor 2 explain the lack of erythropoietin induction in medullary fibroblasts?

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Content

Under physiological conditions, renal erythropoietin (EPO) production is restricted to cortical fibroblasts, although medullary fibroblasts are in principle capable of expressing EPO. Therefore, we wanted to investigate whether differences in the zonal expression or stabilization of the EPO-regulating hypoxia-inducible transcription factor (HIF)-2 might be responsible.

For this purpose, kidney sections from wild-type mice were examined under basal and anemic conditions as well as after genetically or pharmacologically induced HIF-activation. The latter was achieved by multiple administration (8x 90minutes) of the prolyl-4-hydroxylase inhibitor (PHDi) roxadustat. HIF-2 stabilization was determined immunohistochemically. The zonal expression pattern of HIF-2α as well as the induction of EPO expression and other HIF-2 target genes were analysed using RNAscope.

Analysis of HIF2 staining showed that even in severe anemia (hematocrit 20%) HIF-2 stabilization was mainly restricted to cortical fibroblasts, although no obvious zonal differences in the expression levels of HIF-2α mRNA in fibroblasts could be detected. In mice with fibroblast-specific genetic HIF pathway activation, HIF-2 stabilization as well as induction of EPO and the HIF-2 target genes adrenomedullin and RGS4 occured across all kidney zones. After multiple administrations of PHDi, HIF-2 stabilization as well as adrenomedullin and RGS4 expression could be detected across the whole kidney. Interestingly, EPO induction was still restricted to the cortex.

In summary, our findings show that the lack of EPO induction in medullary fibroblasts is not due to insufficient HIF-2 stabilization. Rather, the data could indicate cellular differences in the sensitivity of EPO inducibility or the accessibility of the EPO gene locus.

A 05-03

Melanocortin Receptor Activation in Kidneys

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Content

The melanocortin system comprises five receptors, melanocortin receptor 1 to 5 (MC1R toMC5R) , which regulate a multitude of mechanisms, including melanin synthesis in the skin and appetite control. These receptors are activated by melanocortin peptides derived from the pituitary gland, such as adrenocorticotropic hormone (ACTH) and melanocortin-stimulating hormone (α, β, and γ MSH), which exhibit specificity for the respective MCR. MCRs are expressed in the kidney, but their spatial profile is only roughly known. ACTH, the activator of MC1R, alleviates proteinuria in glomerular nephrosis beyond its function as a glucocorticoid stimulator in the adrenal gland. However, it is not yet known which MCR truly mediates the glomerular protection. Conversely, γ-MSH regulates electrolyte reabsorption in the proximal tubule, thereby influencing blood pressure control and hemodynamics.
In 2020, a novel pharmaceutical agent, setmelanotide, received FDA approval. It is designed to activate MC4R, thereby regulating appetite in monogenetic obesity and in children with Bardet-Biedl syndrome. The impact of this drug on kidney function is not yet known. The objective of this study is to investigate the capability of setmelanotide to activate the ERK1/2 pathway in order to prove any MCR interaction in the kidney. This will be achieved by utilising conditionally immortalised podocytes, HK2 cells and mIMCD3 cells. The aim of this study is to clarify the clinical effects on the kidney of patients undergoing setmelanotide treatment.

A 05-04

Endothelial ANP/GC-A signaling is critically involved in acute ANP-induced stimulation of natriuresis

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Content

Question

Cardiac atrial (ANP) and B-type natriuretic peptide (BNP) are centrally involved in maintaining blood pressure by regulating vascular tone, endothelial permeability and renal sodium/water excretion. These physiological effects are mediated by the membrane-bound guanylate cyclase-A (GC-A). Despite the clear relevance of NP-signaling for kidney function, it is unclear which cell types mediate these effects and if renal microvessels play a role.

Methods

To identify renal target cells of NPs we performed systematical expression analysis of NP receptors in mouse kidneys using mRNA in-situ hybridization. The results led us to investigate the function of isolated perfused kidneys from mice with tamoxifen-induced endothelium-restricted deletion of GC-A (Cdh5-Cre/GC-A$^{flox/flox}$).

Results

In mouse kidneys, podocytes and intrarenal blood vessels express GC-A at high abundance, while no expression was detectable in the tubular system. In the renal medulla, marked expression of GC-A was found in endothelial cells of peritubular capillaries. Infusion of ANP induced a concentration-dependent increase in diuresis, natriuresis and renal blood flow in isolated perfused kidneys. Endothelium-restricted knockout of GC-A reduced total renal GC-A expression by approximately 50 % and abolished the natriuretic response to 100 pM ANP, while enhancing the ANP-induced increase in renal blood flow. In addition, the kidneys of knockout mice have a higher weight and lower mRNA expression of genes associated with endothelial barrier function.

Conclusions

Endothelial GC-A signaling is critically involved in the ANP-induced increase in natriuresis and diuresis. The surprising finding that endothelial GC-A deletion prevents diuretic while augmenting vasodilating ANP effects directs our ongoing studies.

A 05-05

Zinc is associated with serum calcification propensity in chronic kidney disease

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The author has objected to a publication of the abstract.

A 05-06

Inhibition of sphingosine kinase 1 augments osteogenic signaling and calcification of vascular smooth muscle cells

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The author has objected to a publication of the abstract.

A 05-07

Gain-of-function mutation of PDE3A prevents renal signs of chronic kidney disease

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Content

Hypertension is a major risk factor for the development of chronic kidney disease (CKD). Hypertension with brachydactyly is caused by gain of function mutations in the phosphodiesterase 3a (PDE3A) gene and leads – if untreated – to stroke and premature death. Remarkably, however, that patients keep preserved renal function. Here, we investigated the effect of mutant PDE3A on renal manifestation of CKD.

PDE3A-activating (Δ3aa) and litter-mate wild-type (WT) rats were generated and CKD was induced by bilateral renal ischemia through clamping renal arteries for 45 minutes. Four weeks after surgery kidneys were harvested for histological and gene expression analyses.

CKD induction led to a reduction of the area of the Bowman´s capsule ($p<0.05$) and the glomerular capsule area ($p=0.0536$) in WT but not in theΔ3aa rats. The media-to-lumen ratio of renal arteries was significantly higher in sham Δ3aa rats compared to renal arteries of sham WT rats (1.95 vs 1.72). CKD induction led to increased media-to lumen ratio of renal arteries in WT rats but not in Δ3aa rats. Additionally, induction of CKD led to significant renal fibrosis in the kidneys of both WT and Δ3aa rats. However, the fibrotic area in kidneys of Δ3aa rats was less than that of the WT rats. The expression of genes involved in fibrosis showed similar trends in WT and Δ3aa rats.

Our data show that gain-of-function mutation of PDE3A limit the development of CKD-induced histological changes arguing that PDE3A modulation can be a useful approach for prevention of hypertension-associated CKD.

A 05-08

Enalapril protects mice with renin cell-specific Gsα knockout from renal endothelial injury

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Content

Question

Renin production in the renin producing cells (RPC) is essentially regulated by the Gsα/cAMP-pathway. We previously showed that transgenic mice with inducible Gsα knockout (Gsα-KO) in RPC develop renal endothelial injury. However, it remained unclear whether modulation of renin production by enalapril (ACE-inhibitor) or high-salt diet would modulate the severity of the vascular phenotype.

Methods

Mice with an inducible Gsα-KO in RPC and their wildtype controls (WT) were used. Three months after knockout induction with doxycycline, uninephrectomy was performed. Afterwards, mice remained untreated (controls) or were treated with enalapril or high-salt diet for three months. Blood pressure, GFR, plasma renin activity (PRA) and renal vascular resistance were measured, and immunofluorescence staining of endomucin (EMCN, endothelial injury marker) in kidney sections was performed.

Results

Untreated and high-salt-treated Gsα-KO mice showed decreased EMCN-positive area compared to WT, indicating renal microvascular injury. Strongest damage was observed in cortical peritubular capillaries. The injury was more pronounced in males. No significant reduction of EMCN-positive area was observed in enalapril-treated Gsα-KO mice. Blood pressure was significantly lower in enalapril-treated mice of both genotypes compared to untreated animals. Gsα-KO mice showed higher renal vascular resistance than WT. GFR was not affected by Gsα knockout or treatments. PRA was increased by enalapril and decreased by high-salt diet in both genotypes.

Conclusions

Renal endothelial damage in renin cell-specific Gsα-KO mice is effectively counteracted by enalapril. This protective effect likely relies on local mechanisms, as hemodynamic parameters were comparable between genotypes within the treatment groups.

A 05-09

P2Y6-signaling in renal fibrosis

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Content

Extracellular nucleotides act as paracrine or autocrine signals in cells under regular physiological conditions and play an important role in pathophysiology when released as damage-associated molecular pattern signals. In the kidney, various P_2Y receptors have been shown to be expressed in almost all segments of the nephron, vasculature as well as interstitial cells. Since interstitial cells are key players in the development of fibrosis, we aimed to investigate the relevance of interstitial P_2Y receptors in fibrosis.

Therefore, we performed RNA *in-situ* hybridizations of the $G_{q/11}$-protein coupled receptors *P2ry1*, *P2ry2*, *P2ry4* and *P2ry6* on murine kidney sections. We observed co-expression of the interstitial cell marker *Pdgfrb* with *P2ry1* and *P2ry6* mRNA, while *P2ry2* and *P2ry4* mRNA localized to cells within the glomeruli and tubular epithelial cells. Interestingly, the mRNA expression levels of *P2ry6* were upregulated in fibrotic kidneys, suggestive of a role of *P2ry6* in fibrosis.

To functionally analyze the relevance of these receptors in interstitial fibroblasts, we performed Ca^{2+}-measurements of renal isolated fibroblasts using the ratiometric Ca^{2+}-indicator Fura2. Superfusion of cells with ADP or UDP resulted in transiently increased Ca^{2+}-signals suggestive of functionally active $G_{q/11}$-protein coupled P_2Y_1 and P_2Y_6 receptors.

In summary, (fibrotic) kidneys express P_2Y_1 and P_2Y_6 in interstitial fibroblasts and both receptors are functionally active in isolated renal fibroblasts. The relevance of the interstitial P_2Y_1 and P_2Y_6 receptors in fibrosis progression and their potential for pharmacological interference in disease progression will be analyzed in future studies.

A 05-10

Cytoskeletal GTPase septin expression and recruitment to damaged mitochondria by cadmium in the kidney

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Content

Septins are oligomerizing GTP-binding proteins, forming higher-order rings and filaments upon GTP hydrolysis. Recruitment of dynamin-related protein 1 (Drp1) by SEPTIN2 to mitochondrial fission sites and the formation of SEPTIN7 cages around mitochondria for degradation by autophagy have been reported. The nephrotoxic metal

cadmium (Cd) mediates renal cell apoptosis through disruption of the mitochondrial network and generation of reactive oxygen species. Thus, the role of septins in Cd-induced mitochondrial dysfunction was interrogated.
In both rat renal cortex tissue and the rat renal proximal tubule (PT) cell line WKPT, qPCR revealed septin mRNA abundance in the order: *Septin2*>*Septin7*>*Septin9*, which was comparable to human kidney. Cd (5µM, 24h) treatment of WKPT cells resulted in 1.2-fold and 3.1-fold increase in *Septin2/7* and *Septin9* mRNA, respectively. Similarly, SEPTIN2/7/9 proteins by immunoblotting were augmented by up to 1.5-fold after 1 h and up to 1.9-fold after 24 h by 1-5 µM Cd over controls. Increases at 6h and 12h by Cd appeared for SEPTIN2/7 (<1.3-fold). In mitochondria isolated by differential centrifugation from WKPT cells exposed to 5µM Cd for 24h, increases in SEPTIN7 (1.8-fold) and SEPTIN9 (1.2-fold) were observed in the mitochondrial fraction compared to control in immunoblots in parallel to increased rounded mitochondria, determined by live cell imaging of MitoTracker-labeled WKPT cells. In contrast, SEPTIN2 was attenuated by ~30% in mitochondria. In conclusion, Cd elevated septin expression and recruitment of SEPTIN7/9 to mitochondria, and could be the underlying mechanism to removal of damaged and dysfunctional mitochondria by fission and targeting for lysosomal degradation.

A 05-11

Podocyte-derived vesicles as urinary markers of kidney function

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Content

Background: Changes in the integrity of the glomerular filtration barrier (GFB) have been shown to induce the release of albumin-containing, podocalyxin-positive, podocyte-derived vesicles into the urinary space.
Question: We hypothesized that urinary vesicular albumin (vACR) and podocalyxin (vPCR) may serve as biomarkers of kidney function in humans.
Methods: Urinary vesicles were isolated by ultracentrifugation from participants of a prospective study (626 participants, aged >70 years). Urine samples and clinical parameters were collected at baseline (BL: n = 626), and at two follow-ups (follow-up 1: n = 626, +3 years, and follow-up 2: n = 197, +7 years). Concentrations of vesicular variables were normalized to the urinary creatinine concentration. Using SPSS 28.0.0.0, the association between vesicular parameters and clinical parameters (eGFRCys, urinary albumin/creatinine (uACR), alpha-1-microglobulin/creatinine ratio (a1M/Cr)) were determined.
Results: A negative association between eGFRCys and vACR was observed for all time points (p<.001). Linear regression analysis between vACR or vPCR and uACR or a1M/Cr, both adjusted for relevant covariates, showed a positive association between the variables at all timepoints (p<.05). Surprisingly, increased vACR and vPCR concentrations at BL were associated with a reduced increase of uACR over time suggesting that the formation of podocyte-derived vesicles reduces the development of albuminuria (p<.01).

Conclusion: In summary, increased vACR is associated with decreased kidney function and both vACR and vPCR are associated with the degree of albuminuria and tubular dysfunction. Our data suggests that both vACR and vPCR serve as new biomarkers of kidney function and predict the development of albuminuria over time.

A 05-12

Can podocytic extracellular vesicles affect the metabolism of tubular epithelial cells?

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Content

The nephron comprises around 20 cell types with distinct functions and metabolic needs. The epithelial cells of the proximal tubule have high energy demands and operate strictly glucosefree. In progressive kidney disease, this metabolism switches to glucose utilization.

Extracellular vesicles (EVs) are double phospholipid membrane coated nanoparticles that cells secrete under physiological conditions. EVs transport proteins, and RNA for cell-to-cell communication.

Kidney diseases often involve the kidney filter, housing specialized cells such as podocytes, which are unable to regenerate. Following glomerular disease, podocytes can die, resulting in loss of the entire nephron.

We investigated if EVs influence the metabolism of the downstream proximal tubule.

We stressed podocytes through cold ischemia/warm reperfusion (6h/4°C, 24h/37°C), hypoxia (24h/1%O_2), endotoxemia, 300 mg/dL glucose, or puromycin, respectively. We isolated EVs from the supernatant and characterized their surface marker profile to evaluate stress-induced changes using flow cytometry.

We used these EVs to treat proximal tubular epithelial cells (HK2) for 4h or 8 hours. Changes in gene expression were investigated for genes of glycolysis, gluconeogenesis, and pentose phosphate pathway with real time PCR.

The surface marker profile showed differences between EVs from undifferentiated podocytes and their differentiated counterparts. Podocytes stressed with static cold storage show a different marker profile than those in the other groups.

No changes in the expression of metabolic genes were observed, however we plan to use a higher concentration of EVs to verify our results.

In this project, we want to do further investigations including a resazurin based reduction assay and western blots.

A 05-13

Revealing Molecular Insights into New Claudin-10b Mutations in HELIX Syndrome Patients

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Content

Question

Claudin-10b is a tight junction (TJ) protein, playing a crucial role in the paracellular transport of sodium. Homozygous mutations in the gene encoding claudin-10b (CLDN10b) are associated with HELIX syndrome (**H**ypohidrosis, **E**lectrolyte imbalance, **L**acrimal gland dysfunction, **I**chthyosis and **X**erostomia).

We investigated the molecular basis of four independent CLDN10b mutations leading to single amino acid substitutions or loss of residues that were found in HELIX patients for their localisation, interaction and function.

Methods

Immunostaining of transfected HEK293 cells, characterization of protein interaction (Förster resonance energy transfer), and Ussing chamber experiments using stably transfected TJ-deficient MDCK II quinKO cells (Otani *et* al., 2019).

Results

We observed protein localization at the plasma membrane for two variants, similar to the wild type protein, with additionally strong accumulation in intracellular compartments. The other two variants were retained intracellularly.

Protein interaction was significantly compromised in one of the variants that localized at the plasma membrane. In contrast, the second membrane-localized mutant demonstrated interaction characteristics consistent with those observed in the wild-type.

To elucidate mutation-related alterations in the paracellular channel selectivity, we performed Ussing chamber experiments. Expression of CLDN10b mutants in MDCK II quinKO cells resulted in a loss of CLDN10b-specific cation over anion selectivity in both membrane-localized mutants, indicating an incapacity of forming functioning paracellular channels in epithelial cells.

Conclusions

Understanding the molecular mechanisms by which CLDN10b mutations lead to the strong impairment of TJs can provide valuable and potential insights into both the pathology of HELIX syndrome and new therapeutic approaches for patients.

A 05-14

The urine acid/base score and renal disease progression: evidence from pre-clinical animal models

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Content

Question

Loss of kidney function impairs acid excretion. Thus, CKD patients often develop metabolic acidosis, an independent driver of renal damage. Previous studies have used urine ammonium excretion as a marker of diminished acid excretion. However, patients can have low ammonium excretion due to low capacity or low demand for acid excretion. Following this reasoning, we developed a urine acid/base (AB)-score combining urine pH (a marker of the need for acid excretion) and ammonium (a measure of the capacity to fulfil this need). Here, we investigated the AB-score in 2 animal models of renal disease.

Methods

The AB-score was measured in urine from podocin mutant mice and ZSF1 rats. The podocin KO mice have a defective filtration barrier with proteinuria and progressive kidney function loss. The ZSF1 rats have mutations in the leptin gene and develop obesity, nephropathy and hypertension.

Results

Podocin KO mice had decreased AB-scores at week 8 compared to WTs. The AB-score decreased further at week 19 in the KO mice while the score remained high and stable in the WTs.

In ZSF1 rats, the AB-score was reduced at week 16 and 24, with the score decreasing further between week 16 and 24. Again, the controls showed stable, high AB-score values throughout the experiments.

Conclusions

Our developed AB-score decreases with age in two animal models of CKD. The results emphasize a connection between defective acid excretion and renal disease progression. We propose that the AB-score may report on the reduced function of the tubulo-interstitial compartment.

A 05-15

Discrimination of chronic vs. end-stage kidney disease requiring hemodialysis based on untargeted serum proteomics

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The author has objected to a publication of the abstract.

A 06 | Endothelial cell activation

A 06-01

The endothelial important lncRNA *SMANTIS* interacts with RUNX1 in monocytes

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Content

Expression profiling of the endothelial important lncRNA *SMANTIS* showed a much higher expression in peripheral blood mononuclear cells, in particular monocytes, as in endothelial cells. Therefore, we aim to functionally characterize *SMANTIS* in monocytes in respect to its cardiovascular and disease-related function.

During the differentiation of inducible pluripotent stem cells to monocytes, *SMANTIS* expression strongly appeared with the differentiation to monocytes and was lost once monocytes differentiated further into macrophages. RNA-Seq of a (>100) patient cohort with acute myeloid leukemia (AML) displayed a reduction of *SMANTIS* with the degree of AML differentiation. Interestingly, interaction studies showed that *SMANTIS* interacts, primarily through its Alu-element, with the transcription factor RUNX1. RUNX1 is frequently mutated in AML and important for hematopoiesis. RNA-Seq of CRISPR/Cas9-mediated deletion of *SMANTIS* or RUNX1 in THP-1 cells revealed differentially regulated genes associated with cell adhesion. A flow cytometry based attachment assay of THP-1 to HUVEC confirmed increased

binding of the knockout cells to HUVEC supporting the hypothesis that *SMANTIS* and RUNX1 limit monocyte adhesion to endothelial cells. Further, CUT&RUN for RUNX1 revealed around 800 downregulated peaks after *SMANTIS* deletion. Proximity ligation assay gave more insight into the interference of *SMANTIS* in the interaction of RUNX1 to its interaction partner EP300, a co-transcriptional activator. Collectively, *SMANTIS* interacts with RUNX1 and limits cell adhesion of monocytes to endothelial cells.
These data indicate a diverse function of lncRNA *SMANTIS* in the physiological role of monocytes apart from the inflammatory response. *SMANTIS* interaction with RUNX1 might be especially interesting for AML therapy.

A 06-02

The inflammation-regulated microprotein miP-FERMT3 induces cellular senescence and regulates inflammatory processes in human endothelial cells

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Content

We identified 2265 uncharacterized microproteins (miPs) with fewer than 100 amino acids encoded by non-canonical small open reading frames (smORFs) in human endothelial cells. In this study, we investigated the function of one of these miPs, miP-FERMT3. miP-FERMT3 is a 69 amino acid miP that is encoded by a smORF located within the coding sequence of the FERM Domain Containing Kindlin 3 (FERMT3) transcript, but in a different reading frame. The expression of smORF-FERMT3 was upregulated in activated endothelial cells *in vitro* (IL-1β) and *in vivo* (high fat diet and partial carotid artery ligation), suggesting a potential relevance to endothelial cell function. Interestingly, a FLAG-miP-FERMT3 fusion protein expressed in human endothelial cells was localized to centrosomes. Endothelial cells overexpressing miP-FERMT3 exited the cell cycle, showed an enlarged morphology and positivity for senescence-associated β-galactosidase, but remained viable. Whole transcriptome analysis revealed a significant downregulation of genes related to cell cycle progression and marked upregulation of the senescence-related genes cyclin dependent kinase inhibitors 2A and 2B. Furthermore, The IL-1β-induced adherence of monocytes to endothelial cells overexpressing miP-FERMT3 was impaired due to decreased expression of vascular cell adhesion molecule 1 (VCAM-1), a key adhesion molecule involved in inflammation. Taken together, miP-FERMT3 plays an important role in the regulation of endothelial cell cycle progression and senescence and may play a role in endothelial cell dysfunction。

A 06-03

Influence of Calcineurin on Vascular microRNA-Expression and Function

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Content

Question

The serine/threonine phosphatase calcineurin (PPP3) plays an important role in the development of cardiovascular diseases, including processes like hypertrophy, inflammation and remodeling. Three distinct genes (PPP3CA, PPP3CB, PPP3CC) encode the catalytic subunit of PPP3. Results from experiments with PPP3CB knockout animals and PPP3 inhibitors indicate that calcineurin can modulate vascular function by regulation of microRNAs (miRNAs). Aim of this study is to investigate the impact of dysregulated PPP3CB-dependent miRNA expression on vascular function.

Methods/Results

NGS of aortic samples from a PPP3CB knockout (KO) mouse model revealed 76 calcineurin-dependently regulated miRNAs. Most stably regulated miR-148a-3p was analysed by qPCR in aortic vascular smooth muscle cells (VSMCs) from the genetic model as well as with calcineurin inhibitors cyclosporine A (CsA) and tacrolimus (FK506) in WT aVSMCs and human SMCs (HAoSMCs), endothelial cells and fibroblasts. Function of miR-148a-3p was studied regarding its influence on cell viability, proliferation and migration. MiR-148a-3p antagomir-transfection modified proliferation in BrdU ELISA of VSMCs and HAoSMCs. Clones of A7r5 cells, stably overexpressing miR-148a-3p, showed no change in cell viability but proliferation behavior was altered. Using Western blots, we were able to show that miR-148a-3p overexpression reduced PAI-1, a protein facilitating platelet aggregation, inflammation and hypertension.

Conclusions

We identified miR-148a-3p as a PPP3CB-dependently regulated miRNA that potentially modifies vascular smooth muscle cell proliferation and PAI-1 protein expression. With more research, PAI-1, modulated by miR-148a-3p, could be a promising target to treat cardiovascular diseases.

A 06-04

Aging increases hypoxia-induced endothelial permeability and blood-brain barrier dysfunction by upregulating arginase-II

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The author has objected to a publication of the abstract.

A 06-05

Long non-coding RNAs direct the SWI/SNF complex to endothelial cell-specific enhancers

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Content

Background: Long non-coding RNAs modulate the activity and targeting of chromatin remodelling complexes and thereby gene expression. The mechanisms governing the recruitment of these complexes to specific gene promoters are largely unknown. We previously identified the lncRNA MANTIS as a crucial component of the endothelial SWI/SNF complex. Thus, we hypothesise that a broader network of lncRNAs modulates chromatin remodelling through a cell-specific and context-dependent recruitment of the SWI/SNF chromatin remodelling complex to ultimately mediate endothelial cell and vascular function.

Methods and Results: The core ATPase of the SWI/SNF complex, BRG1, is required for normal endothelial cell function. Knockdown of BRG1 in human umbilical vein endothelial cells (HUVEC) reduced proliferative capacity and angiogenic sprouting potential. BRG1 siRNA and RNA-sequencing alongside BRG1 CUT&RUN revealed multiple enriched genes and pathways crucial to endothelial cell function that were significantly differentially expressed after BRG1 knockdown. BRG1 iCLIP identified novel RNA interaction partners of BRG1. This recovered the lncRNAs EPHA1-AS1, CACNA1G-AS1, MALAT1 and NEAT1, which have been implicated in endothelial function. Subsequent

screens with an siRNA library against BRG1-bound lncRNAs revealed that many of these alter BRG1 genomic binding and endothelial function.

Conclusions: Endothelial cells contain a considerable number of functionally important lncRNAs that interact with the SWI/SNF chromatin remodelling complex. BRG1 CUT&RUN and RNA-seq following BRG1-bound lncRNA knockdown will reveal further specific gene targetting mechanisms and programs that depend upon lncRNAs. RedChIP will also be performed to confirm that these lncRNAs directly recruit BRG1 to its genomic targets to mediate endothelial and vascular function.

A 06-06

Human endothelial cell microproteins contribute to the MHC peptide repertoire

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Content

Sterile inflammation plays a central role in the progression of cardiovascular disease. Recently, specific subsets of endothelial cells have been proposed to express MHC molecules and to present antigens that can influence T cell activity. Given that we identified 2265 endothelial cell microproteins (miPs) and miPs were presented on MHC molecules in numerous cell types, we set out to assess the contribution of endothelial cell miPs to the MHC peptide repertoire. Whole transcriptome analysis revealed that interleukin (IL)-1β upregulated the expression of genes associated with antigen processing and presentation in cultured human endothelial cells. The effect was more pronounced in the combined presence of IL-1β, interferon-g and transforming growth factor-β2. A comparable increase was also observed *in vivo*, in partially ligated carotid arteries from ApoE$^{-/-}$ mice subjected to a high-fat diet. Prediction of peptide binding affinity to HLA class I supertypes (NetMHCpan, EL score ≤0.5) revealed 23,070 peptide binders (i.e. autoantigens) derived from 2,222 human endothelial cell miPs. Additionally, 33,617 peptide binders from 1,177 microproteins were predicted to bind to HLA class II (NetMHCIIpan, EL score ≤1), suggesting their potential role in modulating CD4+ T cells response. Taken together, these findings suggest that immunomodulatory endothelial cells may function as non-professional antigen-presenting cells in a context of cardiovascular disease and that the endothelial cell microproteome may contribute to shaping the (auto)antigen repertoire.

A 06-07

Loss of SHP-2 phosphatase activity augments the adhesive phenotype of insulin resistant endothelial cells

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Content

Question

Insulin resistance in type 2 diabetes is associated with vascular inflammation. We previously found that loss of SHP-2 activity results in excessive endothelial activation and inflammation. Therefore, we hypothesized that SHP-2 inactivation may additionally contribute to the inflamed endothelium under insulin resistant (IR) conditions.

Methods

Insulin resistance was induced by high insulin (100 nM) and glucose (15 mM) (HIG) for 24h or 48h. Wild type (WT), dominant negative (CS) or constitutively active (EA) SHP-2 were over-expressed in human umbilical vein endothelial cells (HUVEC). Upregulation of adhesion molecules was detected by flow cytometry and qRT-PCR. Leukocyte adhesion to EC was assessed under flow (1 dyne/cm^2). SHP-2 activity was assessed by immunoprecipitation and measurement of pNPP dephosphorylation.

Results

HIG mediated ICAM-1 and VCAM-1 expression in EC (n=6-12) and in aortic endothelium (n=5) was enhanced after expression of SHP-2 CS ($p>0.05$), compared to SHP-2 WT, whereas expression of SHP-2 EA prevented this ($p<0.05$). Similarly, the adhesion of leukocytes was increased by SHP-2 CS expression compared to SHP-2 WT upon HIG treatment (n=5, $p<0.05$), while SHP-2 EA prevented this (n=5, $p<0.05$). Endogenous SHP-2 activity was impaired by HIG treatment (n=7, $p<0.05$) but was rescued by ROS inhibition (VAS2870, n=3, $p<0.05$), while SHP-2 expression remained unchanged (n=16).

Conclusions

SHP-2 activity seems to prevent endothelial activation and protects the endothelium from the development of an inflammatory endothelial phenotype. Its inactivation by high concentrations of insulin and glucose may thus be a fundamental step in the progression of an inflamed endothelium seen in insulin resistant diabetes.

A 06-08

Identification of proteins associated with global and specific RNA-DNA triplexes

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Content

Background: Interactions of RNA with DNA are principles of gene expression control which recently gained considerable attention. Interactions of long non-coding RNAs (lncRNAs) with DNA occur often through RNA-DNA triplex formation, which is resistant to RNase H. Importantly, recent research highlighted the importance of triplexes for the cardiovascular system, but unlike R-loops, triplex-associated proteins are not known and were identified here.

Methods & Results: To identify proteins associated with specific or global RNA-DNA triplexes, several techniques were established. In a global approach, antibodies against dsDNA were used to immunoprecipitate RNA-DNA triplex-associated proteins in endothelial cells. To identify proteins acting in the triplex of lncRNA *MEG3* with *TGFBR1*, IDAP (Isolation of DNA-associated proteins) was performed, in which synthetically labelled lncRNA oligos mimicking the triplex-forming region of lncRNA *MEG3* were used in combination with the corresponding *TGFBR1* DNA target site. Both approaches yielded more than 100 nuclear proteins associated with RNA-DNA triplexes in RNaseH-dependent manner. The global approach revealed transcription factors, protein scaffolds and chromatin remodelling proteins such as YBX3, WDR5 and CBX3. Interestingly, after IDAP with HEK cells and HUVECs, 178 proteins were pulled down, including DNA topoisomerase TOP3A and the transcription factor SP3. The data suggested that proteins associated with RNA-DNA triplexes are conserved between different cell types.

Conclusion: Immunoprecipitation of proteins bound to dsDNA and IDAP are useful tools for identifying triplex-associated proteins which may scaffold physiologically relevant RNA-DNA interactions in endothelial cells.

A 06-09

Amlodipine impacts on endothelial nanomechanics and function

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Content

Dihydropyridine-type calcium channel antagonists (DHPA) are well-established drugs in the treatment of hypertension by reducing systemic blood pressure. DHPA action is thereby not restricted to vascular-smooth-muscle-cells but also affects endothelial behaviour. Among others, endothelial function can be regulated by changes of the nanomechanical properties of the endothelial cell surface, namely the endothelial glycocalyx (eGC) and the cell cortex, an actin-rich layer 150-200nm beneath the plasma membrane. A healthy, soft endothelial surface can be linked to proper flow-dependent vasodilation. This project aims to identify the impact of DHPA on endothelial nanomechanics and function and seeks to identify possible mechanisms of DHPA on vascular function.

Within an *in vitro* approach, HUVECs were treated for 24h with DHPA Amlodipine (10µM) or with the calcium channel agonist BayK8644 (1µM) and analyzed by Atomic Force Microscopy-based nanoindentation as well as accompanying immunofluorescence stainings. Aortae of atherosclerotic AAV-PCSK9^{DY} mice, treated for 12 weeks with amlodipine (5 mg/kg bw) or vehicle *in vivo*, were analyzed *ex vivo*.

Amlodipine treatment improved endothelial function by increasing eGC height to 141.4nm compared to 127.3nm within the controls. In parallel, cortical stiffness was decreased to 0.93pN/nm compared to 1.13pN/nm within the controls, whereas DHP agonist BayK8644 leads to increased cortical stiffness. DHPA also led to augmented endothelial nitric oxide (NO) production. In line, in AAV-PCSK9^{DY}-mouse aortae amlodipine application led to cortical softening and increased plasma levels of NO.

We conclude, that DHPA directly impacts endothelial behaviour, thus providing a novel explanation for the vasculo-protective effect of DHPA on an endothelium-based level.

A 06-10

Effects of Chronic Inflammatory Activation of Endothelial Cells at Normal Lipoprotein and Cholesterol Levels *In Vivo* and *In Vitro*

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Content

Atherosclerosis is initiated by high LDL-lipoprotein/cholesterol levels leading to inflammatory activation of endothelial cells, subsequent recruitment of leukocytes, proliferation of smooth muscle cells and various cellular transitions leading to plaque formation. Prior work from our group showed that persistent genetic activation of the inflammatory NF-κB pathway specifically in endothelial cells at high lipoprotein/cholesterol levels resulted in an aggravation of the atherosclerotic process. However, it remained unclear, how arteries respond to inflammatory activation of endothelial cells under normal blood lipid levels *in vivo*. This question was addressed using a mouse model bearing inducible constitutively active IKK2-expression specifically in arterial endothelial cells. RNA-sequencing of whole aortic tissue revealed significant upregulation of inflammatory as well as cell death signaling pathways. On the other hand, pathways involved in protein synthesis machinery were found to be downregulated. Angiotensin converting enzyme 2 (ACE2), an important counterplayer of ACE in blood pressure regulation, but also the receptor for SARS-CoV2, was identified as the most upregulated gene. Elevated mRNA and protein levels of ACE2 were also observed in human primary endothelial cells *in vitro* after exposure to inflammatory stimuli. This was accompanied by an increase in apoptosis, although not the same extent as in whole aorta, which might be a consequence of signal amplification from paracrine crosstalk between the cells of the vasculature. The significant upregulation of ACE2 by inflammatory triggers might explain why patients with chronic inflammatory conditions (including obesity) show a higher susceptibility to SARS-CoV2 infection or a more severe course of the COVID19 disease.

POSTER SESSION B

B 01 | Heart failure mechanisms

B 01-01

Cardiac myofilament properties in a mouse model with a dilated cardiomyopathy-causing myosin mutation

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Content

Question

[The myosin mutation F764L is associated with dilated cardiomyopathy (DCM) and has been shown to significantly reduce myosin ATPase activity and Ca^{2+}-dependent force development before onset of the clinical phenotype. From this it can be deduced that the F764L/+ mutation directly impairs myofilament function and that this impairment precedes structural remodeling of the heart. Here we analyzed whether early structural remodeling in mice with F764L/+ myosin mutation includes changes in myofilament passive stiffness and titin.]

Methods

[Fiber bundles from papillary muscles of 10-12-week-old heterozygous F764L/+ mice and wildtype littermates (n=7/group) were used to assess passive and Ca^{2+}-dependent force development, Ca^{2+}-sensitivity, and length-dependence of force activation. SDS-PAGE and Western blotting were employed to analyze the expression and modification of target proteins, including titin.]

Results

[Titin isoform composition was unaltered, with an average of 82%±1% of N2B titin in F764L/+ mice and 81±1% in controls. Western blot analyses demonstrated a significant reduction in the relative phosphorylation of titin S4099 by 16.7±5.6% and of cardiac troponin I (S23/24) by 23.2±5.6%. Despite the observed biochemical modification of the investigated sarcomere proteins in F764L/+ mice, we found no significant impairment in the biomechanical parameters. Passive stiffness, Ca^{2+}-sensitivity, and length-dependent force development were unchanged compared to wild-type mice. There was a trend towards increased maximal Ca^{2+}-induced force development in F764L/+ mice (24.4±3.3 mN/mm^2) compared to controls (19.7±2.6 mN/mm^2).]

Conclusions

[Our observations suggest that in 10-12-week-old mice the F764L/+ myosin mutation causes adaptive changes to myofilament proteins without impairment of myocyte function.]

B 01-02

Potential role of Proenkephalin (PENK) in right heart failure

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Content

Background

Despite many similarities between the left ventricle (LV) and the right ventricle (RV), important differences in development, anatomy, pathophysiology, and clinical management exist. However, the molecular mechanisms of progressive RV failure are incompletely understood.

Methods

To identify RV-specific signatures, we established rat models, which show two slowly developing disease stages (compensated and decompensated) in response to pulmonary artery banding (PAB) or ascending aortic banding (AOB). Gene expression was analyzed by RNA-Sequencing, proteomics analyses, qPCR and Western blot. We also analyzed gene expression in 71 patients with chronic thromboembolic pulmonary hypertension (CTEPH), who were treated by pulmonary endarterectomy.

Results

Among the top regulated genes implemented in RVF progression in PAB rats and patients with CTEPH, was proenkephalin (Penk), the precursor opioid polypeptide hormone of various enkephalins. Interestingly, the expression of Penk correlated strongly with the severity of RV disease in rats and humans. Within the heart, cardiomyocytes show a strong Penk expression. A screening with typical mediators involved in heart failure-associated neurohumoral activation, showed a strong induction of Penk expression in cardiomyocytes in response to angiotensin II, norepinephrine or endothelin-1 stimulation. The contractile response of isolated rat cardiomyocytes was significantly impaired following short-term incubation with Leu-enkephalin. In addition, Leu-enkephalin was able to attenuate the pro-hypertrophic effects of the alpha1-adrenergic agonist phenylephrine.

Conclusions

Penk was among the top regulated genes in rat and human RV failure and its expression correlated with disease severity. It mediates manifold functional effects on isolated cardiomyocytes and Penk expression is induced in cardiomyocytes by neurohumoral activation.

B 01-03

Cardiac conduction velocity increases by increased heart rate or optogenetic-induced depolarization due to a novel distance-to-threshold effect

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Content

Fast conduction velocity (CV) of action potential (AP) is important for proper cardiac function and prevention of lethal ventricular arrhythmia. CV depends on resting membrane potential (RMP) affecting Na^+ channel function, heart rate and extracellular K^+ concentration. To investigate the interaction of these parameters, we established optical voltage mapping with the red-shifted voltage sensitive dye Di-4-ANEQ(F)PTEA in combination with microelectrode recording of membrane potential, electrical pacing and optogenetic subthreshold depolarization. Surprisingly, increasing the pacing frequency from 4 to 5.6 Hz increased CV by 6%, although RMP depolarized by 2-5 mV, which blocked Na^+ channels indicated by slower AP upstroke. To proof that the RMP increase underlies CV acceleration, we generated gradual subthreshold optogenetic depolarization by illumination in a Channelrhodospin-2 mouse model and found that RMP depolarization up to ~8 mV increased CV despite reduction of Na^+ channel availability. However, if the heart rate was even faster (6.7 Hz) or optogenetic depolarization was stronger (10-15 mV) CV was reduced. Increasing extracellular K^+ levels from 4 to 6 mM led to RMP depolarisation of ~8 mV and increased CV, whereas further optogenetic depolarization slowed CV. In contrast, if extracellular K^+ was reduced (2-3 mM), RMP hyperpolarized and CV was reduced but optogenetic depolarization up to 15 mV led to an increase of CV.
Thus, we identified a CV reserve that can be recruited by mild depolarization due to a “distance-to-threshold effect” showing that the normal RMP is not optimal for fastest CV, especially at low extracellular K^+ levels.

B 01-04

Deletion of MAO-B using ROSACreERT2 mice increased weight loss and mortality rate

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Content

Monoamine oxidase B (Mao-B) is a protein of the outer mitochondrial membrane that degrades biogenic amines and leads to the production of reactive oxygen species (ROS). ROS are involved in ischemia/reperfusion injury in cardiomyocytes. To investigate the impact of MAO-B in the whole heart we have crossed ROSACreERT2 mice with MAO-$B^{fl/fl}$ mice and analyzed the efficiency of MAO-B deletion. Doses of 50 mg tamoxifen/ kg body weight for five

consecutive days and additional waiting time of one week resulted in a decrease of MAO-B protein in the heart to 80 %. After ten weeks waiting time, MAO-B protein decreased further to 60 %. Under the same conditions, the amount of MAO-B protein in the liver was already reduced to less than 40 % and to below 5 % after ten weeks. Injection of 100 mg tamoxifen/ kg body weight for ten consecutive days and additional waiting time of tens weeks resulted in a decrease of MAO-B protein in the heart to 40 %. The MAO-B protein content of the cardiomyocytes was only 10 %. MAO-B RNA as well as protein in liver was barely detectable. While injection of 50 mg tamoxifen was tolerated by most mice, injection of 100 mg tamoxifen resulted in a noticeably loss of weight which led to the death or euthanasia of the mice. Examination of liver samples of dead mice revealed an increase in IL6 and IL1β RNA expression, which indicates increased inflammation. Conclusion: Ubiquitous MAO-B deletion by ROSACreERT2 provokes early death in mice.

B 01-05

Leveraging cell-penetrating peptides to cleave titin in intact cardiac muscle tissue

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Content

Question

Titin is an important structural and regulatory protein in striated muscle cells. The titin cleavage (TC) knock-in mouse model, which allows for acute and specific cleavage of titin using Tobacco Etch Virus protease (TEVp), has enabled the determination of the force contribution of titin in permeabilised muscle tissue. However, TEVp is not membrane permeable, preventing its use in intact TC mouse muscle cells. This is problematic as permeabilised samples present inconsistencies in sarcomeric force production properties and a loss of other structural elements, such as the microtubules. Therefore, we aimed to deliver TEVp into intact TC muscle cells while maintaining cellular integrity.

Methods

We established a protocol utilizing cell-penetrating peptides (CPPs) to shuttle TEVp into intact left ventricular muscle tissue of TC and wild-type (control) mice. We tested different TEVp:CPP ratios, concentrations, and incubation times for the well-studied CPPs TAT and PepB2 and analysed the amount of titin cleaved in agarose-strengthened titin gels.

Results

We found PepB2 to be more effective than TAT under all conditions tested. The best results were obtained for a TEVp:CPP-ratio of 1:200 (TEVp: 0.75 µM, PepB2: 150 µM) after 30 minutes of incubation, where 80.8 ± 2.24 % (n=3) of titin was cleaved.

Conclusions

This study establishes a method for manipulating titin function in intact cells, paving the way to a deeper understanding of its role in healthy and diseased hearts. It further opens up the possibility of shuttling other molecules into intact striated muscle cells to modulate cellular function.

B 01-06

Protein Quality Control and Titin turnover during dedifferentiation and redifferentiation of cultured adult rat cardiomyocytes

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Content

Question

Titin is the third filament system in the sarcomere, and disintegration and degradation of titin are major challenges for cardiomyocytes. Here we studied titin turnover and protein-quality-control (PQC) during sarcomeric de- and redifferentiation processes using cultures of adult rat cardiomyocytes (AdRCM).

Methods

AdRCMs were isolated and cultivated for up to 20 days and analyzed by Western blot, immunofluorescence staining with antibodies to PQC associated proteins and titin epitopes, and by proteasome activity assays.

Results

During cultivation, AdRCMs lost their rod shape and sarcomeric structures were gradually decreased. After 6 days many cells had lost their sarcomeric structure, accompanied by elevated titin ubiquitination (+413 ±47%), loss of titin protein levels (-32 ±%) and increased proteasomal activity (+257 ±22%). Although Z-disc titin exhibited early diffuse organization, we did not detect domain-specific disappearance of titin antibody signals, suggesting titin degradation in one. We observed more ubiquitin aggregates and loss of sarcomeres over time. By culture day 12-15, sarcomeric structures were rebuilt, but they did not entirely regain the parallel sarcomere organization observable in freshly isolated AdRCMs. The observed cell morphology was consistent with that of isolated embryonic or neonatal cardiomyocytes. Furthermore, the titin isoform composition shifted toward an increase in the more compliant N2BA isoform (+51 ±2%).

Conclusions

The presented data indicate that sarcomeres and, in particular titin are rapidly degraded during dedifferentiation. The probable mechanisms underlying this degradation involve the proteasome and autophagy. Notably, the observed degradation is not limited to individual domains but appears to affect the entire protein.

B 01-07

Not so different after all: temperature dependence of actin filament sliding along cardiac alpha- and beta-myosin molecules

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Content

Different isoforms of myosin-II motor proteins such as cardiac alpha- and beta-myosin are generally considered to have distinct functional properties such as actin filament sliding velocity. Alpha-myosin is regarded the fast and beta-myosin the slow post-natal cardiac myosin isoform. However, in many species both myosin isoforms can work friendly together in mixed myocardial tissue apparently without interfering with each other's function.

Due to experimental limitations such as increasing protein, myofibril or myocyte damage, only few data exist regarding alpha- and beta-myosin velocities at higher temperatures. This raised the question whether alpha- and beta-myosin might move actin with approximately equal velocity at body temperature.

We used myosin extracted from porcine atrial appendage and ventricular myocardium to gain mainly alpha- and beta-myosin preparations and compared their actin sliding velocities in an *in vitro* motility assay.

Varying the temperature between 15 and 39°C we found that with increasing temperature the difference between actin sliding velocities on alpha- and beta-myosin decreased progressively. While at 15°C, a temperature often used in muscle fiber, -cell, or myofibril experiments, alpha-myosin moved actin filaments with 0.316 ± 0.014 µm/s almost four-times faster than beta-myosin with 0.088 ± 0.004 µm/s, approaching body temperature of 39°C this difference decreased to 1.3-fold faster movement on alpha-myosin (9.760 ± 0.202 vs. 7.743 ± 0.465 µm/s).We conclude that at physiological temperature cardiac alpha- and beta-myosin move actin with almost equal velocity which suggests that in mixed myocardial tissue both myosin isoforms could work together without interfering with each other's function.

B 01-08

Age-dependent changes in contractile properties and protein quality control in a mouse model with hypertrophic cardiomyopathy

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Content

Question: Hypertrophic cardiomyopathy (HCM) is an often inherited disease characterized by ventricular hypertrophy, hypercontractility and reduced diastolic distensibility. Recent studies have implicated stress-signaling and impaired

protein quality control (PQC) as potential mechanisms underlying the HCM phenotype. Here, we investigated PQC mechanisms and contractile properties in an HCM mouse model with MYH7 mutation R453C. Analyses were performed on young (11w) and older (38-40w) mice to test for changes that may contribute to disease onset and progression.

Methods: Fiber bundles from papillary muscles of heterozygous R453C/+ mice and wild-type littermates (n=2-6/group) were used to determine passive and Ca^{2+}-dependent active force and Ca^{2+}-sensitivity. Relative expression and modification of PQC proteins and titin were analyzed by SDS-PAGE and Western blotting.

Results: Biomechanical parameters were unchanged in old R453C mice compared to controls, but young R453C mice were hypercontractile with Fmax=48.5±13.6 mN/mm² compared to 25.4±12.3 mN/mm² in age-matched controls and 22.2±4.1 mN/mm² in old R453C mice. The Ca^{2+}sensitivity of force development was increased with EC_{50}=1.82±0.04 µmol Ca^{2+}, compared to 2.16±0.002 in age-matched controls and 2.21±0.06 in old R453C mice, respectively. Preliminary data indicate a 4% higher relative expression of the stiffer N2B-titin isoform in young compared to old R453C mice, resulting in higher passive tension of skinned fibers. Relative titin and total protein ubiquitination were significantly higher in old R453C mice compared to controls, indicating increased protein turnover.

Conclusions: We conclude that hypercontractility in HCM mice is age-dependent, promoted by titin stiffening and likely accelerates sarcomeric protein turnover in later stages of the disease.

B 01-09

Longitudinal assessment of in vivo and ex vivo rodent cardiac functions in conventional and experimental irradiation

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Content

The irradiation therapy has established itself as an essential part of the tumor therapy. Although, improving technical possibilities or experimental irradiation may be able to hit the target volume more precisely, we still cannot fully rule out a severe effect to the surrounding organs. In thoracic irradiation the heart is often an organ-at-risk. Therefore, we aim to evaluate the effects of conventional and experimental irradiation on the heart. For this purpose, a panel of in vivo and ex vivo methods was established.

In in vivo studies, we investigated changes in cardiac function with an ECG and echocardiography two weeks prior and after a broad-beam irradiation exposure of 30 Gy. Additionally, we did the same examinations with a sham irradiated group. After excision, the organ was characterised in the Langendorff perfusion setup. The living heart was challenged with various doses of carbachol and norepinephrine.

To examine the cardiac conduction system, we analyzed the heart rate, heart rate variability and the amount of arrhythmic events over 24h with the ECG as well as via a simultaneously ECG recording during the perfusion in the

Langendorff system. As surrogate markers for the myocardial function, we measured left intraventricular diameter, thickness of the left ventricular posterior wall and ejection fraction of the heart in the echocardiography.
We established a panel of methods to evaluate the physiological heart functions. We will use these methods to assess potential harmful effects of experimental irradiation regimes, like the microbeam irradiation.

B 01-10

Deep learning instance segmentation model for quantification of infarct size in pigs with myocardial ischemia/reperfusion

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Content

Background: Infarct size (IS) is the most robust endpoint for evaluating the success of preclinical studies on cardioprotection. The gold standard for IS quantification in ischemia/reperfusion (I/R) experiments is triphenyl tetrazolium chloride (TTC) staining, typically done manually. This study aimed to determine if deep learning instance segmentation is a time-saving and valid alternative to manual IS quantification.
Methods: Retrospective data from pig experiments (n=390) with I/R and I/R+cardioprotection were used to cover a wide IS range. IS was analyzed using a standard manual method. High-resolution images (n=3,869) of TTC-stained heart slices were labeled. A supervised deep learning instance segmentation model based on dynamic U-Net architecture was developed and trained. The model underwent five-fold cross-validation (n=221 experiments) and testing on an independent test set (n=169 experiments). Performance metrics (Dice similarity coefficient [DSC], pixel accuracy [ACC], average precision [mAP]) were calculated, and IS from model predictions was compared to manual IS quantification (linear regression, Pearson's r; analysis of covariance).
Results: Performance metrics near one indicated good model performance on cross-validated and test data (DSC: 0.82 / 0.76, ACC: 0.98 / 0.98, mAP: 0.77 / 0.72). IS quantified manually correlated well with IS from predictions (cross-validation: r=0.93; test data set: r=0.90). No significant difference in IS from predictions was observed between I/R and I/R+cardioprotection data. The model reduced IS quantification time from approximately one hour to a few minutes.
Conclusion: IS quantification using a deep learning instance segmentation model is comparable to manual methods and reduces analysis time.

B 01-11

Absence of an inflammatory proteomic signature in HFpEF versus HFrEF human hearts

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Content

Question: Althoughevidence remains limited, low-grade systemic inflammation and endothelial dysfunction may be involved in the pathomechanism of heart failure with preserved ejection fraction (HFpEF). Here, we aimed to identify a cardiac proteomic and phosphoproteomic signature to discriminate HFpEF from heart failure with reduced ejection fraction (HFrEF), compared to nonfailing donor hearts.

Methods & Results: Left ventricular myocardial tissues of explanted or donor human hearts (Medical University Graz) were classified into nonfailing control (Ctrl), HFpEF or HFrEF (mean EF (%): 63 (Ctrl), 62 (HFpEF), 24 (HFrEF); mean age 58.1±9.5; mean BMI 26.6±2.7) and analysed by mass spectrometry (N=6-7/group). Cardiac proteome analysis identified only 19 proteins that exhibited significant regulation in HFpEF but not in HFrEF versus Ctrl hearts. Gene ontology (GO) enrichment analysis revealed that these uniquely altered proteins were predominantly linked to "muscle contraction" and "calcium ion binding". Of significant importance, low-grade systemic inflammation emerged as a disease factor in both human HFpEF and HFrEF hearts. This was corroborated by GO enrichment analysis of significantly regulated proteins in HFpEF and HFrEF versus Ctrl hearts demonstrating altered protein expression associated with “complement activation” and “innate immune response”. Phosphoproteomic alterations in HFpEF and HFrEF versus Ctrl hearts concerned mainly sarcomeric proteins (e.g. titin, desmin), which were predominantly hyper-phosphorylated.

Conclusions: Low-grade systemic inflammation is evident in HFpEF and HFrEF patient hearts, with no distinct inflammatory proteomic signature observed in HFpEF hearts.However, our findings suggest an association between systemic inflammation and sarcomeric protein phosphorylation that is to be explored in future research.

B 01-12

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

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Content

Skeletal muscle excitation-contraction (EC) coupling relies on the conformational coupling between $Ca_V1.1$ on the sarcolemma and RyR1 on the sarcoplasmic reticulum (SR). STAC3 is an adapter protein essential for this process: it is both crucial for the $Ca_V1.1$ functional expression and the conformational coupling between $Ca_V1.1$ and RyR1. STAC3 establishes two distinct interactions with $Ca_V1.1$, one between the SH3-1 domain of STAC3 and the II-III loop of $Ca_V1.1$, and one between the C1/linker region of STAC3 and the proximal C-terminus of $Ca_V1.1$. Additionally, STAC3 was hinted to be the link between $Ca_V1.1$ and RyR1. To determine the importance of each interaction, two STAC3 fragments were generated and reconstituted in a $Ca_V1.1$/STAC3 KO muscle cell line. Electrophysiological recordings revealed STAC3-NT, interacting with the proximal C-terminus of $Ca_V1.1$, is sufficient for $Ca_V1.1$ functional expression and nominal calcium release from the SR. Conversely, STAC3-CT, interacting with the region of the II-III loop of $Ca_V1.1$ specific for skeletal muscle EC coupling, merely enhances EC coupling. Finally, we analyzed whether the SH3-2 domain of STAC3 establishes an additional interaction relevant for EC coupling. However, multiple mutagenesis experiments revealed that the SH3-2 domain is important for stabilizing the SH3-1 interaction with the II-III loop of $Ca_V1.1$. Altogether, these results suggest that STAC3 allosterically modulates $Ca_V1.1$, rather than mediating an interaction with RyR1.

B 01-13

ERC1 enhances skeletal muscle excitation-contraction coupling

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Content

Question

ERC1, a member of the family of CAST/ELKS scaffold protein, supports the structure of presynaptic active zones. ERC1 directly interacts with the CaVβ subunit of voltage-gated Ca2+ channels (VGCC). Importantly, this interaction affects VGCC activity, as ERC1 deletion leads to reduced calcium influx at inhibitory synapses in the hippocampus, the calyx of Held, rod photoreceptors, and pancreatic β-cells. Here, we hypothesized that ERC1, endogenously

expressed in skeletal muscle, might influence the membrane and functional expression of CaV1.1, as well as voltage-induced calcium release from the sarcoplasmic reticulum.

Methods

We performed patch-clamp experiments in (1) HEK cells, in (2) wild-type and in (3) a newly-generated CRISPR/Cas9 ERC1 KO skeletal muscle cell line reconstituted with ERC1 or ERC1-short, an isoform endogenously expressed in skeletal muscle.

Results

In HEK cells both ERC1 isoforms increase CaV1.1 functional expression. On the other hand, in skeletal muscle, ERC1 overexpression decreases CaV1.1 currents, without affecting EC coupling. ERC1-short overexpression does not affect CaV1.1 currents, but significantly increases EC coupling. We therefore generated two ERC1 KO cell lines: one deleting specifically the long isoform and one deleting both. In the cell line deleting specifically the long isoform, both ERC1 and ERC1 short significantly increase EC coupling without affecting CaV1.1 functional expression.Ongoing experiments will reveal the effect of the deletion of both ERC1 isoforms.

Conclusions

The short ERC1 isoform increases voltage-induced calcium release from the sarcoplasmic reticulum. Ongoing experiements will reveal its binding partners in the triads and its therapeutic potential in the treatment of myopaties.

B 02 | Ion channels (epithelia and other systems)

B 02-01

Heteromeric polycystin-2/polycystin-1 and homomeric polycystin-2 ion channels demonstrate distinct properties in a heterologous expression system

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Content

Autosomal-dominant polycystic kidney disease (ADPKD) is caused by mutations in the PKD1 or PKD2 gene coding for polycystin-1 (PC1) or polycystin-2 (PC2), respectively. Conflicting results have been published regarding the ion channel properties of PC2 as a homomer or in complex with PC1. This is partially due to the fact that PC2 and PC2/PC1 produce very low currents in heterologous expression systems. Guided by the available cryo-electron microscopy (cryo-EM) structure of PC2/PC1 complex (PDB ID 6A70), we generated a novel gain-of-function (GOF) PC1 mutant ($PC1^{GOF}$). Coexpression of $PC1^{GOF}$ with a known $PC2^{GOF}$ construct produced PC2/PC1 heteromeric ion channels with high monovalent cation permeability in *Xenopus laevis* oocytes. The formation of PC2/PC1 complexes was confirmed by co-immunoprecipitation experiments. Heteromeric PC2/PC1 channels exhibited significantly altered cation selectivity properties compared to homomeric PC2channels. In contrast to PC2homomers, PC2/PC1heteromers were

permeable for organic cations (DME^+ and DEA^+), but almost impermeable for Ca^{2+}. By varying the expression ratio of $PC1^{GOF}$ and $PC2^{GOF}$ we demonstrated that PC2 preferentially forms heteromeric complexes with PC1 rather than homomeric ion channels. In addition, we re-interpreted the original EM map corresponding to the published PC2/PC1 structure. This analysis suggested that the PC1 pore-forming domain exhibits a canonical TRP-like conformation. This interpretation is consistent with results from experiments involving a covalent modification of $PC1^{GOF}$ by a cysteine modifying reagent (MTSET). Our data suggest that PC2 homomers and PC2/PC1 heteromers are two distinct types of ion channels and likely play different (patho-)physiological roles.

B 02-02

Comparing electrophysiological data in healthy and CF primary human respiratory epithelia with morphological conditions

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Content

Cystic Fibrosis (CF) is an inherited disease that arises from the malfunction of the cystic fibrosis transmembrane conductance regulator (CFTR). This eventually disrupts ion and water homeostasis resulting in more viscous mucus on airway epithelia. To investigate differences in ion transport properties of healthy vs CF, we utilized a modified Ussing chamber technique known as the Multi Transepithelial Current Clamp (MTECC). This innovative approach enables us to investigate the electrical properties, such as transepithelial resistance (R_t) and potential. We can calculate the transepithelial conductance (Ct) across healthy and CF airway cells derived from nasal brushings from these values. The primary cells are cultured under air-liquid interface (ALI) conditions to recreate pseudostratified epithelium in vitro. Our investigation uncovers significant differences in Ct between healthy cells and those affected by CF, with healthy cells exhibiting notably higher conductance levels while CF cells with lower conductance. Moreover, our analysis of immunofluorescence staining with ALI filter cryosections shows a contrast in cell layer thickness, with healthy cells presenting thicker layers compared to CF cells. Intriguingly, these findings correlate with a lower R_t observed in healthy cells and a higher R_t in CF cells. We propose that increased thickness of healthy cell layers allows more functional ion transport systems, facilitating smoother ion flow, and thereby lowering resistance. Conversely, thinner CF cell layers likely possess fewer functional transport systems, disrupting ion flow and elevating resistance. Unraveling these cellular differences holds promise for a deeper understanding of CF and paves the way for targeted therapeutic interventions.

B 02-03

Analysis of the transport mechanism of SLC26A6

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Content

Solute Carrier Family 26 (SLC26) genes encode multifunctional anion exchangers and channels that transport a broad range of substrates.SLC26A6 is a Cl^-/HCO_3^- exchanger that also mediates transport of divalent anions (sulfate, oxalate). Electrogenic transport was proposed in some studies, with a 1:2 stoichiometry for monovalent exchange. In other studies, the transport mode was reported as electroneutral. Thus, transport mode and stoichiometry are still controversial. In this study, we aim to solve this controversy and clarify the transport mechanism of SLC26A6.
Transport currents were measured to identify electrogenic transport modes while bicarbonate: chloride exchange was also recorded by fluorometry with the pH-sensitive dye pHrodo. Currents were recorded by whole-cell patch-clamp in CHO cells and by 2-electrode voltage clamp from *Xenopus* oocytes expressing SLC26A6. Transport substrates (oxalate, sulfate, bicarbonate) were applied to the extracellular solution while varying the concentration of counter-transported chloride. Membrane localization of the GFP-fused constructs was checked by confocal microscopy.
CHO cells and *Xenopus* oocytes expressing mouse SLC26A6 yielded robust transport currents in the presence of divalents. Smaller but robust currents were detected in the presence of HCO_3^-, indicating electrogenic Cl^-/oxalate (1:1), Cl^-/sulfate (1:1), and Cl^-/HCO_3^- (1:2) exchange. Electrogenic Cl^-/HCO_3^- exchange was suppressed by millimolar concentrations of extracellular Cl^-, indicating that 1 Cl^- : 2 HCO_3^- stoichiometry is inhibited competitively by extracellular Cl^-. This competition by Cl^- may explain differences in previously published stoichiometries. Moreover, electroneutral 1:1 Cl^-/HCO_3^- exchange may be the predominant transport mode under physiological conditions, where substantial extracellular Cl^- is present.

B 02-04

Zinc blocks the HCNL1 proton channel

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Content

Ion channels can be activated or inhibited by pharmacological agents. A common modulator of channel activity is zinc (Zn^{2+}). In particular, proton channels were shown to be sensitive to Zn^{2+}: Zn^{2+} blocks the proton channel Hv1, and Zn^{2+} blocks or potentiates proton channels of the OTOP family. Here, we tested the Zn^{2+} sensitivity of the recently discovered

hyperpolarization-activated HCNL1 proton channel. HCNL1 was heterologously expressed in *Xenopus laevis* oocytes and recorded using the two-electrode voltage clamp technique or excised marco patches. We show that HCNL1 is inhibited by micromolar concentrations of extracellular Zn^{2+}. Our data show that Zn^{2+} slows activation and deactivation kinetics and shifts the voltage dependence of activation to more negative potentials. Voltage-clamp fluorometry experiments show that Zn^{2+} alters voltage-induced conformational changes in HCNL1. Interestingly, HCNL1 is also inhibited by intracellular Zn^{2+}. The HCNL1 proton channel is expressed in zebrafish sperm; Hv1 is expressed in human sperm. Zn^{2+} has been suggested to play an important role in the male human reproductive system. It is tempting to speculate that Zn^{2+} might also have a physiological role in the male zebrafish reproductive system.

B 02-05

p53 regulates TMEM206 in a p21-dependent manner and controls acid-induced cell death in HCT116 colorectal cancer cells

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Content

Acid-induced chloride-flux might play a role in cancer and inflammation, where tissue acidification is prevalent. In 2019, TMEM206 was identified as the key component of the acid-sensitive outwardly-rectifying anion channel (ASOR), mediating chloride flux at low pH. Localizing to the plasma membrane, TMEM206 contributes to cellular processes like acid-induced cell death. Since over 50% of human cancers carry loss-of-function mutations in the p53 gene, we aimed to analyze how TMEM206 is regulated by p53 and its role in acid-induced cell death in HCT116 colorectal cancer cells. To answer this question, we generated p53-deficient HCT116 cells and assessed TMEM206-mediated Cl^--currents and transcriptional regulation using patch-clamp and dual-luciferase reporter assays, respectively. To assess the contribution of TMEM206 in p53-mediated acid-induced cell death we performed cell death assays.

In our study, we observed increased TMEM206 mRNA levels and currents in HCT116 p53-knockout cells. This phenotype can be rescued by transient overexpression of p53. TMEM206 promoter activity is not altered by p53, but knockout of p21 increases Cl^--currents. In p21-deficient cells, p53 overexpression does not reduce TMEM206 currents; conversely, p21 overexpression decreases currents compared to the control group. In the acid-induced cell-death assay, p53-deficient HCT116 cells showed decreased cell death, while TMEM206-knockout cells displayed a small increase compared to parental cells. In p53-knockout cells this effect of TMEM206-knockout is absent, suggesting that TMEM206 in cell death lies within the p53 pathway.

These observations suggest that p53 regulates TMEM206 in a p21-dependent manner. In addition, TMEM206 contributes to acid-induced cell death in a p53-dependent manner.

B 02-06

The relationship between extracellular and intracellular pH in glioblastoma cancer stem cells – influence of acid transporters

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Content

Glioblastoma multiforme (GBM) is the most aggressive brain tumor in adults. The tumor microenvironment (TME) of GBM is acidic due to the accumulation of metabolic byproducts. Despite the acidic TME, GBM cells can maintain an intracellular pH (pH_i) that allows them to thrive. However, the mechanisms of pH_i regulation in GBM cells under acidic stress are poorly understood.

In this study, we preconditioned two GBM-derived stem cell lines (GSCs), R54 and R8 for two weeks at an acidic pH of 6.6 to mimic chronic acidic stress. Additionally, we used a two-hour preincubation at pH 6.6 to mimic acute acidic stress. Subsequently, we measured pH_i with a ratio-metric, fluorescent dye.

RNA sequencing revealed that different transporters associated with acid extrusion, especially the Na^+/HCO_3^--cotransporters (NBCs) SLC4A7, SLC4A5, and SLC4A8, were upregulated under chronic acidic stress. To assess their role, we measured pH_i of GSCs with and without inhibition of different acid-base transporters. Following chronic acidic stress, GSCs had a slightly higher pH_i when measured at pH 7.4, compared to their control preconditioned at physiological pH, suggesting an increased capacity to extrude acid. Acute acidic stress did not result in a change in pH_i. However, contrary to our expectations, the pan-NBC inhibitor S0859 increased pH_i at pH 7.4 and 6.6. To further reveal the role of NBCs in pH_i regulation of GSCs, we generated R54 GSCs with a knockout of different NBCs. We are currently analysing these knockout cells. Our results will enhance our understanding of pH_i regulation in GBM.

B 02-07

Targeting the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ in pancreatic ductal adenocarcinoma

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Content

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in Western countries. The molecular mechanisms that give rise to PDAC are far from being clear and have not yet delivered efficient therapies. PDAC is linked to the physiology and microenvironment of the exocrine pancreas. In PDAC, the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ is massively overexpressed and indicates a poor prognosis. Despite some *in vitro*

information on the role of $K_{Ca}3.1$ channels on individual cell types of PDAC, its role is still poorly characterized, and *in vivo* data are missing.
In vivo experiments were performed using the$Kras^{LSL-G12D/+}Trp53^{fl/fl}Pdx1^{Cre/+}$ (KPfC) PDAC mouse model. Mice were treated with vehicle, gemcitabine, the $K_{Ca}3.1$ inhibitors TRAM-34 and maurotoxin or a combination of an inhibitor with gemcitabine. Retrieved pancreata were sliced, stained, and analyzed to assess tumor size and the extent of fibrosis. This was complemented through the evaluation of $K_{Ca}3.1$ inhibition on migration in a spheroid model of PDAC.
The inhibition of $K_{Ca}3.1$ in combination with gemcitabine leads to a moderate decrease in tumor size and a reduction of gemcitabine-induced fibrosis. In mixed spheroids, $K_{Ca}3.1$ inhibition in combination with gemcitabine decreases the invasive potential and induces cell death. This is accompanied by a change from an elongated to a round morphology of the migrating cells. More interestingly, the inhibition of plasma membrane $K_{Ca}3.1$ by maurotoxin decreases the invasive potential of the spheroids more efficiently than the combination of TRAM-34 and gemcitabine suggesting that plasma membrane and intracellular $K_{Ca}3.1$ have distinct effects in PDAC.

B 02-08

Do TMEM175 channels conduct protons and K^+ ions at the same time?

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Content
Question
Originally TMEM175 was described as lysosomal K^+ channel but later this view has been challenged as functional and structural analysis suggested that TMEM175 may function as K^+ channel at neutral pH but as highly selective H^+ channel with strongly reduced K^+ conductance under acidic conditions that are typical for lysosomes.
Methods
Here, we studied the ion selectivity and gating of TMEM175 channels heterologously expressed in HEK293 cells or *Xenopus laevis* oocytes with patch clamp measurements in the whole cell mode as well as in the outside-out and inside-out patches.
Results
Using a voltage pulse protocol designed to avoid H^+ accumulation we resolved the time-course of pH activation and after correcting for the effect of pH on the H^+ conductance we estimated the pH dependent change in open probability. Our analysis suggested an apparent pKa value of about 5.5 for the pH sensing mechanism of TMEM175. Furthermore, our measurements revealed that a low extracellular pH not only activated the H^+ conductance but concurrently also increased the K^+ (and Cs^+) conductance.

Conclusions

These observations suggest that extracellular acidification induces a conformational change in TMEM175 that enhanced both the H^+ as well as the K^+ conductance suggesting that the pore can conduct both type of ions simultaneously.

B 02-09

Experiments in the student laboratory course: effects of snacking on blood glucose levels

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Content

Question

Students frequently snack on ice-tea powder. The following experiment was performed in a physiological laboratory course for medical students to evaluate the physiological effects of this practice.

Methods

Six volunteers from each group were asked to refrain from eating before the course. After an instruction on laboratory safety, blood glucose concentration was self-measured using a commercial testing kit, after which three students consumed 83 g of ice-tea powder (ice-tea group) or an equicaloric snack of (unsalted) peanuts. Further values were obtained after 0.5, 1 and 2 hours. Data were pooled with results from previous groups.

Results

In the ice-tea group, blood glucose concentration (mg/dl) rose significantly ($p < 0.001$) from 103 ± 6 to 153 ± 8 (0.5 h), dropping to 115 ± 5 ($p < 0.001$) after 1 h, and 98 ± 4 after 2 h (13 males, 13 females). In the peanut group, values were 97 ± 6, 97 ± 4, 95 ± 3, and 94 ± 2 ($p < 0.05$ versus baseline, 12 males, 14 females). No sex-related differences emerged in choice of snack or blood sugar levels. Male students volunteered to participate more frequently.

Conclusions

The peak in blood glucose concentration seen in the group of students who consumed the high sugar snack was expected. Possible reasons for the small drop in the blood glucose concentration in the peanut group were discussed, such as the possible secretion of incretins (GIP or GLP-1). It will be interesting to see if this result is reproducible in further courses.

B 03 | Ion channels (sensory and other systems)

B 03-01

Pathophysiology of mutations of a cation channel of the transient receptor potential superfamily found in patients with primary aldosteronism

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Content

The steroid hormone aldosterone is produced in the glomerulosa cells of adrenal cortex. Aldosterone increases sodium reabsorption and potassium secretion in the kidneys and is significantly involved in the long-term regulation of blood pressure. Around 10% of cases of high blood pressure are due to autonomous aldosterone production, known as primary aldosteronism. We identified mutations of a cation channel of the transient receptor potential superfamily (here called "*TRP-Aldo*") in aldosterone-producing adrenal adenomas of patients with primary aldosteronism. Physiologically, the stimulation of aldosterone production in glomerulosa cells by angiotensin II and increased plasma K^+ levels is mediated by a depolarization of the cell membrane and an increase in cytosolic Ca^{2+} activity. HEK293 cells expressing the mutant form of *TRP-Aldo* had a depolarized membrane potential and showed increased Na^+-dependent inward-currents. Basal cytosolic Ca^{2+}-activity measured with fura-2, was increased in both HEK293 cells and adrenal HAC15 cells expressing the mutant *TRP-Aldo* channel. In addition, the mutant cells showed signs of impaired intracellular Ca^{2+} handling by exhibiting greater cytosolic Ca^{2+} changes when the extracellular Ca^{2+} concentration was altered. The pathological Ca^{2+} permeability is likely caused by activation of voltage-dependent Ca^{2+}-channels and by a direct Ca^{2+} current via *TRP-Aldo*. In conclusion, increased basal activity of mutant *TRP-Aldo* generates a signal that probably drives autonomous aldosterone secretion in patients with primary aldosteronism.

B 03-02

Distinct mechanisms of inhibition in human TWIK-Related Spinal Cord K+ (hTRESK) channels

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Content

The hTRESK channel was the last discovered member of the two-pore domain potassium (K_{2P}) channels and is amongst the least understood. It has been implicated in migraine and chronic pain, making it an attractive

pharmacological target. Its study is complicated by lack of a crystal structure and of channel openers. However, hTRESK is inhibited by a plethora of substances that activate other K_{2P}s, including cellular lipids. In order to gain insights into the gating and regulation of hTRESK, we explored the mechanism(s) through which various inhibitors act.

Methods

We used giant excised patches from *Xenopus* oocytes to characterize pharmacological substances and endogenous lipids. We investigated binding regions and inhibition mechanisms through competition experiments with Quaternary Ammonium (QA+) ions and by exchanging K+ for Rb+ as the permeating ion. Additionally, we used cysteine and alanine mutagenesis to study the involvement of residues at a putative constriction at F145/F352.

Results & Conclusion

All inhibitors studied competed with QA+ ions, showing that they bind in the pore close to the QA+ binding site. Surprisingly, we found that F145C and F352C had differential impact on their inhibitory effects. Notably, certain inhibitors showed a Rb+-dependence; as the only known structure in ion channels that can discriminate between different permeating ions is the selectivity filter, we conclude that their effect is mediated through an allosteric mechanism that couples binding in the pore to changes in the selectivity filter. Thus, our study classifies diverse inhibitors into 2 distinct mechanisms of action, i.e. pore block vs. allosteric inhibition.

B 03-03

TWIK-related spinal cord potassium (TRESK) channel conductance and action potential frequency are diminished by ceramide synthase 1 and C18:0-ceramide

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Content

The background leak channel TRESK (gene *KCNK18*) is implicated in pain disorders and nociception, and mutations lead to altered excitability in sensory nociceptive neurons by modulating calcium and sodium currents. We hypothesized ceramide synthases (CerS; gene *LASS*), which generate the sphingolipid ceramide, contribute to the regulatory lipid microenvironment for TRESK activity. Transient TRESK overexpression in HEK293 cells reduced *LASS1* mRNA and LASS1 protein by ~35% and ~15%, respectively, but not other *LASS* isoforms. Heterologous hCerS1 expression or delivery of its product C18:0-ceramide (10pM) reduced outward-directed ion currents by ~80% at +100mV in whole-cell patch-clamp recordings in HEK293 cells stably expressing TRESK (HEK-TRESK). Single channel recordings evidenced attenuated TRESK conductance with hCerS1 (control 25.4pS vs. hCerS1 16.5pS) whereas open probability was unaffected. Plasma membrane TRESK expression determined by surface biotinylation and immunoblotting was unchanged in hCerS1-transfected HEK-TRESK cells. In single-cell current-clamp measurements in human nociceptive neurons derived from induced pluripotent stem cells, exogenous C18:0-ceramide (10pM) lowered action potential frequency by ~25% and half-height width (control 0.49ms vs. C18:0-ceramide 0.30ms)

with concomitant increase in amplitude (control 50.8mV vs. C18:0-ceramide 70.7mV). Intriguingly, mouse dorsal root ganglion tissue harbored >50% less *Lass1* and >1,000-fold more *Kcnk18* mRNA compared to spinal cord neuronal tissue by qPCR, suggesting TRESK-inhibiting C18:0-ceramide is minimized in nociceptive neural tissue to promote TRESK activity and pain transmission. In summary, CerS1 and its biosynthetic metabolites most likely alter plasma membrane lipid composition to inhibit TRESK rather than perturb its trafficking, presenting a novel mechanism underlying pain threshold and nociceptor activation.

B 03-04

Oxidative environment modulates TRPM8

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Content

Question

Transient receptor potential melastatin subtype 8 (TRPM8) is an outwardly rectifying non-selective cation channel. As a multimodal nociceptor, it is involved in the sensation of cool temperatures as well as various “cooling agents”, such as menthol. Several TRP channels have been shown to be sensitive to an oxidative environment, resulting in altered responses towards ligands and environmental cues, or even direct activation. However, respective evidence for TRPM8 is lacking.

Methods

We investigated the effect of oxidative environment using H_2O_2, which can oxidize cysteine and methionine amino acid residues as well as Chloramine-T, which preferentially oxidizes methionine. HEK293T cells were transfected with human TRPM8 and studied using patch-clamp electrophysiology. The effect of oxidative agents on the biophysical properties as well as cooling- and menthol-evoked responses of human TRPM8-transfected cells at -60 mV was quantified.

Results

Compared to the control treatment, H_2O_2 (10 mM) led to a decrease in cold-evoked peak inward currents (22% at 2 min and 57% at 4 min) and menthol-evoked (300 µM 28% at 4 min) peak inward currents in human TRPM8-transfected cells. In addition, chloramine-T (1 mM) caused a rapid and even more pronounced decrease of menthol-evoked inward currents along with a gradual decrease in voltage-dependent channel activation measured at +100 mV.

Conclusions

We conclude that oxidative agents decrease both cold- and menthol-evoked currents of human TRPM8. This may be due to changes in oxidation-prone amino acid residues leading to a rightward shift in the voltage dependence of channel activation.

B 03-05

Hyperforin, an activator of TRPC6 channels, elicits biphasic effects on contractility of adult rat cardiomyocytes

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Content

Question

TRPC are cation channels activated by diverse means. Altered expression and function of TRPC have been implicated in cardiac disease. There are multiple TRPC isoforms in myocardium, but the physiological roles of individual TRPC isoforms remain elusive. We tested the hypothesis that activation of TRPC6 is involved in regulation of cardiomyocyte contractility.

Methods

Isolated adult rat cardiomyocytes were electrically-stimulated and sarcomere shortening was recorded on an IonOptix setup. Confocal calcium imaging was performed on cardiomyocytes loaded with Fluo-4/AM. Hyperforin, a compound from St. John's Wort, was used as a selective activator of TRPC6 channels.

Results

In ventricular myocytes, hyperforin elicited a concentration-dependent (0.1–0.3–1µM) positive-inotropic and positive-lusitropic effect. At 1µM, hyperforin increased fractional shortening (FS) within a few minutes by 33±4% (n=28, $P<0.0001$ vs control), whereas time-matched control cells exhibited a reduction in FS. In some cells, the positive-inotropic effect was followed by a steep decline or loss of contractility. The fraction of cells exhibiting hyperforin-induced decline/loss of contractility increased in a concentration-dependent manner (0% at 0.1µM, 14% at 0.3µM, 48% at 1µM). BI-749327 (100nM), an inhibitor of TRPC6, did not affect contractility (n=21). Store-operated calcium entry (SOCE), induced by re-addition of calcium to calcium-depleted cardiomyocytes, was increased >2-fold by hyperforin (5µM, n=20). Qualitatively similar results were obtained in atrial myocytes.

Conclusion

Hyperforin elicits biphasic effects on cardiomyocyte contractility and increases SOCE. The positive-inotropic effect of hyperforin may be explained by increased calcium influx via TRPC6. The mechanisms underlying the decline of contractility remain to be determined.

B 03-06

Investigation of TRPM4 in cancer hallmark functions in 2D & 3D colorectal cancer models

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Content

Colorectal cancer (CRC) is one of the top three occurring types of cancer worldwide and is characterized by high mortality rates. Many proteins are dysregulated in CRC, including the transient receptor potential melastatin 4 channel (TRPM4). TRPM4 is a non-selective, calcium-activated cation channel, which is linked to different pathologies i.e., cardiac malfunctions, immune diseases, and CRC. Recently, our in vitro analysis on established CRC cell lines showed a differentiated regulation of TRPM4 expression in CRC as well as a role for TRPM4 in intracellular vesicles. One important hallmark of cancer is cell migration, crucial for metastasis and the progression of the disease. It also constitutes the major reason for death for CRC patients. We generated TRPM4 knock-out clones of the CRC cell lines Lovo and DLD1 with the CRISPR/Cas9 technique and we are investigating how TRPM4 affects cellular functions. For our experiments, we use 2D cell cultures, but also 3D tumorspheres, to mimic the patient tumor environment. Initial experiments with the Lovo cells show that knock-out of TRPM4 leads to higher proliferation and migration in 2D. Knock-out of TRPM4 also reduced the size of 3D cancer tumorspheres of the Lovo cells and changed tumorspheres' outgrowth. In conclusion, TRPM4 contributes to CRC hallmark functions in 2D and 3D cellular systems and could potentially be a putative target in CRC therapy.

B 03-07

Unraveling the selectivity of ionic pathways of TRPM3 channel isoforms

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Content

Like all members of the TRP family, tetrameric TRPM3 channel complexes have four voltage-sensing-like domains (VSLD) and a central ion-conducting pore. In TRPM3 channels, due to alternative splicing, there are two versions of the central pore, which differ by the insertion of 12 amino-acids, thereby changing the biophysical and pharmacological properties profoundly. For instance, the short-pore isoform is activated by pregnenolone sulfate (PS) while the long-pore isoform is opened by clotrimazole. Additionally, the opening of a Na^+-preferring, non-canonical pore in the VSLD

of short-pore, but not long-pore, TRPM3 channels was reported when co-applying PS and clotrimazole at hyperpolarized membrane potentials.
Here, using HEK293T cells for heterologous expression, we investigated the selectivity of ionic pathways through TRPM3 channel variants by recording reversal potentials in the whole-cell patch-clamp configuration.
Clotrimazole alone or co-applied with PS did not shift the reversal potential of TRPM3 currents in any splice variant. However, the expected shifts in reversal potentials were found when co-expressed sodium-selective FaNaCh channels were opened. Furthermore, clotrimazole did not affect the block of short-pore TRPM3 channels by extracellular Na^+, a unique biophysical property of the central pore of these channels. Finally, we confirmed that short-pore TRPM3 channels have higher permeability ratios for divalent cations, compared to the long-pore isoform, regardless of the agonist employed.
These results show that the clotrimazole-induced conductance is non-selective for cations and has exactly the same selectivity profile as the central pore. These data do not support the existence of a separate non-canonical ionic pathway through TRPM3.

B 03-08

Human TRPA1 single nucleotide polymorphisms

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Content

Question

Mutations in ion channels can lead to functional changes, resulting in either loss or gain of function. Specifically, activation of the TRPA1 channel has been found to induce pain in humans. The dbSNP database lists five missense single nucleotide polymorphisms (SNPs) in the TRPA1 gene—R3C, R58T, E179K, K186N, and H1018R — appearing in over 10% of the general population. This study aimed to compare these genetic variants to the most common TRPA1 form in terms of their effects on known activation mechanisms.

Methods

Using site-directed mutagenesis, human TRPA1 channel variants were generated and expressed in HEK293t cells, and their responses were analyzed with calcium microfluorimetry. Responses to both electrophilic (AITC and JT010) and non-electrophilic (Carvacrol and PF-4840154) agonists were tested. Given that TRPA1 variants usually occur heterozygously and can form channels with mixed TRPA1 monomers, cotransfection experiments were conducted with the most common form and each SNP, using varying agonist concentrations. Voltage dependent activation of TRPA1 variants was also probed.

Results & Conclusions

Results showed that each agonist elicited concentration-dependent activation of human TRPA1 channels, with the R58T variant consistently exhibiting a higher EC50 than other variants. The other SNPs did not significantly alter

sensitivity to the pharmacological agonists. Further research is needed to determine if individuals heterozygous for the R58T mutation might experience reduced TRPA1 responsiveness.

B 03-09

Modulation of Acid-Sensing Ion Channel 3 by the thyroid hormone T3

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Content

Acid-sensing ion channels (ASICs) are proton-gated Na^+ channels predominantly expressed in the nervous system. ASIC3 is an important ASIC subunit in the peripheral nervous system and plays an important role in the pain pathway. The thyroid hormone, triiodothyronine (T3), plays a key role in the regulation of metabolism and growth. T3 binds to nuclear receptors and is believed to act primarily via the regulation of protein expression. However, few studies have addressed its direct effects on ion channels.

We expressed rat ASIC3 (rASIC3) in HEK cells and found that micromolar concentrations of T3 strongly potentiated the proton-activated currents of rASIC3 (EC_{50} > 100 µM at pH7). Mechanistically, T3 increased the proton affinity of rASIC3, shifting activation curves to a more alkaline pH, resulting in the potentiation of both transient and window currents. Strikingly, for human ASIC3 (hASIC3), T3 induced a strong sustained current at neutral pH (EC_{50} > 10 µM); nanomolar concentrations of T3 (10 – 100 nM) were sufficient to induce ASIC currents. hASIC3 currents elicited by T3 were selective for Na^+ (E_{rev} = +31.2 mV) and inhibited by amiloride (IC_{50} = 264.6 µM), a canonical ASIC inhibitor.

While plasma concentrations of free T3 are normally in the picomolar range, we speculate that upon release of T3 from thyroid follicular cells, T3 could reach concentrations high enough to activate ASICs. We found that ASIC3 is indeed expressed in a human thyroid follicular epithelial cell line. We are currently investigating the effects of T3 on ASIC3 in this cell line.

B 03-10

Piezo1 as protector in endothelial stiffening and vascular inflammation

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Content

Endothelial ion channels like the mechanosensitive Piezo1 are crucial regulators of the blood pressure. Due to their localization, the mechanical properties of the endothelial cell surface are maintained through direct interaction with structural cellular components. It is known that both the endothelial glycocalyx (eGC) and the cell cortex, an actin-rich layer underneath the plasma membrane, are very dynamic and associated with proper endothelial function. Chronic mechanical stiffening of the endothelial cell (EC) surface leads to endothelial dysfunction and vascular inflammation. Here we hypothesize that eGC and Piezo1 interact as endothelial mechanosensors, maintaining the endothelial behavior under inflammatory conditions.

To investigate the anti-inflammatory effects of Piezo1 on endothelial nanomechanics, ECs were treated with TNF-α and the Piezo1-agonist Yoda1. The nanomechanical properties of the eGC were quantified using Atomic Force Microscopy-based nanoindentation in Piezo1-depleted ECs (CRISPR-Cas9-induced) as well as endothelial specific Piezo1-K.O. mouse aortas.

TNF-α-induced inflammation of ECs led to severe eGC damage. We observed that this effect could be reversed by simultaneous activation of Piezo1 (by Yoda1). Cell adhesion experiments demonstrated that Yoda1 also inhibits the TNF-α-mediated increase in monocyte adhesion, which could be verified by monocyte wash-away assay. In contrast, depletion of Piezo1 abolished the effect of Yoda1 after TNF-α treatment.Based on the present data, we propose that Piezo1 is a crucial regulator of endothelial behavior under inflammatory conditions. Its activity enhances has vasoprotective effects especially in terms of improvement of the eGC integrity. Thus, Piezo1 may serve as a pharmaceutical target to prevent the development of cardiovascular pathologies.

B 03-11

The MedEdCloud®: A cooperation for quality-assured digital media

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Content

Question

[Digital media are an indispensable part for teaching and learning in medical education. A single faculty can hardly provide resources to create media for the entire curriculum despite the existing expert knowledge. Purchasing media is expensive. Individual lecturers face barriers when searching for and using high-quality media. Therefore, we have established a digital cooperation platform enabling the exchange and use of high-quality digital teaching media between medical faculties and lecturers.]

Methods

[Based on the open-source data management system Pimcore, we created the front and backend structures for a media database. We implemented Elastic Search for efficient searching and Medical Subject Headings (MeSH) for keywording. Members of the cooperating faculties can easily log in to the platform via SSO.]

Results

[Apart from 120 common media formats, the MedEdCloud® can display specific formats (virtual microscopy, 360° images, virtual patient cases). Media can be downloaded or integrated directly into learning management systems (LMS). To ensure the high quality of the provided media, a process has been implemented that enables structured quality control by editors.]

Conclusions

[The MedEdCloud® is a portal for quality-assured teaching and learning media in medical education. It enables all cooperating partners to share, view, and download media or integrate them directly into their own LMS. The use of Pimcore as the software basis ensures long-term operations at reasonable costs. The MedEdCloud® is a joint project of the medical faculties in Baden-Württemberg and open to other medical faculties to join the cooperation.]

B 03-12

Thermal hypoalgesia as a marker for diabetic neuropathy in larval zebrafish – TRPV1-dependent nociception as a model to study loss of small fiber function in diabetes

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Content

QuestionDiabetic peripheral neuropathy (DPN) affects 50% of diabetic patients leading to sensory loss indicated by increased warmth detection and heat pain thresholds (HPT). We developed novel methods to investigate TRPV1-dependent nocifensive heat responses and temperature preference in larval zebrafish, with which we determined the sensory phenotype in models of diabetes mellitus regarding DPN.

Methods

We injected embryos with trpv1, pdx1, glo1, or control morpholino oligonucleotides, or immersed larvae in glucose or mannitol, or methylglyoxal from 24-96 hours post fertilization. Larvae were treated for hyperglycemia with PK11195. We randomly applied heat stimuli of different intensities (150ms, 18-88 mW) with a near-infrared diode laser, and recorded behavior in a thermogradient (18-36°C) for 30 minutes.

Results

TRPV1 knockdown (62.5 ± 6.5 mW [mean±SD]) significantly increases HPT compared to controls (28.6 ± 4.4 mW). Both pdx1 knockdown (50.1 ± 4.9 mW) and glucose incubation (10 mM: 42.3 ± 4.7 mW) elevate HPT compared to controls (33.3 ± 3.6 mW), or mannitol incubation (10 mM: 33.5 ± 3.6 mW), which was prevented by PK11195. Glo1 knockdown (45.5 ± 4.9 mW) and methylglyoxal incubation (50 mM: 47.3 ± 4.9 mW) increase HPT. Both pdx1 and glo1 morphants prefer higher temperatures in the thermogradient than controls.

Conclusions

We demonstrate nocifensive behavior can be quantified in larval zebrafish. Heat nociception depends on zebrafish TRPV1 and its genetic ablation results in distinct thermal hypoalgesia. Further, we confirmed thermal hypoalgesia observed in DPN patients in all tested zebrafish diabetes models, opposed to thermal hyperalgesia observed in most rodent models.

B 04 | Blood and hypoxia

B 04-01

Propofol effects hypoxia-inducible factor signaling in human leukocytes under physiological oxygen exposure

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Content

Propofol is used for anesthesia and is dissolved in soybean oil. Side effects include hypotension, respiratory depression, and immune/nervous system abnormalities. Long-term use may cause the often fatal propofol infusion syndrome (PRIS). Propofol treatment is associated with altered accumulation of hypoxia-inducible factor (HIF) in leukocytes. The precise regulation of the HIF pathway is critical for an adequate immune response. The mechanism of propofol-induced changes in the HIF pathway and its effect on leukocytes is still unknown. A better understanding of the potential immunologic side effects of propofol on human leukocytes is needed to improve patient outcomes during prolonged anesthesia.

The effects of different oxygen concentrations (21%, 8%, 1%) on HIF pathway in human peripheral blood mononuclear cells (PBMCs) were investigated by Western Blot (protein) and qPCR (gene). Furthermore, we analyzed the influence of clinically relevant concentrations of propofol and its solvent soybean oil on HIF of PBMCs.

Our data indicate an activation of the HIF pathway under hypoxic conditions and a slight decrease in HIF-1α accumulation after propofol/soybean oil treatment under hypoxia.

We also plan to analyze leukocyte function under propofol treatment by measuring oxygen consumption rate and extracellular acidification rate of living cells in a time-dependent manner (Seahorse Extracellular Flux Analysis). Further studies are needed to improve the safety and usability of the drug for patients undergoing prolonged anesthesia and to elucidate possible immunomodulatory effects of propofol.

B 04-02

Bone morphogenetic protein 9 induces hypoxia-inducible factor-1α accumulation in human leukocytes

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Content

Bone morphogenetic protein 9 (BMP9) is a regulatory cytokine of the transforming growth factor beta (TGF-β) superfamily. BMP9 is involved in angiogenesis and recently, BMP9 has also been suggested to play a role in the immune response. The hypoxia-inducible factor (HIF) helps cells to adapt to low oxygen supply. Studies have shown that leukocytes lacking HIF-1α are dysfunctional.

Patients with mutations affecting genes of the TGF-β/BMP9 signaling pathway (Hereditary Hemorrhagic Telangiectasia (HHT)) exhibit dilated abnormal vessel structures and an impaired immune response. Our group recently demonstrated that HHT leukocytes have decreased expression of the *HIF1A* gene and HIF-1α protein. However, detailed information on how BMP9 affects HIF-1α expression in human leukocytes is still missing.

We isolated human peripheral blood mononuclear cells (PBMCs) from buffy coats and treated them with BMP9 (0, 6, 12 ng/ml) for 4 and 24 hours under varying oxygen conditions. Our results indicate that BMP9 leads to HIF-1α protein accumulation in a concentration-dependent manner under hypoxic conditions. After 24 hours of treatment, *HIF1A* gene expression is increased by BMP9. Based on our assumptions, we expected that a decreased level of the HIF-1α degrading prolyl hydroxylase 2 (PHD2) would explain the accumulation. Nevertheless, our results indicate that there is no change in the levels of PHD2 mRNA and protein. This implies that HIF-1α is regulated through a different mechanism in BMP9 treated PBMCs.

Our findings will help to understand the overall impact of BMP9 on HIF-1α in leukocytes and thus its role in the immune response.

B 04-03

Reduced vacuolar ATPase protects mice from Friend virus infection – an unintended but instructive effect in *Hif-2a^{fl}* mice

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Content

During acute viral infections, innate immune cells invade inflamed tissues and face hypoxic areas. Hypoxia-inducible factors (HIFs) adapt cellular responses towards these conditions. We wanted to investigate the effects of a loss of HIF-2α in macrophages during acute Friend murine leukemia retrovirus (FV) infection in C57BL/6 mice using a Cre/loxP system. Remarkably, mice with a floxed *Hif-2a (Hif-2a^{fl})* did not show any signs of FV infection, independent of Cre activity. This prevented a detailed analysis of the role of macrophage HIF-2α for FV infection but allowed to study a model of unexpected FV resistance. *Hif-2a^{fl}* mice showed a significant decrease in the expression of the *Atp6v1e2* gene encoding for the E2 subunit of the vacuolar H^+-ATPase, which resulted in a decreased acidification of lysosomes and limited virus entry into the cell. These findings highlight that the insertion of loxP sites is not always without functional consequences and has established a phenotype in the floxed *Hif2a* mouse, which is not only unexpected, but unwanted and it is of relevance for the use of this mouse strain in (at least virus) experiments.

B 04-04

The potential role of metabolic dysregulation as an early pathophysiological mechanism in the context of age-related macular degeneration

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Content

Age-related macular degeneration (AMD) is the most common cause of vision loss in people over the age of 50. The most common subtype, dry AMD, is characterized by hypoxia and oxidative stress, which was previously linked with the degeneration of retinal pigment epithelium (RPE) cells. Hypoxia leads to the accumulation of hypoxia-inducible factors (HIFs), in particular HIF-1 and HIF-2, which are dimeric transcription factors with an oxygen-labile α-subunit and a constitutively-expressed β-subunit. Chronic stabilization of HIFs is associated with a poor metabolic situation.

Since AMD was previously linked with metabolic dysregulation in RPE cells and photoreceptors, our aim was to find a link between hypoxia, oxidative stress, and metabolic dysregulation in RPE cells.
For this purpose, we conducted siRNA-mediated knockdowns of the α-subunits of HIF-1 (*HIF1A*) and HIF-2 (*HIF2A*) in a human RPE cell line and treated these cells with sodium iodate ($NaIO_3$), which induces oxidative stress, under normoxic (21% O_2) and hypoxic (1% O_2) conditions. Treatment effects on mitochondrial respiration and glycolysis were analyzed using a Seahorse Bioanalyzer. While under normoxic conditions, $NaIO_3$ treatment led to impaired mitochondrial respiration, under hypoxic conditions mitochondrial respiration was upregulated by $NaIO_3$ compared to control groups. Furthermore, *HIF1A* knockdown resulted in a lack of glycolytic switch under hypoxia, resulting in highly increased mitochondrial capacity. In contrast, *HIF2A* knockdown groups had the lowest mitochondrial capacity.
Taken together, our model enables the investigation of the molecular mechanisms leading to metabolic dysregulation in AMD and validate HIF-based therapeutic approaches to improve mitochondrial function in early AMD.

B 04-05

Xenotopic expression of alternative oxidase challenges disease paradigms but also reveals potential hazards for its use in humans

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Content
Question
The mitochondrial electron transport chain (ETC) is the central building block for oxidative phosphorylation (OXPHOS), but its redox reactions also keep central metabolic circuits operational and generate heat. Disruption of electron flow through the ETC thus impairs the viability of cells and organisms and has been identified as the underlying cause of a heterogeneous group of diseases and conditions ranging from heart failure and diabetes mellitus to cancer and ageing.
Methods
Plants, yeast and some lower organisms, but not insects and vertebrates, harbor a unique enzymatic mechanism that confers resistance to stress associated with ETC dysfunction: alternative oxidase (AOX). Of note, even in cells that naturally lack AOX, it is sorted into the mitochondrial compartment where it becomes catalytically active upon xenotopic expression.
Results
Here the various effects of AOX are described in mammalian cells, fruit flies and mice to which it was transferred to challenge disease paradigms. Interestingly, in some cases AOX showed remarkable rescue effects, while in other cases it had no effect or even aggravated a condition despite seemingly similar conditions or disease etiologies.
Conclusions
Thus, AOX has demonstrated to be a valuable genetic tool for deciphering the pathomechanistic role of ETC dysfunction and may eventually become a therapeutic approach for some of the deadliest medical threats.

B 04-06

Multi-modal regulation of the ciliogenesis-associated function of androglobin

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Content

Androglobin (ADGB) is a highly conserved protein with a unique domain structure: it contains a rearranged globin domain, embedded between a calpain-like protease domain at the N-terminus and a mostly uncharacterised C-terminus. The globin fold is interrupted by a calmodulin-binding IQ motif. Adgb knockout mice display a variety of phenotypes suggesting a link to ciliogenesis, but the underlying mechanistic insights remain mostly unknown. Therefore, we aim to further understand a) the transcriptional regulation of the *ADGB* gene and its association with intracellular pathways and b) the impact of the different protein domains on ADGBs function.

A dual luciferase assay screening approach identified MYBL2 as putative candidate for transcriptional regulation of the *ADGB* locus. We verified this interaction by induction of endogenous ADGB mRNA expression and chromatin immunoprecipitation (ChIP) assays. Furthermore, CRISPR- and mutagenesis-based methods by blocking or mutating *in silico* predicted binding sites revealed a 60 bp region within the *ADGB* promoter which is essential for MYBL2 binding. The transcriptional regulation of *ADGB* via MYBL2 indicates a potential link to the cell cycle and its regulation.

We generated several mutated domains of the ADGB protein and are currently investigating their consequences on the putative function of ADGB during different phases of the cell cycle and ciliogenesis, by targeting morphological and quantitative differences in cilia, as well as for differences in mRNA and protein expression of ciliogenesis related genes and proteins. These approaches will enable us to delve deeper into the mechanistic insights of ADGB involvement in cilia formation and function.

B 04-07

Long-term storage of lecithin-modified nanoscale oxygen carriers after lyophilization

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Content

Question

Allogenic blood transfusions are essential in modern medicine but limited and critically discussed due to potential risk of infection. Perfluorocarbon (PFC)-based artificial oxygen carriers offer a promising alternative due to their high oxygen capacity, available for intravenous use through emulsification. However, colloidal instability of PFC/water emulsions opposes long-term storage stability, which is crucial for clinical application. Lyophilization provides a gentle drying method, ensuring a stable formulation during long-term storage.

Methods

The nanoemulsion consisted of a perfluorodecalin core and a mixed shell of albumin and lecithin (LENOX emulsion). Emulsification was done via Microfluidizer®, a high-pressure homogenizer. After synthesis, different concentrations of trehalose were added as a cryoprotectant, and the product was lyophilized following an established protocol. Particle size and oxygen capacity were analyzed after storage for 24 h and 14 days using dynamic light scattering and the Oroboros oxygraph O2k respirometer, respectively.

Results

The results revealed that 24 h post-lyophilization, the particle size did not exceed that of the non-lyophilized LENOX stored at 4 °C for the same duration (control emulsion). Unlike the lyophilized particles, the particle size of the control emulsion exhibited significant growth over 14 days. Additionally, the oxygen capacity of the lyophilized particles remained unaffected after 14 days, compared to both, the control emulsion and freshly synthesized LENOX, demonstrating the preservation of functionality.

Conclusions

The data suggest that lyophilization maintains emulsion stability without compromising functionality. Hence, it presents a promising long-term storage method for artificial oxygen carriers, crucial for potential clinical applications.

B 04-08

Automated blood cell recognition and morphological quantifications: AI-based algorithm with an explainable output

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Content

Traditional histologic studies of blood cells are still widely used to diagnose various diseases, although alternative high-throughput methods produce much higher volumes of data. Techniques such as blood smears rely on the expertise of a physician rather than advanced computational methods. This limitation is why, despite progress in microscopy, pathology and computer science, this traditional technique is typically used only in special situations. We aim to advance blood smear analysis by using an AI algorithm to automatically classify blood cells, thereby increasing the data yield of the technique while maintaining its simplicity. Our custom algorithm segments and identifies neutrophils, eosinophils, monocytes, lymphocytes, erythrocytes, and platelets. Unlike previously published algorithms, our code provides explainable AI output that clarifies the features used to identify the highlighted blood cell. As discussed in our recent publication (J. of Mol. Pathology, 2024; 5(1): 28-44), our classification method focuses on the quantification of multidimensional morphological features based on entropy and contrast of gray-level co-occurrence matrices (GLCMNs), quantifications that are unattainable by mere human observation, classical high-throughput methods, or previously published AI techniques. Changes in internal cellular structures associated with disease states can be clustered using these multidimensional GLCM measurements. We conclude that AI-based automated blood cell microscopy has the potential to capture subtle cellular changes prior to the manifestation of a clinically relevant disease state, thereby facilitating and enhancing the data that can be extracted from traditional blood smears.

B 04-09

Nuclear actin shaping the transcriptional response to hypoxia parallel to the HIF pathway

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Content

Actin, a highly conserved and abundant eukaryotic protein involved in the formation of the cytoskeleton, also plays crucial roles in nuclear processes such as transcriptional regulation. Nuclear actin dynamics have been proposed as a mechanism by which cells respond to environmental cues and organize their cellular response. This notion aligns

with the finely regulated levels of nuclear actin and its shuttling between the nucleus and cytoplasm. We find a significant decrease in the nuclear actin pool under hypoxic conditions (1% O_2) across various cell types. Importantly, this reduction occurs independently of HIF (hypoxia-inducible factor)-1, the transcriptional master regulator of the hypoxic response. Fluorescence recovery after photobleaching (FRAP) experiments reveal that hypoxia also disturbs actin's nucleo-cytoplasmic shuttling, slowing import and increasing export. ATP availability, essential for directed nuclear transport, diminishes in hypoxic cells, consistent with reported reduced mitochondrial ATP-concentration under hypoxia. Importantly, reducing cellular ATP-levels under normoxic conditions led to a drop in nuclear actin, suggesting that decreased energy availability may contribute to the observed decrease in nuclear actin. Moreover, our data reveal partial co-localization of nuclear actin with active RNA-polymerase II, indicating a potential role for nuclear actin in modulating gene expression under hypoxia. RNAseq results support this hypothesis, showing changes in the hypoxic transcriptional response when nuclear actin levels are maintained.
In summary, our results demonstrate altered nucleocytoplasmic shuttling of actin, coinciding with decreased ATP availability in hypoxia. We propose that, alongside the classical HIF pathway, nuclear actin dynamics may contribute to the fine-tuning of the hypoxic transcriptional response.

B 05 | Vascular pathophysiology

B 05-01

High density lipoprotein - bile acid conjugates a synergistic interaction restoring endothelial function

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Content

Question

Patients suffering from obesity display symptoms of endothelial dysfunction and exhibit overall increased cardiovascular morbidity and mortality rates. According to current studies weight loss is able to improve cardiovascular morbidity but not mortality. A comparison of serum and tissue samples from patients suffering from obesity prior and post gastric bypass surgery as well as lean controls identified a para-physiologic elevation in systemically circulating bile acids (BAs) unique to gastric bypass patients. A substantial amount of these BAs was found in the high density lipoprotein (HDL) serum fraction. Leading to the question if this increase in systemically circulating BAs alters endothelial cell (EC) function and if this HDL-BA interaction improves HDL quality?

Methods

HDL was isolated from human serum, and conjugated with BAs. Following a verification of the stability of the conjugates via mass spectrometry *in vitro* experiments were conducted, observing the influence of HDL (obese and lean), BAs and the conjugates on primary human aortic endothelial cells.

Results

HDL from obese donors failed to protect ECs from apoptosis, did not reduce TNFα induced inflammatory marker expression or induce NO production in ECs. BAs alone helped to maintain a quiescent EC phenotype by reducing glucose uptake, migration and proliferation. However, conjugates of obese HDL with BAs succeeded in restoring HDL function to comparable levels of lean donor HDL.

Conclusions

HDL-BA conjugates restore the cardiovascular protective function of dysfunctional HDL and following further research might provide completely new treatment aiding life style intervention and possibly reducing cardiovascular mortality.

B 05-02

RyR2 sensitivity to luminal [Ca^{2+}] and its implications for elementary calcium signaling in arterial vascular smooth muscle cells

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Content

Question: Ca^{2+}-dependent relaxation is mediated by localized Ca^{2+} release events through type 2 ryanodine receptor (RyR2) channels in the sarcoplasmic reticulum (SR). We studied two mutant RyR2 mice, i.e. RyR2-R4496C and RyR2-E4872Q, to explore RyR2 gating and its implications for elementary calcium signaling in arterial VSMCs. **Methods:** We investigated spontaneous calcium (Ca^{2+}) sparks in isolated mesenteric artery VSMCs. **Results:** Preliminary data predict that blocking T-type $Ca_v3.2$ channels inhibits Ca^{2+} sparks in RyR2-R4496C and RyR2-E4872Q VSMCs similarly to WT. Additionally, blocking L-type $Ca_v1.2$ channels results in less effective inhibition of Ca^{2+} sparks in RyR2-R4496C VSMCs and more effective inhibition in RyR2-E4872Q VSMCs compared to wild-type (WT). The findings illuminate the functional behavior of both RyR2 mutations in cardiomyocytes. The RyR2-R4496C mutation is characterized as a gain-of-function (GoF), enhancing RyR2's sensitivity to both cytosolic and SR luminal calcium ([Ca^{2+}]). In contrast, the RyR2-E4872Q mutation is recognized as a loss-of-function (LoF), which results in reduced activation by luminal [Ca^{2+}], even though the E4872 residue is located in the cytosolic domain. **Conclusions:** Direct or cytosolic activation of RyR2 in VSMCs is predicted to be normal in both RyR2-R4496C and RyR2-E4872Q mutants. The differences in indirect RyR2 activation suggest that both the R4496C and E4872Q mutations alter RyR2 sensitivity to luminal [Ca^{2+}]. Given that both RyR2 mutations are implicated in causing ventricular tachycardia in humans, therapeutic strategies targeting pathogenic LoF/GoF RyR2 variants must account for their definitive effects on peripheral vascular resistance, particularly in scenarios where direct RyR2 activation remains fully intact.

B 05-03

Elastic and plastic behaviour of ex vivo abdominal aortic walls and aneurysms

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Content

The estimated risk of rupture of abdominal aortic aneurysm (AAA) is the aneurysm diameter and growth rate. Individual factors like gender, age, mean blood pressure and other geometry features of the AAA are currently not taken into account. Finite element method-based (FEM-based) postprocessing of CT-images towards the calculation of the Peak Wall Stress and Peak Wall Rupture Index has been proposed to assist the clinical decision making. To further strengthen this approach a broader base for the used biomechanical properties of AAA tissue is necessary. We here determine the Young´s Modulus and tensile strength of porcine aortas and ex vivo material from human aneurysms. For the porcine aortas we find differences in longitudinal and circumferential samples. For the circumferential samples the Young´s Modulus is higher and there are distinguished peaks compared to the longitudinal samples where the peak is much wider. For the latter, from the same patient two samples are analyzed: one sample from the area of maximal Peak Wall Rupture Risk and one of an area with a low wall tension. From these human samples we find significantly reduced values of the Young´s Modulus and the tensile strength in the area of maximal Peak Wall Rupture Risk as compared to the area of low wall tension. These findings underline the potential of an FEM based decision making.

B 05-04

Critical role of p38 MAPK – NFAT5 – SGK1 signaling in vascular smooth muscle cell calcification

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The author has objected to a publication of the abstract.

B 05-05

Caldesmon expression determines pre-glomerular vascular reactivity and serves as a marker for vascular aging

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Content

Here we explore the role of acto-myosin-tropomyosin-binding protein, caldesmon (CaD) in the regulation of pre-glomerular vascular tone in human and murine renal arteries. Expression of CaD, myosin-light-chain (MLC_{20}), a-smooth-muscle-actin (a-SMA) and the targeting subunit of the myosin-light-chain-phosphatase, MYPT1 were determined in human renal arteries (h-RA) from patients who underwent surgical nephrectomy/enucleation or murine interlobar arteries from young and old animals (y-IA and o-IA) obtained from wild type animals (WT), or animals carrying a global mutation for CaD (CaD+/-). Endothelial function, reactivity to a_1-agonist, phenylephrine, inhibitor of RhoA-associated protein kinase (ROK), Y27632 and activator of the soluble guanylate-cyclase, cinaciguat were tested in IAs by wire-myography. In h-RA, CaD-expression negatively correlated with advanced age, while the expression of MLC_{20} and MYPT1 correlated positively. CaD-mutation reduced the protein by ~50% in IAs from CaD+/- at both ages, but had no effect on the expression of MLC_{20}, a-SMA and MYPT1. Consistent with the findings in patients, CaD-expression was also reduced in o-IAs. CaD-mutation did not affect acetylcholine-reactivity in all groups. Inhibition of endogenous NO by L-NAME had no effect on acetylcholine-relaxation in y-IAs. L-NAME completely blunted this effect in WT o-IA and attenuated acetylcholine-relaxation in o-IA from CaD+/- by 50%. At both ages, phenylephrine-induced contraction was unaffected by CaD-mutation or L-NAME. At all groups, mutation led to rightward-shift of Y27632-relaxation and was without effect on cinaciguat-sensitivity. Present study supports the view that CaD-expression determines endothelial reactivity and sensitivity to ROK of pre-glomerular renal vasculature and seems to be an important marker for vascular aging.

B 05-06

Impact of Secreted modular calcium binding protein (SMOC) 1 on foam cell formation and atherosclerosis development

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Content

Atherosclerosis is a, inflammatory disease characterized by the formation of lipid-laden plaques. Lipoprotein uptake by macrophages to result in the generation of foam cells and the initiation of vascular inflammation play an important role in this process. Here we assessed the role of the secreted modular calcium binding protein (SMOC) 1, a modulator of TGF-β signalling and lysosome formation on foam cell formation and atherogenesis. In in vitro studies assessing the uptake of dil-oxLDL, macrophages from SMOC1+/- mice accumulated more material than cells from wild-type littermates. This was partly attributed to the inability to form functional lysosomes. To determine the impact of SMOC1 deletion on foam cell formation and atherogenesis, we generated mice lacking SMOC1 selectively in myeloid cells ($SMOC1^{\Delta LysM}$ mice). In vitro more ox-LDL was retained in macrophages from $SMOC1^{\Delta LysM}$ mice, which also expressed higher levels of the endosomal marker CD68 and the lectin-like oxLDL receptor compared to the appropriate $SMOC1^{fl/fl}$ wild-type mice. Next, atherogenesis was initiated using AAV8-PCSK9 combined with partial left carotid arterial ligation and feeding ahigh fat diet. In this model, plaque burden was significantly higher in $SMOC1^{\Delta LysM}$ mice. Our data indicate that SMOC1 is necessary for formation of the functional lysosomes in macrophages treated with ox-LDL. Its myeloid specific deletion leads to the enhanced foam cell formation, and accelerated atherogenesis.

B 05-07

Effect of calcineurin (PPP3CB) on basal and AngII-induced renal alterations

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The author has objected to a publication of the abstract.

B 05-08

C-type natriuretic peptide/cyclic GMP signaling counter-regulates metabolic remodelling and hyperproliferation of lung pericytes from patients with Pulmonary Hypertension

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Content

Question

Vascular remodeling in Pulmonary Hypertension (PH) is accompanied by metabolic alterations of vascular smooth muscle and endothelial cells contributing to their hyperproliferative phenotype. Whether such disease-inherent alterations also affect microvascular pericytes is unknown. We investigated the functional and metabolic properties of lung pericytes from PH patients and the impact of C-type natriuretic peptide (CNP)/cGMP signaling.

Results

Under culture conditions, PH pericytes displayed increased proliferation, glucose uptake, and glycolysis in response to Platelet-derived growth factor-BB (PDGF-BB). CNP significantly reduced PDGF-BB-induced proliferation and glucose uptake in both PH and healthy pericytes. Interestingly, CNP decreased glycolysis only in PH pericytes, indicating a unique regulation of glycolytic pyruvate utilization. Indeed, metabolomic analyses revealed a distinct metabolic pathway in PH pericytes, ultimately enhancing de novo pyrimidine synthesis. Notably CNP, via cGMP, counter-regulated these changes. Studies in cultured murine precision-cut lung slices confirmed that CNP inhibits PDGF-BB-induced proliferation and metabolic gene expression of pericytes *in situ*. Supporting the clinical relevance of these findings, immunohistochemistry showed a strong upregulation of the involved metabolic machinery in lungs from PH patients. Concurrently, CNP mRNA expression was significantly downregulated.

Conclusions

Lung pericytes from PH patients have unique metabolic alterations which drive their excessive proliferation and thereby microvascular thickening. Based on the inhibitory effect of *exogenous* CNP in vitro, the diminished expression of endogenous CNP in PH lungs might contribute to the progression of these changes. Consequently, administration of exogenous stabilized CNP might inhibit the metabolic and functional changes of lung pericytes and thereby attenuate the progression of PH.

B 05-09

From physiological research to rational effective therapy of small vessel disease and tissue hypoxia.

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Content

As already reported extracellular potassium ion activity $[K^+]_0$ exerts dual effects on smooth muscle cell membrane voltage. At 3 to 20 mM increasing hyperpolarization and dilatation of arterioles with increasing microflow occur. Above 20 mM potassium ion induced depolarization and constriction with decreasing microflow and tissue hypoxia/anoxia occur, until no flow at 50 mM.

With daily intravenous injection of 6 mM Magnesium within 15 to 20 seconds repolarization can be achieved until all arterioles are again dilated.

As arterioles possess heat sensors reacting only to changes of blood temperature the onset of heat indicates increase of microflow in organs.

Therefore, we determine the partial circulation times in seconds from the beginning of injection until appearance of heat in different regions.

If these circulation times are prolonged or if no heat appears within one minute microflow is decreased. In addition the patients are instructed to respire oxygen 5l/min at least 6-8 hours daily. Oxygen does not constrict arterioles, but besides the appropriate increase in arterial blood pO2, oxygen even ameliorates capillary flow probably by improving blood fluidity.

In more than 2000 patients with severe cerebral, myocardial and peripherical diseases treated in the last 30 years the prolonged circulation times decreased to normal, microflow improved and the symptoms of illness were reduced or even disappeared.

Conclusion:

Disturbed microflow and tissue hypoxia are main pathophysiological mechanisms in severe diseases. Magnesium-Oxygen-Therapy is an effective treatment additionally to usual therapies, without negative side effects.

B 05-10

The endocannabinoid anandamide mediates anti-inflammatory effects through activation of NR4A nuclear receptors

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Content

Background and purpose: Endocannabinoids are lipid mediators, which elicit complex biological effects that extend beyond the central nervous system. Tissue concentrations of endocannabinoids increase in atherosclerosis, and for the endocannabinoid N-arachidonoyl-ethanolamine (anandamide, AEA), this has been linked to an anti-inflammatory function. In this study, we set out to determine the anti-inflammatory mechanism of action of AEA, specifically focusing on vascular smooth muscle cells.

Experimental approach: RNA-sequencing, RT-qPCR, LC-MS/MS, NanoBit, ChIP, microscale thermophoresis, NMR structural footprinting, Gal4 reporter gene assays and loss of function approaches in cell and *ex vivo* organ culture were used.

Key results: AEA pretreatment attenuated the cytokine-mediated induction of inflammatory gene expression such as CCL2. This effect was also observed in preparations obtained from cannabinoid receptor knockout mice and after pertussis toxin treatment. The anti-inflammatory effect of AEA required preincubation, suggesting an effect through gene induction. AEA increased the expression of the nuclear receptors NR4A1 and NR4A2. Knockdown and knockout of these receptors blocked the AEA-mediated anti-inflammatory effect in cell culture and aortic organ culture, respectively. Conversely, NR4A agonists (CsnB, C-DIM12) attenuated inflammatory gene expression. AEA was binding to NR4A and mutations in NR4A attenuated this effect. The interaction of AEA with NR4A caused recruitment of the nuclear corepressor NCoR1 to the CCL2 promoter, resulting in gene suppression.

Conclusion and implications: By binding to NR4A, AEA elicits an anti-inflammatory response in vascular smooth muscle cells. NR4A-binding by AEA analogs may act as novel anti-inflammatory agents.

B 06 | Control of gene expression & signalling

B 06-01

Non-coding genetic variation contributes to cardiovascular physiology and pathology via disrupted RNA:DNA:DNA triple helix formation

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Content

Hypothesis:

RNA:DNA:DNA triple helix (triplex) formation modulates the epigenetic landscape of the cell. The contribution of triplex formation to human physiology and disease remains unknown. We hypothesise perturbed triplex formation disrupts gene networks which contribute to cardiovascular disease.

Methods:

SNPs from the NHGRI GWAS Catalogue were mapped onto predicted triplex-forming regions of ncRNAs. Disease-relevance of triplex SNPs and predicted target genes were compared to stratify triplex interactions with potential relevance to disease. Additionally, lncRNA-centred triplex-gene-disease networks were constructed from predicted triplex formation between lncRNAs and disease-associated genes. These networks allow testing of how perturbations of lncRNA gene expression could influence downstream gene regulation and, as a consequence, progression of associated diseases.

Results:

Triplex targets of 962 SNP-harbouring human lncRNAs were predicted. SNP associations were compared to ontologies of target genes, identifying potential triplex networks. One candidate SNP-harbouring RNA was *RP11.936I5*, which could be linked to erythropoiesis via the target gene *ARID1B.*

A network-based approach linked lncRNA *PVT1* to cardiovascular disease. *PVT1* is predicted to interact with promoters of *LDLR, RBPJ, CXCL12, ADD1* and other cardiovascular disease-associated genes. *PVT1* expressionis dysregulated in patients with coronary artery disease, raising the question of whether therapeutic targeting of *PVT1* could influence pathophysiology of cardiovascular diseases.

Conclusions:

Variation in ncRNA sequence or expression may lead to aberrant RNA-DNA interactions, and represent a novel mechanism by which non-coding variation contributes to human diseases. LncRNA candidates representing each of these two paradigms will be further interrogated for their cardiovascular-relevance molecular and biophysical techniques.

B 06-02

Identification of Toll-like Receptor Interactions

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Content

Introduction

Toll-like receptors (TLR), induce an innate immune reaction by recognizing damage- and pathogen associated molecular patterns. This might result in an inflammatory process. A variety of autoimmune diseases and other sterile inflammations have been linked to the activation of TLRs via DAMPs such as high-mobility group box 1 (HMGB1). Hence, uncovering the full mechanisms of TLR signaling is vital to better understand a multitude of diseases and develop targeted therapies. Ligand recognition is based on the formation of TLR dimers. Those dimers can be both homo- and heterodimers, depending on the TLR in question. The formation of heterodimers has been previously described and is considered to expand the receptors ligand spectrums. However, not all TLR receptor combinations have yet been discovered. Uncovering further receptor combinations and their ligands may help understand the pathogenesis of diseases.

Methods

In this study, Luciferase Complementation Assay (LCA) for TLRs was established to examine which TLR are expressed in physical proximity to each other on the cell membrane. We examined the cell membrane receptors TLR1, 2, 4, 5, 6, and 10. TLR-LCA-fusion proteins were cloned and TLR receptor function was checked by using NF-κB reporter cell assays. To perform the LCA the plasmids were transiently transfected into HEK293T cells. 48h post-transfection the cells bioluminescence was measured over 24 hours.

Results

In an all-(TLR) against all Matrix Experiment we find previously unknown TLR interactions.

Conclusions

The potential TLR interactions might be more complex than known in the literature.

B 06-03

Eplerenone reduces Cirrhosis associated changes of hepatocyte Glucose and Lipid Metabolism

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Content

Question

Studies suggest that mineralocorticoid receptor (MR) activation enhances the progression of liver cirrhosis. Hypoxia, common in liver cirrhosis, induces ligand-independent MR activation in hepatocytes in vitro. Herein, we investigate the impact of hepatocyte MR activation by hypoxia.

Methods

GOterm enrichment analysis was performed on livers from rats treated for 12 weeks with or without CCl_4 and for the last four weeks with or without eplerenone (MR antagonist). Furthermore, we investigated if hypoxia mimicks those changes and alters glucose and lipid metabolism in primary rat hepatocytes (pRH) and human HepG2 cells.

Results

In rats eplerenone reverses the downregulation of genes annotated to the GOterm “Monocarboxylic acid metabolic process” (PPARα, PDK4, AMACR, ABCC2 and Lipin1). In pRH and HepG2 cells, hypoxia reduces the mRNA and protein content of those proteins. This effect can be partially reversed by eplerenone but is not mimicked by aldosterone under normoxic conditions. Those proteins are key regulators of hepatocyte carbohydrate and lipid metabolism. Therefore, we analyzed glucose consumption, lactate production and lipid accumulation in HepG2 cells. As expected, hypoxia increased glucose consumption and lactate production in HepG2 cells. This effect was partially abrogated by eplerenone treatment. Furthermore, hypoxia associated lipid accumulation in hepatocytes is at least partially provoked by MR activation.

Conclusions

Our findings suggest that non-physiological MR activation by hypoxia plays a role in dysregulating glucose and lipid metabolism in hepatocytes, thereby contributing to the development of liver cirrhosis. Hence, MR antagonists have therapeutic potential in the treatment of liver diseases, beside their diuretic effects.

B 06-04

Impact of Nitric Oxide on Mineralocorticoid Receptor Signaling

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

Content

Introduction

The mineralocorticoid receptor (MR) is a key regulator of sodium and potassium balance, blood pressure regulation, and fluid volume homeostasis in response to its principle ligand aldosterone (aldo). This study aims to investigate the regulatory effects of nitric oxide (NO), a signaling molecule with cardioprotective properties, on mineralocorticoid receptor signaling and the underlying molecular mechanisms.

Methods/Results

To investigate the role of NO on transcriptional MR activity, we performed SEAP-GRE and luciferase reporter gene assays, and we could confirm that for its inhibitory effect the CDEF domains are sufficient. With biotin switch assay we demonstrated that NO can lead to S-nitrosylation of MR-CDEF. We investigated potential PTM cites in the MR, mutated cysteine sites to serines. MR activity and expression was tested for the mutants. Our results suggest that NO may interact and potentially inhibit MR function via the DNA binding domain. We further analyzed NGS data of MR overexpressing HEK cells stimulated with aldo and/or NO donor (SNAP). We identified aldo-regulated genes and confirmed their expression by RT-qPCR. Furthermore, we used Ingenuity Pathway Analysis (IPA) to identity a network of pathways affected by both aldo and NO, with notable involvement of the senescence pathway.

Conclusion

We demonstrated that NO inhibits MR activity, induces posttranslational modifications, and thereby influences downstream cellular responses. Moreover, mutational analysis of MR's key domains revealed distinct functional consequences, highlighting the intricate sensitivity of MR signaling to NO modulation. These findings suggest a novel role for NO in modulating cellular aging processes via MR signaling pathways.

B 06-05

Characterization of a high affinity fluorescent phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) probe

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Content

Dynamic changes in the concentration of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), a component of the inner leaflet of the plasma membrane, control many cellular processes, e.g. the function of certain ion channels. Therefore,

monitoring such changes in living cells is desirable. This can be achieved using genetically encoded fluorescently labeled $PI(4,5)P_2$-binding probes such as PLCδ1-PH-GFP and tubby-GFP. Since these changes can putatively occur over a wide range of concentrations, the affinity of the probe should be matched to the range relevant for the question at hand. Previously, we demonstrated that tubby-GFP has a lower affinity than PLCδ1-PH-GFP. Additionally, in that study we constructed and characterized a set of tubby-GFP mutants with reduced affinity.
To supplement the set of available probes with a higher affinity variant, we inserted a second PLCδ1-PH domain into the PLCδ1-PH-GFP probe, expecting an increased affinity.
The apparent $PI(4,5)P_2$ affinity of this tandem-PH-domain probe was measured in living cells semi quantitatively using voltage dependent degradation of $PI(4,5)P_2$ by a voltage sensitive phosphatase (VSP). $PI(4,5)P_2$ dependent translocation of the GFP-tagged probe was observed by total internal reflection microscopy (TIRF-M). Besides affinity, the binding and unbinding kinetics of $PI(4,5)P_2$ probes are relevant when monitoring rapid concentration changes. To this end, we used TIRF-M at high frame rates to measure unbinding kinetics at a temporal resolution that is sufficient to obtain the relevant time constants.
Indeed, we observed an increased affinity and slower unbinding for the tandem PH-domain probe in comparison to the single PH-domain probe.

B 06-06

Interactions of Quinone Derivates with Human Organic Cation Transporters 1-3 and Plasma Membrane Monoamine Transporter: Implications for Antimalaria Drug Pharmacokinetics

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Content

The interactions between quinone derivatives and human organic cation transporters (hOCT1-3) and the plasma membrane monoamine transporter (PMAT), which belong to the SLC22 and SLC29 gene families, may influence the pharmacokinetics of antimalarial drugs. In this study, we investigated the inhibitory and uptake properties of 17 quinone derivatives on hOCT1-3 and PMAT. Our results demonstrate that hOCT1 is moderately to strongly inhibited by all quinone derivatives, with IC50 values ranging from 2.14 to 37.17 µM. hOCT2 is similarly inhibited by all quinones except dihydroartemisinin. Additionally, most quinones inhibited MPP+ uptake via hOCT3, while PMAT showed moderate to no inhibition by these compounds. In terms of substrate properties, most quinone derivatives were not substrates of hOCT1-3 and PMAT. However, certain compounds exhibited moderate uptake: primaquine was taken up by hOCT1-3 and PMAT, cinchonidin by hOCT2, hOCT3, and PMAT, and mepacrine by hOCT2. These findings suggest that the interactions between quinone derivatives and these transporters may contribute to their pharmacokinetic profiles and potentially their therapeutic effects in malaria treatment. Further investigation is required to confirm the involvement of hOCT1-3 and PMAT in the efficacy of antimalarial drugs. Understanding these interactions could aid in optimizing antimalarial drug design and therapy.

B 06-07

Pericellular potassium gradients reported by a genetically encoded extracellular indicator

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Content

High frequency repolarization may lead to pericellular K^+ accumulation which could depolarize cardiomyocytes stabilizing ventricular tachycardia. Methods for precise K^+ measurement in the narrow pericellular space do not exist yet. We have developed an extracellular FRET-based genetically encoded potassium ion indicator with three different mutations to prevent N-glycosylation.

Surprisingly, the half-maximal effective concentration (EC_{50}) of the three mutations were significantly lower in isolated proteins (2.9, 8.3, 14.4 mM) than expressed in HEK293 cells (7.7, 22.1 and 25.2 mM) or in cardiomyocytes (10.1, 33.5 and 34.7 mM), respectively. This may be due to an active K^+ import and diffusion limitation by the pericellular matrix. Accordingly, EC_{50} values in cells were significantly reduced and closer to isolated proteins either after enzymatical removal of the pericellular hyaluronic acid or when expressing the sensors at 30 or 60 nm distance to the cell membrane with a α-helix. Furthermore, we used patch-clamp to control K^+ efflux in GIRK1/2 overexpressing HEK293 cells. In the presence of the GIRK channel opener ML293, depolarization (+40 mV) led to K^+ efflux and increased FRET ratios compared to hyperpolarization (-80 mV) without K^+ efflux. Importantly, this effect was even larger after preventing K^+ import through the Na^+/K^+-ATPase by Ouabain and was absent without ML293.

Thus, the pericellular space represents a K^+ diffusion barrier and the local K^+ concentration is highly affected by K^+ efflux and influx. In the future, our sensors will allow to investigate the effects of fast electrical activity on pericellular K^+ in cardiac tissue or the brain.

B 06-08

Effect of tirzepatide, a GIPR and GLP1R dual agonist, on endothelial cell function and metabolism

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Content

Question

The obesity pandemic is a growing health problem, as obesity is accompanied by several co-morbidities, including cardiovascular disease (CVD). One aspect of CVD in obesity is endothelial cell dysfunction, the first step leading to atherosclerosis. Recently, incretin- based therapies for weight management were approved. Tirzepatide is a dual

agonist of glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1) receptors. Tirzepatide lowers blood glucose and blood pressure, leads to a significant weight reduction and improves dyslipidemia. Both GIPR and GLP1R are expressed on endothelial cells, however the effect of tirzepatide on endothelial cells is not known.

Methods

Human umbilical vein endothelial cells were treated with different concentrations of tirzepatide (1-500 nM). Cell proliferation was assessed using high content screening; nitric oxide (NO) production was measured using the fluorescent DAF-FM diacetate dye, and mitochondrial function was measured using the Seahorse Cell Mito Stress Test.

Results

Tirzepatide had no significant effect on cell proliferation. Basal NO production of cells treated with tirzepatide was either similar or slightly reduced compared to control cells, depending on the concentration. However, acetylcholine-induced NO production in tirzepatide-treated cells was higher compared to control. Moreover, Cell Mito Stress Test assay showed a trend towards a reduced basal and ATP-linked respiration of cells exposed to tirzepatide. Further experiments will be performed to investigate the effect of tirzepatide on glycolysis.

Conclusions

Based on the current results, we hypothesize that tirzepatide treatment leads to a state of metabolic quiescence in endothelial cells.

B 06-09

RADIAnT: Unified identification of statistically-robust RNA-DNA interactions from diverse data types

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The author has objected to a publication of the abstract.

B 07 | Tumorphysiology

B 07-01

The acidic tumor microenvironment hampers tumor cell proliferation via Ca^{2+} signaling

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Content

Question

The role of the tumor microenvironment in tumor progression is more and more appreciated. Yet the acidification of the tumor mass, due to metabolic changes and due to the abnormal tumor vasculature, and its impact on proliferation is not fully understood.

Methods

Therefore, proliferation in prostate tumor cells (Dunning R-3327 AT-1) was analyzed by propidium iodide and BrdU staining. Effects of acidification on tumor cell transcriptome were studied by sequencing and subsequent analysis with Qiagen Ingenuity Pathway Analysis. Changes on protein level as well as protein phosphorylation were assessed by Western blotting, while changes in intracellular free calcium ($[Ca^{2+}]_i$) and reactive oxygen species (ROS) were analyzed by using Fura-2-AM and H_2DCFDA, respectively.

Results & Conclusions

We could show that acidic extracellular pH leads to reduced number of cells in S phase and increased cell number in G_0/G_1 phase. These changes were concomitant with distinct changes in the tumor cell transcriptome, associated with reduced expression of genes important for cell cycle progression, while genes involved in cellular senescence were elevated. The observed effect on proliferation was not based on changes in mTOR or c-Myc signaling, nor on increased level of ROS. Rather changes in $[Ca^{2+}]_i$ were critical for transcriptional regulation of cell cycle-relevant genes in tumor cells.

Thus, we could show a new mechanistic link between Ca^{2+} signaling and the proliferation of tumor cells in an acidic tumor microenvironment.

B 07-02

Interaction of Connexin 43 with SHP-2 controls focal adhesion turnover during cell migration

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Content

Question

We previously demonstrated that Cx43 enhances endothelial cell migration, and that interaction with the tyrosine phosphatase SHP-2 is essential for this process. Migration is characterized by a fast focal adhesion (FA) turnover with small focal complexes (FC) and the disassembly of large FAs. Since the FA-protein paxillin is a target of SHP-2, we here analysed the effect of Cx43 and SHP-2 on the regulation of cell migration and FAs.

Methods

SHP-2 was downregulated by a specific siRNA. FAs of Cx43-expressing, or Cx-deficient (CTL) HeLa cells were analysed by immunofluorescence stainings and western blots of phospho- paxillin. A potential binding site of SHP-2 in Cx43 (SH2 domain) was mutated by site-directed mutagenesis and the migration of transfected cells was analysed.

Results

Phospho-paxillin containing FAs of CTL were larger and located both centrally and at the membrane, whereas HeLa-Cx43 formed FC with small FAs at the membrane. SHP-2 activity was significantly increased and phospho-paxillin decreased in Cx43-expressing cells, compared to CTL ($n=4$, $p<0.05$). Downregulation of SHP-2 increased phosphorylation of paxillin and FA distribution in HeLa-Cx43 ($n=2$). Mutation of the potential SHP-2 binding site of Cx43 significantly decreased cell migration ($n=4$, $p<0.05$).

Conclusions

Our results demonstrate that the interaction of SHP-2 with Cx43 potentially via the SH2-domain seems to be crucial for an enhanced dephosphorylation of the SHP-2 target paxillin which is required for a fast focal adhesion turnover and associated with a less stress fibres formation. This may be an underlying mechanism for the previously shown increased migration of Cx43-expressing cells.

B 07-03

NoxO1- a novel regulator of EGFR signaling and trafficking

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The author has objected to a publication of the abstract.

B 07-04

Deletion of the deubiquitinase OTUB1 affects AKT-dependent signaling

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Content

Heart failure (HF) with preserved ejection fraction (HFpEF) accounts for about 50% of all HF patients with increasing prevalence. To date, there is no pharmacological treatment option and underlying molecular mechanisms of HFpEF are largely unclear. We previously observed that mice with constitutive heart-specific *Otub1* deletion develop a phenotype comparable to HFpEF with reduced cardiac output, increased left ventricular mass, extended isovolumetric relaxation and contraction time, and preserved ejection fraction. We therefore aimed to assess how *Otub1* deletion affects heart tissue homeostasis and function, starting with analyses in mouse embryonic fibroblasts (MEFs) with *Otub1* deletion (*Otub1*$^{-/-}$). Sustained AKT activation has previously been reported to lead to pathological cardiac hypertrophy and heart failure and OTUB1 has been shown to affect AKT activation in T cells. Therefore, the effect of FCS removal and re-introduction on AKT phosphorylation (pAKT) was analyzed. *Otub1*$^{-/-}$ MEFs showed increased basal pAKT independent of FCS. Interestingly, ERK phosphorylation was in turn decreased following FCS re-introduction in *Otub1*$^{-/-}$ MEFs. Following stimulation with insulin, pAKT was also enhanced in MEFs lacking *Otub1*. Therefore, *Otub1* deletion has a profound effect on AKT phosphorylation, which may at least in part be a mechanism that underlies the observed impact of *Otub1* deletion on heart homeostasis and the development of features comparable to human HFpEF.

B 07-05

2,3-Bisphosphoglycerate mutase (BPGM) expression in clear cell renal carcinoma cells supports cellular resistance.

Philipp N. Becker[1], **Robert Labes**[1], Claudia Czopek[1], Gohar Ter-Avetisyan[1], Kameliya Rögner[1], Vera A. Kulow[1], Anica Högner[1], Andreas Patzak[1], Michael Höpfner[2], Michael Fähling[1]

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The author has objected to a publication of the abstract.

B 07-06

Characterization of the novel Heat Shock Protein 90 Inhibitor 246TMP-3SF5 as potential new treatment option for hepatocellular carcinoma

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Content

Question:

Often overexpressed Heat Shock Protein (HSP) 90 is a promising new target, with treatment options for advanced hepatocellular carcinoma (HCC) still limited. We evaluated novel HSP90 inhibitors for their antiproliferative efficacy and mechanisms of action.

Methods:

Anticancer effects of HSP90 inhibitors were assessed in HepG2 and HuH-7 cells. Antiproliferative effects were measured dose- and time- dependently using crystal violet staining. Unspecific cytotoxicity was examined by measuring lactate dehydrogenase (LDH) release, while apoptosis, cell cycle, and anti-migratory effects were analyzed by flow cytometry, Western Blot and scratch assays.

Results:

Among the eight novel HSP90 inhibitors initially tested, 246TMP-3SF5 (TMP) exhibited the most pronounced antiproliferative effects, with IC_{50} values of 1.3 ± 0.2 µM in HepG2 and 2.2 ± 0.3 µM in HuH-7 cells. LDH release of TMP-treated cells was not increased by more than 5 % as compared to untreated controls after 6 and 24 hours, thereby excluding unspecific cytotoxic effects as predominant mode of action. However, a significant increase of up to 30 % of cells in sub-G1 phase together with an increase in caspase-3 activation and concomitant poly-(ADP-ribose)-

polymerase cleavage, indicated a strong induction of apoptotic cell death. Furthermore, a cell cycle disruption and reduced migratory potential of HCC cells upon treatment with TMP were observed, as evidenced by scratch assays.

Conclusions:

246TMP-3SF5 is a promising novel HSP90 inhibitor demonstrating significant antiproliferative, apoptosis-inducing and cell cycle-disrupting effects in HCC cells, meriting further investigations to fully elucidate underlying mechanisms of action and suitability for urgently needed treatment of advanced HCC.

B 07-07

Orai1 mediates collagen release and ECM deposition from pancreatic stellate cells in pancreatic cancer

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Content

Pancreatic ductal adenocarcinoma (PDAC) is characterized by excessive collagen-rich connective tissue, predominantly secreted by cancer-associated fibroblasts (CAFs), including pancreatic stellate cells (PSCs). We hypothesized that the Ca^{2+} channel Orai1, known to mediate PSC proliferation and transforming growth factor β1 (TGF-β1) secretion, is involved in PDAC fibrosis and linked to collagen secretion. We conducted immunohistochemistry on murine PDAC (KPfC) slices and revealed Orai1 expression in CAFs. To assess collagen production, we developed a high-throughput *in vitro* fibrosis assay that is based on collagen staining with Sirius Red or the collagen-binding peptide CNA-35-tdTomato. Using small interfering RNA (siRNA) and the Orai1 inhibitor Synta-66, we demonstrated that Orai1 regulates collagen secretion of PSCs but not of NIH-3T3 fibroblasts. Moreover, we found that TGF-β1 and vitamin C promote collagen deposition from PSCs. Physiological levels of vitamin C induce a drastic increase of the intracellular $[Ca^{2+}]$ in PSCs with Orai1 channels being involved in this process. In conclusion, our study introduces a robust *in vitro* assay for fibrosis and identifies Orai1 as a critical ion channel involved in PSC-driven fibrosis.

B 07-08

Establishing a ruminal 3D cell culture model

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Content

Question

The ruminal epithelium of cattle has the capacity to absorb Ca^{2+} via a menthol-sensitive pathway. For further research, an optimal cell culture model is desirable.

Methods

Ruminal epithelial keratinocytes were isolated and cultured on filters as previously described and studied via transepithelial electrical resistance measurements, transmission electron microscopy and immunohistochemistry. Alternately, cells were first cultured in a 3D organoid culture using selective media. Commercial RNA sequencing was used to compare gene expression.

Results

Model epithelia developed resistances of 400 to 900 $\Omega \cdot cm^2$, showed a multilayered structure with cell-cell contacts resembling tight junctions and stained for Claudin-1, Claudin-4 and TRPV3. RNA sequencing yielded strong expression levels for Claudin-1, Claudin-4 and various cytokeratins typical for the esophagus (KRT13, KRT19, KRT17, KRT14, KRT5, KRT6A) in all preparations. Reads for the collagens COL1A1, COL1A2 and COL3A1 were above 6000 in the classical model, above 200 in the native tissue but below 10 in the 3D organoid model. Mean reads (± SEM) for menthol-sensitive cation channels in the classical model, organoid model or native preparation were as follows: TRPV3: $858^a \pm 190$; $924^a \pm 16$, $1650^b \pm 127$; TRPA1: $2^a \pm 1$; $0.3^a \pm 0.3$; $14^b \pm 2$ and TRPM8: $0^a \pm 0$; $0^a \pm 2.9$; $0^a \pm 1.4$ ($n=4$, $p<0.05$).

Conclusions

Ruminal cells cultured both using the classical and the 3D organoid approach differentiate into barrier-forming epithelial models, with the latter possibly containing less fibroblasts. TRPV3 is highly expressed and the most likely candidate gene mediating the effects of menthol.

B 07-09

NHE1, NHE3 and NHE8 cooperatively modulate migration of single colorectal carcinoma (HT29-MTX) cells in the presence and absence of HCO_3^-

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Content

In colorectal cancer (CRC), excessive activity of NHE1 (Na^+/H^+ exchanger 1) correlates with increases in cytosolic pH (pH_i), tumor growth, motility and chemoresistance. In contrast, expression of mucosal-protective NHE3 and NHE8 is reduced in human CRC. In mice, malfunction of NHE3 or NHE8 increases CRC growth. Whether metastasis is affected is still unknown.

An 80% NHE8 knockdown and the corresponding mock-control of the human CRC cell line HT29-MTX were compared. Immunostaining showed an increased occurrence of NHE3 in migrating NHE8 knockdown cells, particularly in bleb-like protrusions, consistent with a higher NHE3 mRNA expression. NHE1 remained unaffected. Inhibiting NHE3 with tenapanor or NHE1 with BI-9627 decreased single-cell migration (velocity, distance) in both cell lines. Combined use of tenapanor and BI-9627 had an additive effect. In the presence of tenapanor and BI-9627, and a concomitant absence of HCO_3^-, NHE8-deficiency reduced migration even further. While pH_i was higher in the NHE8 knockdown, the Na^+-dependent pH_i recovery from a cytosolic acidification, measured in the nominal absence of HCO_3^-, was not different. The intracellular buffering capacity did not differ either, but inhibiting NHE1 unmasked a higher “tenapanor-sensitive” pH_i recovery-component in the knockdown. In both the control and the NHE8 knockdown, NHE1-inhibition decreased the pH_i recovery by 90%. Additional NHE3-inhibition caused a further decrease with the effect being more pronounced in the knockdown.

In summary, NHE1, NHE3, NHE8 and the availability of HCO_3^- stimulate CRC cell motility. Increased NHE3 activity may compensate for a disabled NHE8. In terms of single-cell motility, NHE8-deficiency does not increase malignancy.

B 07-10

K_{Ca}3.1 channels regulate processes underlying metastasis of non-small-cell lung cancer cells by modulating cellular mechanics.

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Content

We study the role of K_{Ca}3.1 channels for cellular mechanics and their role in the processes underlying the metastatic cascade of non-small cell lung cancer (NSCLC) cells. Together with biochemical aspects also mechanics shapes the tumour progression. A mechanical interplay between circulating tumour cells and the endothelium is crucial for their extravasation. We showed earlier that overexpression of K_{Ca}3.1 channels promotes cellular aggressiveness and therefore worsens clinical outcome.

We used Peak Force Quantitative Nanomechanical atomic force microscopy (PFQNM-AFM) to study the topography and mechanics of endothelial cell monolayers. We prepared polyacrylamide gels mimicking the stiffness of endothelial cells. They were used for modeling the intravascular migration of metastasising NSCLC cells. Live-cell imaging of NSCLC cell lines were taken on soft (<1kPa) and stiff (around 10kPa) substrates, as well as in a 3D ECM-like matrix. Proliferation and viability were measured via luminescence ATP assay. The impact of K_{Ca}3.1 channel function was verified pharmacologically with senicapoc.

Our PFQNM-AFM maps show a high variability of elasticity of endothelial cells, especially along cell-cell contacts. Migration velocity, translocation and proliferation are lower on soft than on stiff substrates. Inhibiting K_{Ca}3.1 channels softens NSCLC cells. Additionally, combined treatment with osimertinib and senicapoc decrease migration parameters of NSCLC by around 60% and viability by 40%.

We show, that variations of substrate stiffness as observed in endothelial layers steers migration of NSCLC cells. K_{Ca}3.1 stiffens NSCLC cells, which could increase their migratory abilities. Our data provides new evidence to understand the role of mechanics in processes underlying metastasis.

POSTER SESSION C

C 01 | Cardiac Metabolism

C 01-01

Sex-Specific Cardiovascular Responses to Chronic High-Salt Diet

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Content

Excessive dietary salt intake has been linked to elevated blood pressure (BP) and can have adverse effects on target organs, including the blood vessels, heart, kidneys, and brain. Population studies indicate a higher prevalence of salt-sensitive hypertension in women. To investigate sex-specific responses to prolonged high salt intake, 6-month-old male and female mice were fed an 8% high-salt diet (HSD) for 14 months. A separate group was reverted to a normal diet (ND) after seven months. BP and cardiac function were monitored over time using tail cuff plethysmography and magnetic resonance imaging. Vascular function and vascular expression changes were evaluated at the conclusion of the study by wire myography, quantitative polymerase chain reaction (qPCR), and histology. After 14 months of HSD, only female mice exhibited higher BP compared to age-matched ND controls. Male HSD-fed mice exhibited altered cardiac parameters, including higher stroke volume, left ventricular (LV) end-diastolic volume, and LV mass. Additionally, aortic alterations were observed, including increased wall thickness and the predominance of synthetic smooth muscle markers. The mesenteric arteries of HSD-fed male mice exhibited a diminished response to potassium chloride. Alpha-1 adrenergic activation resulted in significantly higher vessel tension in HSD-fed female compared to HSD-fed male mice. Following diet reversal, cardiac alteration normalized in males as evidenced by a reduction in stroke volume and LV mass. The data collectively indicate that chronic high salt loading elicits pronounced sex-specific responses. Diet reversal improves cardiovascular health in both sexes, which further emphasizes the importance of moderating salt intake.

C 01-02

Lipid droplet accumulation in isolated cardiomyocytes affects titin-based cardiomyocyte properties

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Universitätsklinikum Düsseldorf, Cardiovascular Physiology, Düsseldorf, Germany

Content

Question

Cardiac lipid droplets (LD) in type 2 diabetes mellitus patients are associated with heart failure and impaired cardiomyocyte function. In our current study, we investigated how LD accumulation affects sarcomere structure and passive mechanical properties of isolated cardiomyocytes.

Methods

We established an in vitro model to induce LDs in isolated cardiomyocytes from adult C57BL/6N mice, by incubating cells for 4-6 h with non-esterified fatty acids (NEFA) or DMSO/BSA controls. For microscopic analysis, cells were fixed and LipidTOX stained. Furthermore, Perilipin expression and relative phosphorylation of titin PEVK domain were determined by Western blot analysis. Passive tension was measured in cardiomyocytes by stretching the cells from 1.9 µm to 2.3 µm sarcomere length.

Results

We found a 25% increase in the proportion of total area covered by LD in NEFA-treated cells compared to DMSO/BSA controls. LD size was increased in NEFA-treated cells with diameters of 0.87±0.16 µm, compared to 0.67±0.24 µm in controls. This finding was supported by Western blot analyses showing increased expression of Perilipin 2 and 5 in NEFA-treated cells. Relative phosphorylation of PKC were unchanged. Relative titin PEVK phosphorylation was increased at S12022 by around 20 % compared to controls. Consequently, we observed an up to 1.47-fold increase in cardiomyocyte passive tension in NEFA-treated cells, compared to DMSO/BSA controls.

Conclusions

We conclude that LD accumulation mediates titin phosphorylation and increases cardiomyocyte passive tension. The results suggest that lipid uptake causes alteration of sarcomere distensibility, which may contribute to the development of cardiomyocyte dysfunction in patients with diabetes.

C 01-03

Altered skeletal muscle function in a diabetic mouse model with diet-induced lipid accumulation

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Content

Question: Type-2 Diabetes mellitus is associated with impaired skeletal muscle function, however, the pathomechanisms and the role of intracellular lipid accumulation are poorly understood.

Methods: Soleus muscles were isolated from 20-weeks old C57/BL6J mice with high fat/high sucrose diet (DIO) and streptozotocin (STZ)-induced diabetes (DIO-STZ, n=7), mice with STZ-induced diabetes with standard chow (STZ, n=9) and in untreated controls (n=5), and functional measurements were performed using a Myodynamic Muscle-Strip-System. Immunhistochemical and Western blot analyses were performed to determine fiber-type composition and intramuscular lipid accumulation.

Results: Bodyweight was reduced in STZ mice (24.4±0.5g) compared to DIO-STZ (27.5±0.7g) and controls (29.9±0.2g). Soleus muscle weight was comparable among groups, but the proportion of fast twitch fibers (type IIa) was significantly higher in soleus from DIO-STZ (60.1±0.4%) than in STZ (47.7±0.4%) and control mice (48.6±0.1%). Maximum isometric tetanic force was decreased to 20±2,2 mN/mm^2 in DIO-STZ mice compared to 52±6,9 mN/mm^2 in STZ, and 48±11,2 mN/mm^2 in control mice. In DIO-STZ mice, relative PLIN2 and PLIN5 expression was significantly increased to 140.5±7.6%, and 174.6±3.9% of control levels, respectively, suggesting elevated intramuscular lipid uptake.

Conclusions: STZ-induced diabetes shows no significant changes in muscle function during the observation period. High-fat/high sucrose diet in DIO-STZ mice is associated with increased intramuscular lipid uptake and impaired skeletal muscle function, even in the absence of obesity.

C 01-04

Positive inotropic effects of glucose-dependent insulinotropic polypeptide in the human atrium

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Content

Question

[Glucose-dependent insulinotropic polypeptide (GIP) is a comprised of 42 amino acids. GIP is formed in the intestinal tract. GIP can increase insulin secretion from the pancreas. GIP acts via GIP receptors. GIP receptors can stimulate adenylyl cyclases and increase cAMP levels. The physiological role of GIP in the mammalian heart is unclear. ..]

Methods

[.We performed contraction experiments in isolated paced (1 Hz) left atrial preparations of mice. In addition, force of contraction was studied in isolated electrically driven (1 Hz) human right atrial preparations from patients undergoing bypass surgery. ..]

Results

[.We noted that in paced human atrial preparations 100 nM and 300 nM GIP increased force of contraction. This effect was attenuated by subsequently applied Pro2GIP, an antagonist at GIP receptors. In the presence of 0.1 µM cilostamide, a phosphodiesterase 3 inhibitor, the positive inotropic effect of 100 nM GIP was augmented. The effect of GIP could be washed out. The positive inotropic effect of GIP could be attenuated by 1 µM H89, an inhibitor of the cAMP dependent protein kinase, nifedipine and ryanodine.

Conclusions

[.We conclude that a physiological function of GIP in the human heart resides in a positive inotropic effect. This effect is probably mediated by elevation of cAMP. It remains to be elucidated whether GIP can increase the heart rate or ventricular inotropy in humans and whether such effects are attenuated in failing human hearts. ..]

C 01-05

PKC Induces T-System Loss mediated by PKD/NFκB-Driven Macropinocytosis in Cardiac Myocytes

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Content

Question

In heart failure, the loss of T-tubule (TT) integrity impairs excitation-contraction coupling. Protein kinase C (PKC) activation was suggested to contribute to TT remodeling, but downstream signaling and mechanisms remain unclear.

Methods

Isolated rat cardiomyocytes were treated with the PKC activator phorbol 12-myristate 13-acetate (PMA) +/- inhibitors targeting PKC and downstream factors PKD or NFκB. TTs were assessed via Di-8-ANEPPS staining and confocal microscopy. To measure TT loss, we calculated the mean TT distance (dTT). To investigate processes of TT loss, fluorophore-conjugated dextrans were added to the extracellular solution.

Results

PMA caused significant TT loss, which was prevented by broad-sprectrum PKC inhibition (dTT in µm 1.69±0.01 in PMA vs 0.85±0.03 in PMA+BISXI, $p<0.001$), PKD inhibition (dTT in µm 1.43±0.18 in PMA vs 0.54±0.02 in PMA+CRT0066101, $p<0.01$), and NFκB inhibition (dTT in µm 1.5±0.11 in PMA vs 0.9±0.09 in PMA+BMS-345541, $p<0.05$). PMA stimulation led to increased dextran internalization, showing TT shapes and absence of membrane staining. Internalization was attenuated by PI3K inhibition (volume ratio 0.0087±0.0003 in PMA vs 0.0055±0.0007 in

PMA+PI103, p<0.01), pitstop and amiloride (0.0087±0.0003 in PMA vs 0.0013±0.0004 in PMA+Pitstop, p<0.001; 0.01±0.0007 in PMA vs 0.0059±0.001 in PMA+amiloride, p<0.01). Dynasore, an inhibitor of dynamin and clathrin-mediated endocytosis, showed less efficacy in reducing internalization (volume ratio 0.0091±0.0007 in PMA vs 0.0073±0.0006 in PMA+dynasore, p<0.05). Linear regression revealed a positive relationship between TT loss and dextran internalization across multiple conditions and pharmacological agents (p<0.01, R^2=0.54).

Conclusions

PMA induces TT loss via PKC/PKD/NFκB signaling, presumably mediated by macropinocytosis.

C 01-06

Endothelium-derived SMOC1 protects against loss of cardiac function after myocardial infarction

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Content

Myocardial infarction (MI) and its consequences are a major health challenge and treatment option that promote cardiac regeneration are lacking. Transforming growth factor (TGF)-b plays an important role in repair after cardiac injury but is detrimental if prolonged as it induces endothelial-to-mesenchymal transition (EndMT) as well as cardiac fibrosis. We analysed multiple single cell sequencing datasets after MI and observed increased expression of secreted modular calcium binding (SMOC) 1 exclusively in endothelial cells undergoing EndMT. This is relevant as SMOC1 interferes with TGF-b signalling. To investigate the impact of SMOC1 on cardiac regeneration we generated mice lacking SMOC1 specifically in endothelial cells ($SMOC1^{\Delta EC}$) and subjected them to MI using a minimally invasive approach. In the absence of endothelial cell-derived SMOC1 the cardiac infiltration of macrophages, monocytes and T cells (in particular CD4+ T cells) in $SMOC1^{\Delta EC}$ mice in comparison with wild-type littermates 1 and 3 days after MI. The lack of SMOC1 had an negative impact on heart function and 14 days after MI, there was a highly significant impact on the ejection fraction which decreased to ~10-15%. In these mice, histological analysis revealed a markedly increased infarct area in $SMOC1^{\Delta EC}$ mice with development of ventricular aneurysm. Our results indicate that endothelial cell-derived SMOC1 plays a protective role in the heart as its leads to a detrimental outcome after MI.

C 01-07

Activation of the glucocorticoid receptor improves Ca^{2+} release and contractile force in human cardiac slice culture

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The author has objected to a publication of the abstract.

C 01-08

High fat high sucrose diet in combination with low dose streptozotocin leads to heart failure with preserved ejection fraction in mice

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Content

Patients with diabetes have a up to three-times higher risk of developing heart failure with preserved ejection fraction (HFpEF), which is associated with high morbidity. However, the pathophysiology and cardiac metabolic changes in HFpEF are still incompletely understood, both in non-diabetic and diabetic conditions. To study the impact of diabetes on heart failure development, we analyzed metabolic parameters and cardiac function in: 1. a hyperinsulinemic high fat/high sucrose mouse model (DIO), 2. a type 1 diabetic mouse model after low-dose streptozotocin treatment (STZ), and 3. a model with the combination of both treatments (DIO-STZ). DIO-STZ and STZ mice exhibited marked hyperglycemia (chow: 162±22 mg/dl; DIO-STZ: 464±68 mg/dl; STZ: 344±96 mg/dl), hypoinsulinemia and glucose intolerance. Echocardiographic analyses revealed a HFpEF phenotype with reduced cardiac output (chow: 33.7±1.8 ml/min; DIO-STZ: 18.9±3.9 ml/min), normal ejection fraction (chow: 64.1±2.9%; DIO-STZ: 62.9±6.2%) and decreased end-diastolic volume (chow: 90.7±5.4 µl; DIO-STZ: 59.6±8.0 µl), accompanied with increased left ventricle filling pressure (chow: 3.3±1.2 mmHg; DIO-STZ: 7.4±3.2 mmHg) in DIO-STZ mice. In clear contrast the indication for HFpEF development was not found in DIO as well as STZ animals. Furthermore, we analyzed the expression profile of heart tissues from all three mouse models using RNA sequencing and were able to demonstrate, among other things, the activation of mitochondrial fatty acid beta-oxidation pathways through pathway analysis in DIO-STZ animals. In summary, the DIO-STZ model provides a non-genetically modified alternative for investigating HFpEF in a diabetes model.

C 01-09

Diabetes mellitus associated HFpEF development disturbs left ventricular geometry

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Content

The diagnosis of heart failure with preserved ejection fraction (HFpEF) is commonly based on clinical scores as the HFE-PEFF score. Here, a reduction in global longitudinal strain (GLS) presents one criterion of HFpEF diagnosis. From a geometric point of view, a reduction in GLS could only be reconciled with a preserved EF if the global ventricular shape is altered.

To investigate the effect of HFpEF development on ventricular shape, we used a HFpEF mouse model with high fat/high sucrose diet (DIO) and streptozotocin (STZ)-induced diabetes (DIO-STZ, n=9). DIO-STZ animals developed a strong HFpEF phenotype with reduced cardiac output, decreased end-diastolic volume and increased LV-filling pressure. GLS was reduced in DIO-STZ compared to control animals (-12±4% vs. -16±3%)

To determine LV-geometry, length (apex-base), height (anterior-posterior) and width (septal-lateral) during diastole and systole were determined, and fractional shortening (FS-length, FS-height, FS-width) as well as sphericity indices (length/heigth, and height/width) were calculated. During diastole all dimensions were reduced in DIO-STZ compared to chow treated controls (n=8) reflecting the reduced end-diastolic volume, and sphericity indices were unchanged. However, during systole length/height sphericity in DIO-STZ was increased by 14 %, and height/width was decreased by 10 %. These differences were attributable to an increase in FS-height (32±4% vs. 27±2%) whilst FS-length was reduced and FS-width was unchanged.

In summary, Diabetes mellitus associated HFpEF results in disturbances of LV-geometry pointing towards the hypothesis that alterations in systemic metabolism causes regional differences in myocardial adaptations.

C 01-10

Diabetes mellitus associated HFpEF is accompanied by an increased myocardial fatty acid oxidation capacity

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Content

Heart failure with preserved ejection fraction (HFpEF) represents a disease with high morbidity and mortality. Epidemiological data show that Diabetes mellitus is associated with an increased incidence of HFpEF, but the underlying pathophysiological mechanisms are poorly understood. Partly responsible for this inadequate

understanding is a shortage of appropriate preclinical mouse models to investigate disease mechanisms. We demonstrated that a combined treatment of mice with high fat/high sucrose diet (DIO) and streptozotocin (STZ)-induced diabetes cause a strong HFpEF phenotype as seen by reduced cardiac output, decreased end-diastolic volume and increased LV-filling pressure. Here, we investigated the cardiometabolic disturbances in this DIO-STZ HFpEF mouse model. DIO-STZ mice showed an increased expression of PDK4 compared to healthy controls. Furthermore, genes associated with fat metabolism (e.g. CD36, CPT1b, or CPT2) were strongly upregulated, suggesting alterations in myocardial substrate metabolism. Extracellular flux analysis of intact ventricular tissue slices using glucose as well as palmitate as substrates revealed an increase in oxygen consumption rate (OCR) by 40% in DIO-STZ animals, whilst non-mitochondrial OCR was unchanged. In addition, pharmacological inhibition of CPT1 by etomoxir reduced maximal OCR to a larger extent in DIO-STZ myocardium indicating an improved capacity for long-chain fatty acid oxidation (61±5% vs 46±8%). In contrast, no difference in the capacity for glucose oxidation was observed. In summary, the DIO-STZ mouse model, which presents the functional phenotype of HFpEF as well as a disturbed myocardial substrate metabolism, might be a promising tool to investigate the causative role of altered metabolism as driver of HFpEF.

C 02 | Neuroscience (Systems)

C 02-01

Directed and acyclic synaptic connectivityin the human layer 2-3 cortical microcircuit

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Content

The computational capabilities of neuronal networks are fundamentally constrained by their specific connectivity. Previous studies of the cortical connectivity have been mostly carried out in rodents; however, whether the principles also apply to the evolutionary expanded human cortex is unclear. Here we studied network properties within the human temporal cortex using samples obtained from brain surgery. We analyzed multi-neuron patch-clamp recordings inlayer 2-3 pyramidal neurons and identified substantial differences compared to rodents. Reciprocity showed random distribution, synaptic strength was independent from connection probability and connectivity of the supragranular temporal cortex followed a directed and mostly acyclic graph topology. Application of these principles in neuronal models increased dimensionality of network dynamics suggesting a critical role for cortical computation.

C 02-02

Under environmental stress, electrical synapse plasticity contributes to behavioural adaptation in *C. elegans*

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Content

To survive, animals dynamically adapt their behaviour to ever changing environmental conditions. The functional output of neural circuits is therefore shaped by many factors, including previous experience, sensory input or metabolic state. How do neural circuits integrate these diverse factors to generate adaptive behaviour? While numerous studies have investigated the roles of neuromodulatory signalling and chemical synapses in the plasticity of neural circuit function, how electrical synapses, formed by gap junctions, contribute to neural circuit and behavioural plasticity is poorly understood.

The nematode Caenorhabditis elegans provides the opportunity to explore how sensory responses are modulated by environmental context in the living organism. We focussed on the neural circuit regulating oxygen homeostasis to investigate the impact of electrical coupling on O_2 responses during short and long-term exposure to environmental extremes. This circuit generates well-defined and reproducible behavioural responses to ambient O_2. Despite the circuit's structural simplicity, oxygen responses are highly plastic and shaped by previous experience and environmental context.

To explore how electrical coupling contributes to context-dependent plasticity, we created a strain in which the O_2 sensors can only signal via gap junctions to downstream neurons, and assayed oxygen responses while the animals were exposed to long or short-term environmental stresses such as high [CO_2] or elevated temperature. We found a number of contexts where these environmental stresses alter O_2 responses. Some of these contexts regulate behaviour independently of electrical coupling, while specific contexts appear to change behavioural output by modulating gap junction coupling.

C 02-03

Glomerulus-specific inhomogeneity of the resting activity in the olfactory bulb of mice.

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Content

Glomeruli are signal-processing units of the olfactory bulb (OB), playing a key role in many OB computations including contrast enhancement, gain control, and odorant-selective habituation. In mice, a typical glomerulus receives inputs from ~1000 olfactory sensory neurons (OSNs) all expressing the same type of odor receptor. Because the chemoreceptive properties of different OSNs overlap, it is largely assumed that the combinatorial pattern across the set of corresponding odor-activated glomeruli forms the basis for odor perception and identification. This logic, however, assumes that odor-evoked OSN signals are projected onto a homogeneous layer of "resting" glomeruli.
By using *in vivo* two-photon Ca^{2+} imaging in awake head-restrained mice expressing a FRET-based Ca^{2+} indicator Twitch-2B under the chronic cranial window, we challenge the above view by showing that the "resting state" of the glomerular layer of the OB is characterized by inhomogeneous, glomerulus-specific patterns of endogenous activity, forming a basal glomerular activity map. Such maps were stable at least across weeks. Using different experimental approaches we found that they are sustained by endogenous spiking of OSNs, air flow-stimulated OSN firing, modulatory cholinergic projections to the OB and, to a lesser extent, by the odor environment. Remarkably, in a mouse model of Alzheimer's disease, the loss of glomerular map inhomogeneity occurred before the amyloid plaque deposition and was associated with an impairment of odor-driven behavior.
These results add an important layer to the signal-processing network of the OB, likely acting by increasing variance in the system via glomerulus-specific functional heterogeneity.

C 02-04

***In vivo* awake Neuropixels recordings of putative dopamine neurons in the substantia nigra reveal distinct firing patterns related to movement**

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Content

Dopamine (DA) neurons in the substantia nigra (SN) are vital for cognition, motor control, and reward processing, with dysfunction linked to Parkinson's disease. To explore their role in motor control, we conducted extracellular *in vivo* Neuropixels recordings in awake, head-fixed mice on a treadmill, with simultaneous movement tracking using a camera

and a position sensor. Among the recorded ~3000 units (N=11), 48% (n=1500) showed increased (n=882, +6.0 ± 6.3 Hz) or decreased (n=618, −3.8 ± 4.4 Hz) firing rates during movement onset (-2 to -1s vs. 0 to +1s), while 42% (n=1296) responded to movement cessation with increased (n=579, +4.8 ± 6.3 Hz) or decreased (n=717, −5.4 ± 6.0 Hz) firing activities. Sixty-four percent (n=1988) exhibited changes during both phases. As a first attempt, ~8% (n=251) were identified as putative DA SN neurons based on firing rates (≤ 10Hz) and spike waveform shapes (spike-half-width ≥ 200 µs), with tracks in the SN identified by DiI and post-hoc TH staining. Among them, 44% (n=110) responded to movement start, with 26% (n=65, +3.0 ± 3.5 Hz) increasing and 18% (n=45, −1.4 ± 1.2 Hz) decreasing their firing rates. Additionally, 34% (n=85) responded to movement termination, with half (n=41, −2.9 ± 5.8 Hz) decreasing and the other half (n=44, +1.5 ± 1.0 Hz) increasing their firing rates. Fifty-six percent (n=141) responded to both transitions. These initial results are in accordance with previous studies, indicating unique firing patterns in SN DA neurons during self-paced movements. Future steps aim to refine DA SN neuron identification.

C 02-05

Interhemispheric synaptic inputs to neocortical pyramidal cells with dendritic versus somatic axon origin

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Content

Recent data show that a significant fraction of cortical pyramidal neurons has an axon originating from a basal dendrite (axon-carrying dendrite, AcD) rather than from the soma. In the rodent hippocampus, these AcD cells convey privileged synaptic excitation especially in network states with strong perisomatic inhibition (Hodapp et al., 2022). We have recently shown that hippocampal AcD cells receive stronger commissural input than neurons with somatic axon origin (non-AcD) (Stevens et al., 2023).

AcD cells are also present in the neocortex, though seemingly less frequent than in the hippocampus (~20% compared to ~30-50%; Thome et al., 2014, Wahle et al., 2022). However, nothing is known about the network integration of neocortical AcD versus non-AcD cells. We therefore studied commissural inputs in the mouse primary motor cortex (M1), an area with strong interhemispheric connections. Channelrhodopsin 2 was expressed in presynaptic neurons of adult mice by viral injection into the contralateral M1 and whole-cell patch clamp recordings were performed in coronal slices of the M1. The vast majority of all recorded cells responded with strong inward currents to optogenetic activation of presynaptic inputs. Repetitive activation of commissural inputs at 20Hz showed prominent paired-pulse depression, without overt differences between both morphological subtypes. Reconstruction of recorded neurons revealed that 16/64 (i.e. 25%) were AcD neurons, while 42 had their axon emerging from the soma and six neurons showed a shared root of a basal dendrite together with the axon. Further analysis is required to uncover potential functional or structural asymmetries between both cell types.

C 02-06

The K_{ATP} channel opener Levcromakalim can influence neuronal excitation and Cortical Spreading Depolarization (CSD) in adult rats

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Content

Question

Migraineurs reported migraine after systemic application of the K_{ATP} channel opener Levcromakalim. Here, we tested its modulation of cortical excitability and potential interaction with CSD in rats.

Methods

In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.), we recorded the electrocorticogram (ECoG) of 400 and 1200 µm in treated and untreated brain areas. KCl-microinjection induced CSD, and CSD-related direct current (DC) potential shifts, changes in extracellular potassium concentration, and regional cerebral blood flow were monitored continuously for 4 hours. Levcromakalim was administrated either topically onto cortical surface or intravenously at 10^{-5} M or 10^{-6} M. Subgroups were either pretreated topically with the selective CGRP receptor antagonist BIBN4096BS (10 nM) and then received Levcromakalim systemically, or received CGRP at 10^{-7} M topically together with Levcromakalim systemically.

Results

Topical Levcromakalim did only slow CSD propagation. Intravenous Levcromakalim at 10^{-6} M had no effect on CSD, but at 10^{-5} M it induced pathophysiological changes in the ECoG (sharp waves, ictal activity, seizures). This activity needed a trigger (KCl-induced CSD). No spontaneous CSD were seen. Pretreatment with BIBN4096BS prevented ECoG changes caused by intravenous Levcromakalim. Intravenous Levcromakalim together with topical CGRP at 10^{-7} M significantly reduced CSD amplitudes, augmented the ECoG changes observed after Levcromakalim alone, and induced spontaneous CSD. Importantly, a trigger-CSD was required.

Conclusions

In conclusion, Levcromakalim has the potential to increase cortical excitability, but its effects are modulated by CGRP. Additional triggers, such as a CSD wave, are required to manifest this excitability in the ECoG.

C 02-07

The Janus Kinases (JAK1/JAK2) contribute to mechanonociception in C-fibers but not in Aδ-fibers in the normal and inflamed knee joint of the rat

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Content

Question

Patients treated with JAK-inhibitors report on pain reduction. Here we tested, whether such inhibitors can act directly on mechanonociceptors of the normal or of the acutely inflamed joint.

Methods

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 C- or Aδ-nerve fibers supplying the knee joint and classified by their conduction velocity (<1.4 m/s or <10 m/s, respectively). The JAK-inhibitor Baricitinib was injected into the joint cleft (100 ng in 0.1 ml) either alone or as pretreatment followed by either 20 ng interleukin-6 (IL-6) + 20 ng soluble interleukin-6-receptor (sIL-6R) or by 5 ng TNFα. Recordings were performed in either healthy knees, or acutely inflamed knees (7 hours after injection of kaolin/carrageenan into the joint cleft).

Results

Baricitinib injected into the healthy knee joint did not change responses of C- or Aδ-fibers to noxious mechanical stimulation. In C-fibers Baricitinib prevented the sensitization by IL-6+sIL-6R and reduced sensitization by TNFα, but evoked antinociception after injection of TNFα in Aδ-fibers. Baricitinib reduced significantly the responses of sensitized C-fibers of acutely inflamed joint to noxious mechanical stimulation, but had no effect in Aδ-fibers.

Conclusions

We conclude that JAK-signaling is important for neuronal IL-6 signaling, but plays a minor role in TNFα signaling in sensitizing C-fibers. In contrast, the TNFα signaling in Aδ-fibers is controlled by JAK.

C 02-08

Activation of nociceptive muscular afferents may promote a bilateral flexion reflex pattern in the feline spinal cord

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Content

Question

The classical spinal flexor reflex pattern may be modulated by different conditions. We now demonstrated, that muscle pain promotes a transient bilateral flexion reflex response to group III/IV muscle afferents in the feline spinal cord.

Methods

Group III/IV afferents of the gastrocnemius-soleus (GS) muscle were selectively activated by local intra-arterial injections of KCl. Bilateral reflex transmission to PBST (posterior biceps-semitendinosus) and GS was investigated by bilateral monosynaptic reflex testing under four different conditions of the left GS muscle: controls; strong eccentric muscle stretch; acute carrageenan induced myositis; sub-acute ("chronic") myositis induced by infiltration of GS with complete Freund's adjuvant 9-12 days before the terminal experiment. Terminal experiments were performed in anaesthetised and then anaemically decapitated cats artificially ventilated, spinalised at C1 and paralysed.

Results

Although Group III/IV stimulation evoked ipsilaterally the flexion reflex under all conditions, the contralateral responses did not follow a general extension reflex pattern. In control experiments only 9% of tests revealed a contralateral inhibition of PBSt flexor motoneurones and less than half induced a small contralateral facilitation of the extensor GS. Initially, following eccentric GS contraction (about 1hour) or induction of an acute carrageenan myositis (about 2 hours) a bilateral facilitation of PBST reflex effects occurred. In 'chronic' myositis about 90% of tests revealed a bidirectional i.e. from the pre-treated to the un-treated side and, less distinct vice versa, predominance of crossed facilitation of PBSt but no longer any crossed facilitation of GS, instead, in about 50% of tests inhibition of transmission to GS was observed.

C 02-09

Cenobamate affects epileptiform activity in a rat glioma model

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Content

High-grade gliomas have one of the worst survival prognoses of all common human tumor diseases. Frequently, glioma patients also suffer from tumor-associated seizures. Even under successful standard treatment, patients' quality of life is often worsened by adverse effects of anti-epileptic drugs (AEDs). This underscores the need for novel AEDs that offer improved seizure management with fewer side effects and in addition, may also be of use in drug resistant epilepsy. Therefore, we investigated the effects of cenobamate in a preclinical rat glioma model. Cenobamate, a novel AED, appears to inhibit persistent sodium currents and exhibits GABAergic properties.

To examine cortical electrical dynamics, we employed local field potential (LFP) measurements on cortical brain slices ex vivo. Initially, two disinhibition models inducing epileptiform events were assessed. The model displaying the most consistent activity after wash-in, and warm-up phases was selected for further experiments. Subsequent assessments involved LFP measurements on cortical slices from rats with stereotactically implanted F98 gliomas and controls undergoing sham operations. A subset of these slices was treated with cenobamate or solvent. We employed deep learning models based on EfficientNetV2 and WaveNet to detect epileptiform events, complemented by power spectral density (PSD) analyses to investigate more subtle electrical activities.

Our disinhibition models allowed us to differentiate electrical activity across all experimental groups. Preliminary results indicate that cenobamate affects the duration of epileptiform events and alters the PSD. Further investigations will utilize multi-electrode arrays ex vivo to analyze effects of cenobamate on excitability.

C 02-10

Unveiling the Significance of K^+ Channel-Mediated Delayed Maturation of Hippocampal Neurons in Developmental and Epileptic Encephalopathy

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Content

Question

Developmental and epileptic encephalopathies (DEEs) are primarily driven by genetic mutations that alter neuronal excitability. Recently, potassium (K^+) channels such as $K_V1.2$, encoded by the KCNA2 gene, were identified as a cause

of DEE. This study delves into the mechanisms of the $K_v1.2$-mediated DEE, particularly the effect of a gain-of-function mutation in KCNA2 on neuronal maturation.

Methods

We used Golgi-Cox staining to analyze the somatodendritic morphology of hippocampal inhibitory interneurons in one-month-old mutant and control mice. Additionally, CA1 excitatory (pyramidal) neurons were analyzed to assess the specificity of impairment. To do so, two-photon microscopy-aided visualization, and Huygens and Imaris software were utilized for morphological analyses. Immunohistochemistry and confocal microscopy assessed synaptic density and pre-/postsynaptic marker co-localization. VGAT and gephyrin markers helped to visualize GABAergic synapses, while co-labeling for VGLUT and Homer provided insights into excitatory synapse formation.

Results

The CA1 interneurons in the oriens and radiatum layers of KCNA2 gain-of-function mice exhibited delayed morphological maturation, with significant reductions in dendritic arbor, length, Sholl analysis intersections, convex hull parameters, and soma size compared to wild-type mice. However, no significant differences were observed in GABAergic interneuron parameters within the stratum lacunosum-moleculare between the two groups. This lack of difference may be attributed to the intricate regulation of inhibitory interneuron development and synaptogenesis specific to this layer.

Conclusions

Our study highlights the profound impact of KCNA2 mutations on neuronal development, likely underlying the development of DEE. This advances our understanding of DEE pathophysiology and lays the groundwork for developing novel treatment strategies.

C 02-11

Integrated Computational and Electrophysiological Approaches to Optimize Deep Brain Stimulation in Movement Disorders

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Content

Objective: To integrate large-scale computational modeling with electrophysiological insights to optimize deep brain stimulation (DBS) protocols for movement disorders, focusing on Parkinson's disease and dystonia.

Methods: We employed a large-scale computational model encompassing the basal ganglia, thalamus, and connected cortical areas. The model is based on the Hodgkin-Huxley dynamics and includes anatomically defined structural

connectivity. This framework simulates the effects of DBS on the subthalamic nucleus (STN) and globus pallidus internus (GPi), underpinning the motor dysfunctions observed in Parkinson's disease and dystonia. We introduce a novel biomarker measuring thalamic spatiotemporal activity as the ratio of spiking versus burst firing, guiding the adaptation of stimulation protocols to mimic healthy neuronal activity.
Results: The computational model demonstrates that DBS frequencies >130 Hz restore thalamic activity to near-normal levels by influencing the macroscopic properties such as synchronization index, mean synaptic activity, and response efficacy. Electrophysiological assessments in dystonic hamsters reveal that GPi-DBS modifies cortico-striatal and cerebellar interactions, evidenced by changes in synaptic potentials and medium spiny neuron activity. These findings suggest that DBS exerts a dual effect: direct modulation of targeted nuclei and indirect regulation via network-wide synaptic adjustments.
Significance: By combining computational modeling with electrophysiology, we elucidate complex neurobiological mechanisms of DBS, advancing our understanding of its therapeutic impact on motor network dynamics. This integrative approach not only refines current DBS applications but also assists in the personalized tuning of stimulation protocols to improve clinical outcomes for patients with movement disorders.

C 02-12

Effects of Neuropeptide Y on hippocampal sharp-wave ripples and gamma oscillations in vitro

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Content

Neuropeptide Y (NPY) is an abundant neuromodulator in the mammalian brain, including the hippocampus . It acts through five G-protein-coupled receptors (R), from which Y1 and Y2 R are most widely expressed. NPY is involved in fear reduction, the regulation of stress responses and emotional memory processes. The hippocampus is involved in these actions. At the same time, it exhibits different patterns of oscillatory network activity, which are associated with specific memory processes. Sharp wave-ripples (SPW-R) are associated with memory consolidation, while gamma rhythms with memory encoding.
Combining these findings, we hypothesized that the effects of NPY on anxiety and fear memory may be associated with changes in hippocampal network oscillations. We examined the two dominant patterns (SPW-R and gamma oscillations) in mouse brain slices, containing the ventral-to-intermediate hippocampus. NPY caused a decrease of SPW-R activity, predominantly in the CA1 subregion of the hippocampus. The effect could be replicated with a selective Y2 R agonist, but not by activating Y1 R. In contrast, carbachol-induced gamma oscillations were not strongly affected by NPY. Our results are suggestive of selective Y2R-mediated actions of NPY on network processes supporting memory consolidation, but not memory acquisition.

C 02-13

Catecholaminergic modulation of spike timing-dependent plasticity along the dorso-ventral gradient of the mouse hippocampal CA1 region

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Content

Recently, heterogeneous expression of neurotransmitter and neuromodulator receptors were reported along the longitudinal hippocampal axis, suggesting a gradient for catecholaminergic LTP modulation from dorsal to ventral hippocampus. However, the role of catecholamines in regulating spike timing-dependent plasticity (STDP) along the longitudinal hippocampal axis has not been systematically investigated.

We used low repeat (6x at 0.5 Hz) canonical (1EPSP/1AP) or burst (1EPSP/4AP) STDP protocols to induce t-LTP in slices from dorsal (DH), intermediate (IH) or ventral (VH) hippocampus. To investigate catecholaminergic effects, D1 receptor (D1R; 10µM SCH23990) and D2R (10µM Sulpiride), as well as α-Adrenergic (α-AR: Prazosin; 10µM) and β-Adrenergic receptor (β-R: Propranolol; 10µM) antagonists were applied. Combining optogenetic stimulation with patch-clamp recording we used eNpHR3.0-EYFP mice crossed with Th-IRES-cre mice to control catecholamine release at different time points during t-LTP induction.

6x1:4 t-LTP was blocked by either D1 or D2R antagonism in VH, but remained unaffected in DH. In contrast, blockade of 6x1:1 t-LTP required co-application of D1- and D2R antagonists in DH and VH, whereas in IH t-LTP induced by both protocols depended on D1 and/or D2Rs. Moreover, in DH, 6x1:1 t-LTP was blocked by α-AR inhibition, whereas 6x1:4 t-LTP remained unaffected by α-AR/β-AR antagonism. Interestingly, in VH, both STDP paradigms were insensitive to adrenergic antagonists. Optogenetic inhibition of catecholamine release revealed dependence of both t-LTP paradigms on dopaminergic/adrenergic signaling at distinct time points before and during t-LTP induction.

Our study reveals an STDP paradigm-dependent gradient for catecholaminergic modulation of t-LTP along the longitudinal axis of the mouse hippocampus.

C 02-14

Targeting Norepinephrine Neurons of the Locus Coeruleus: A Comparison of Model Systems and Strategies

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Content

The locus coeruleus (LC) noradrenergic (NE) system is involved in a plethora of physiological and pathophysiological processes. Refining our understanding of LC function largely relies on selective transgene expression in molecularly defined cells, allowing targeted manipulation and readout of noradrenergic neurons. Here, we performed a side-by-side comparison of the most commonly used strategies to genetically access the LC, including different cre driver lines and promoter-mediated transgene expression. We report differences between these strategies in terms of transgene expression efficacy and molecular specificity. Notably, we found no behavioral alterations performing anxiety tests and memory tasks in cre-expressing mice of any mouse line as compared to wild-type littermates. Finally, to further ease the investigation of LC-NE function, we created a suite of constructs, including reporter proteins, calcium indicators, and optogenetic actuators whose expression is mediated by the previously described PRS×8 promoter. These constructs allow for monitoring and manipulation of LC-NE activity either in wild-type mice, or in combination with tissue-specific manipulations of different cre driver lines. The results of our study are crucial for the interpretation of results from previous experiments using the respective targeting strategies, as well as for the design of future studies.

C 02-15

Neuroprotective Effects of Omega-3 Enriched Maternal Feeding in a Rat Model of Traumatic Brain Injury

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The author has objected to a publication of the abstract.

C 02-16

Effects of Ketamine/Xylazine Combination and Thiopental anesthesia on various electrophysiological recordings.

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The author has objected to a publication of the abstract.

C 02-17

Taste-associative learning to induce behaviorally conditioned analgesia in a rat model of inflammatory pain – A model for placebo analgesia?

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Content

Question

The eminent impact of our mind on the sensation of pain manifests in effects of nocebo and placebo responses. While effects of placebo analgesia are well documented in humans, it is not clear to which extent similar effects can be observed in animals. Here, we apply an established protocol of taste-associative learning to induce behaviorally conditioned analgesia.

Methods

In a model of Complete Freund's Adjuvant (CFA)-induced paw edema, symptoms of inflammatory pain are induced. Ibuprofen (100 mg/kg, i.p.) is used for repeated analgesic treatment. Drug application is performed immediately after presentation of a novel sweet taste of saccharin. When rats are re-exposed to the taste and injected with a *placebo* (solvent, i.p.) a conditioned response is evoked.

Results

The CFA-induced paw edema is accompanied by a consistently enhanced mechanical and thermal sensitivity over the examination period of eight days. Treatment with ibuprofen on every other day leads to a drug-induced analgesia. Applying the taste-associative learning paradigm with saccharin and ibuprofen, a conditioned taste avoidance (CTA) is observed during the acquisition phase indicative for the association between taste and pharmacological effect. When

rats are re-exposed to the taste and injected with a placebo (retrieval), a conditioned response is detectable by means of an attenuated mechanical and thermal sensitivity.

Conclusions

Having established an animal model of conditioned analgesia in rodents we will be able to analyze neuro-behavioral mechanisms that are involved in placebo effects. Application of classical conditioning paradigms may help to support conventional therapies in humans and animals.

C 03 | Ion channels (cellular function)

C 03-01

Anti-inflammatory and analgesic effects of novel α9α10 nicotinic acetylcholine receptor agonists

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Content

Nicotinic acetylcholine receptors (nAChRs) are not only expressed by the nervous system but also by innate immune cells including mononuclear phagocytes. These cells play pivotal roles in host defense against infection but also in numerous diseases characterized by exuberant inflammation. nAChRs are best known to function as ligand-gated ion channels in neurons. Recent evidence suggests that nAChRs do not generate ion channel currents in immune cells to modulate inflammation and inflammatory pain. We are only beginning to understand the underlying noncanonical mechanism. In addition to α7 nAChRs, the subunits α9 and α10 might be novel targets for the treatment of inflammation and inflammatory pain. This prompted us to investigate the effects of novel selective α9α10 nAChR ligands based on a N,N-diethyl-N'-phenylpiperazine (diEPP) scaffold in *in vitro* models for inflammation and an *in vivo* model for inflammatory pain. The ligands were electrophysiologically characterized with populations of human α7 or α9α10 nAChRs in *Xenopus* oocytes using the two-electrode voltage-clamp technique to establish their relative selective for α9*nAChR. Ligands like *p*CF3- (interacts with α7, α9, α10) and *p*CN-diEPP (α9α10-specific) reduced both lipopolysaccharide- and ATP-induced cytokine production by mouse bone marrow-derived monocyte/macrophage, human peripheral blood mononuclear cells and monocytic/macrophage-like THP-1 cells. In the same line, *p*CN-diEPP was also a potent analgesic in a mouse model of inflammatory pain, and this effect was independent of α7 nAChR

subunits. Our findings highlight the utility of synthetic α9α10 nAChR agonists for the treatment of inflammatory diseases including ATP-induced sterile inflammation and for the development of non-opiate analgesics.

C 03-02

New insights into the gating mechanisms of Orai calcium channel paralogs

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Content

In humans, there are three paralogs of the Orai Ca^{2+} channel, which lie at the heart of the store-operated calcium entry (SOCE) machinery. While the STIM-mediated gating mechanism of Orai channels is still under active investigation, several artificial and naturally occurring mutations are known to cause constitutive activity of the human Orai1 channel. Surprisingly, little is known about the conservation of the gating mechanism among the human Orai2 and Orai3 paralogs and orthologs in other species.

Here, we find that the mutation H134A in transmembrane helix 2 (TM2), previously reported to activate human Orai1, also activates corresponding Orai2 and Orai3 mutants, likely via a similar mechanism. Conversely, this cross-paralog conservation does not apply to another activating “ANSGA” nexus mutation in TM4 intracellular helical extension of human Orai1, which is believed to mimic the STIM1-activated state of the channel. Through systematic investigation of the mechanistic basis of these differences, we identified two critical positions: H171 and F246 in human Orai1, which may be crucial for the channel activation triggered by the “ANSGA” mutations in Orai1. However, mutations of the same residues still allow gating of Orai1 by STIM1, suggesting that the ANSGA mutant of Orai1 may not be a precise surrogate for the STIM1-activated state of the Orai1 channel. Our results shed new light on these important gating checkpoints and show, that the gating mechanism of the Orai paralogs is likely affected by multiple factors, such as TM4-TM3 coupling, which may not be evolutionarily conserved.

C 03-03

Store-operated Ca^{2+} entry in the human placenta

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Content

Dysregulations in Ca^{2+} signaling and altered Ca^{2+} homeostasis have been associated with severe placental pathologies, including gestational diabetes (GDM), intrauterine growth restriction (IUGR) and preeclampsia (PE). However, the underlying mechanisms of intracellular Ca^{2+} signaling and placental Ca^{2+} transfer, including the expression of proteins involved in placental Ca^{2+} regulation and their function in pregnancy complications remain largely unknown.

This study investigated the expression of Ca^{2+}-release activated Ca^{2+} (CRAC) channels, composed of Orai1-3 subunits and the ER Ca^{2+} sensor proteins stromal interaction molecules (STIM1 and 2) in primary human trophoblasts, placental cell lines and tissues derived from healthy and pathological placentae using RT-qPCR. Further, we analyzed store-operated Ca^{2+} entry (SOCE) in healthy primary trophoblast cells and immortalized trophoblast cells with Fura-2AM-based Ca^{2+} imaging.

We found that the mRNA expression of STIM and Orai proteins is increased upon syncytium formation. Primary human trophoblasts showed a high expression of Orai3, suggesting that Orai3 is the predominant isoform. In placental tissue affected by GDM, IUGR and PE the expression of Orai3 was significantly higher in PE and moderately elevated in GDM and IUGR compared to healthy controls. Upon spontaneous syncytialization of primary trophoblasts Fura-2AM-based detection of SOCE was elevated. Similarly, in BeWo cells we observed changes in SOCE upon forskolin-induced syncytium formation.

Our data provide evidence for SOCE in human trophoblasts cells. Although the role of CRAC channels in placental tissue is poorly understood, their involvement may have consequences for transplacental Ca^{2+} transport and potentially contribute to the pathophysiology of placenta-related pregnancy complications.

C 03-04

C-terminal truncation does not abolish ion channel function of human polycystin-2 heterologously expressed in *Xenopus laevis* oocytes

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Content

Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in the PKD1 or PKD2 genes encoding polycystin-1 (PC1) or polycystin-2 (PC2), respectively. Molecular pathomechanisms of ADPKD remain incompletely understood. Among known ADPKD-associated PC2 mutations, truncating mutations are predominant. This prompted us to investigate the impact of a complete C-terminal truncation (PC2_I704X) on PC2 ion channel function. PC2 constructs were heterologously expressed in *Xenopus laevis* oocytes. Ion channel function was assessed in the absence of extracellular divalent cations using the two-electrode voltage clamp technique. Biotinylation and western blot analysis were used to detect PC2 protein at the cell surface. Electrophysiological measurements showed that sodium inward currents mediated by PC2_I704X were significantly higher than those mediated by wild type PC2. This can be explained by higher cell surface expression of PC2_I704X probably due to removal of an endoplasmic reticulum retention motif within the C-terminus. Similar results were obtained with an established gain-of-function (GOF) PC2 construct (L677A N681A, PC2_AA). Indeed, the truncated GOF mutant (PC2_AA_I704X) showed about twofold larger sodium inward currents and significantly increased cell surface expression compared to PC2_AA. In summary, our data demonstrate that C-terminal truncation enhances PC2 currents most likely by increasing channel expression at the cell surface. It is tempting to speculate that ADPKD caused by C-terminally truncating PC2 mutations is not simply due to an inability of truncated PC2 to form functional homomeric ion channels but probably involves impaired interaction and ion channel function of PC2 and PC1 as heteromeric complex.

C 03-05

Mass spectrometry-based protein–protein interaction of organic cation transporter 3 (OCT3)

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Content

Organic cation transporter 3 (OCT3) is a polyspecific transporter for small organic cations including neurotransmitters, drugs and xenobiotics and a member of the solute carrier (SLC) family member. OCT3 is a low-affinity, high-capacity transporter and contributes to monoaminergic signaling. Its broad distribution in mammalian tissues includes the heart, liver, skeletal muscle, placenta and brain. OCT3 has been implicated in cardiac contractility, thermogenesis, liver

fibrosis and tumorigenesis. In the brain, OCT3 is participating in monoaminergic transmission, mood- and stress-related modulation, and the vulnerability in neurodegeneration – and has therefore been implicated as a novel therapeutic target for the treatment of depression.
The functions of SLC transporters are regulated by oligomerization, protein-protein interactions and post-translational modifications such as phosphorylation. To elucidate the regulation of OCT3, a comprehensive proteomic analysis was performed to identify OCT3-interacting proteins.
The YFP-tagged human OCT3 proteins overexpressed in HEK293 cells were immunoprecipitated by using anti-GFP nanobody-conjugated beads for on-bead tryptic digestion followed by mass spectrometry.
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) identified 424 proteins as interacting proteins with OCT3, including proteins involved in ATP-metabolizing processes. These corresponding genes have been shown to be up-regulated in adipose-specific Oct3 knock-out mice to compensate for the loss of ATP production (Song et al., 2019).
The comprehensive OCT3 interactome study provides novel and functionally important associated proteins of OCT3. These may be of high relevance for the functional activity of the transporter.

C 03-06

The bile acid-sensitive ion channel (BASIC) affects the morphology of pancreatic islets via regulating the transcription factor paired box homeodomain 6.

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The author has objected to a publication of the abstract.

C 03-07

A small molecule PAH antidote binds in the pore cavity of TASK-1 K_{2P} channels and prevents the X-gate from closing

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Content

The TWIK-related acid-sensitive two-pore domain K^+ (K_{2P}) channel TASK-1 represents an interesting drug target due to its pathophysiological role in human disease states like neurodevelopmental, metabolic and cardiovascular disorders like pulmonary arterial hypertension (PAH). A reduced TASK-1 K_{2P} channel activity causes cell depolarization, which

in turn increases vascular constriction facilitating PAH. Hence, pharmacological TASK-1 activators are promising tools to treat respiratory malfunctions. Indeed, the phospholipase A (PLA) inhibitor ONO-RS-082 has been reported to activate TASK-1 and thereby reduce the development of PAH in mice.
Here, we report that the effect of ONO-RS-082 on TASK-1 does not involve PLA inhibition but that ONO-RS-082 is a direct dose-dependent activator of homomeric TASK-1 as well as heterodimeric TASK-1/TASK-3 channels. Our results suggest that ONO-RS-082 binds in the pore cavity below the selectivity filter (SF) and thereby prevents the lower gate (i.e., the X-gate) from closing. Furthermore, we found that TASK-1 activation by ONO-RS-082 critically depends on the extracellular K^+ concentration with an extracellular K^+ raise from 4 mM to 8 mM causing a more than ~170 % stronger activation suggesting that also the SF is involved in the activation mechanism of ONO-RS-082.Overall, we provide evidence that direct TASK-1 K_{2P} channel activation can be achieved by small molecules that bind in the pore cavity which bears major implications for future design of PAH drugs.

C 03-08

Deciphering the Role: Exploring the channel activity, localisation and interactome of hCoV-229E ap4 viroporin

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The author has objected to a publication of the abstract.

C 04 | Oxygen and HIF

C 04-01

Impact of the hypoxia-inducible factor in pancreatic ductal adenocarcinoma on T cell function and differentiation in the tumor microenvironment

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Content

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer death worldwide. While immunotherapy has been successful in treating other malignancies, it has been not in PDAC. A major reason is the

immune evading nature of the tumor microenvironment (TME) of PDACs. The immune evasive properties of the TME are exacerbated by the extent of hypoxia in cancers. The abundance of cancer-associated fibroblasts (CAFs) in PDACs make the cancer stroma rich and often hypoxic. Under hypoxia, metabolic reprogramming occurs toward a glycolytic phenotype with different catabolites, altered cytokine signaling profiles, and a higher extent of cell death with subsequent release of ADP. The alterations in the TME are detrimental to T cell effector function and fate. The hypoxia-inducible factor (HIF), which consists of an oxygen sensitive α- and a constitutively active β-subunit, plays a key role in orchestrating these modifications.

Our research focuses on how HIF activation in PDAC affects T cells in the TME.

To model hypoxic tumors, we generate PDAC-CAF-spheroids with hypoxic cores, which we co-culture with T cells from healthy volunteers. We created CAF and PDAC HIF-α knockout cell lines to compare them to wild-type cells in our co-culture model. Using RNA sequencing, flow cytometry, and Seahorse flux analysis, we are investigating changes in T cell functionality and metabolism. With LEGENDplex™ we analyze the altered composition of catabolites and cytokines in the medium/TME.

Here we describe the successful establishment of a model to study the impact of HIF in PDAC on T cells in the TME.

C 04-02

Comparative analysis of the hypoxia impact on adenovirus serotypes for the identification of favorable oncolytic vector candidates

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Content

Effective cancer treatment using human oncolytic adenoviruses (HAdV) relies on the viral spread from infected to uninfected cells. To date, there has been limited success in clinical studies in the use of replicating adenoviruses due to the negative hypoxia impact on adenoviral replication. Hypoxia, the condition characterized by low oxygen levels, is frequently observed in solid tumors. In contrast, adenoviruses infecting tissues that are typically exposed to normal ambient oxygen concentrations. Nevertheless, in studies investigating the impact of hypoxia on adenoviral replication, researchers overlooked the potential variations among different HAdV serotypes.

Currently, the International Committee on Taxonomy of Viruses (ICTV) has identified 54 types of HAdV, organized into seven species designated as A through G. It is important to note that HAdV types recognized as distinct by the ICTV are deemed sufficiently different to meet the allocation criteria.

In this study, our aim was to assess the impact of hypoxia on 40 HAdV serotypes spanning species A through G. Initially, we utilized the hypoxia-mimicking agent Roxadustat to simulate the effects of hypoxia on virus replication. Consistent with earlier observations under 1% O_2 conditions, Roxadustat diminished the replication of HAdV5 in HEK293A cells. Moving forward, our aim is to evaluate 40 serotypes to pinpoint candidates that exhibit reduced sensitivity to hypoxia compared to HAdV5.

C 04-03

Prolonged oxygen supply in cell culture by novel extended-release oxygen carrier

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Content

Question

Organ damage due to lack of oxygen (hypoxia) is a common issue, especially in emergencies and surgeries where blood flow stops. Previous versions of our artificial oxygen carriers (LENOX) couldn't release oxygen for long without recharging. This study presents an improved LENOX that provides sustained oxygen even when blood flow is interrupted.

Methods

We tested the properties and function of the new **e**xtended-**r**elease **o**xygen **c**arrier (E-ROC) in a cardioplegic solution (Custodiol). Oxygen release was measured over time using PreSens system under hypoxic conditions without cells. We compared the oxygenation using preoxygenated E-ROC, Custodiol alone, and LENOX over 4 hours by analyzing HIF-1α via Western blot, ROS production, lactate and cell viability as markers for cell status.

Results

As compared to LENOX preoxygenated with the same amount of oxygen oxygen release from E-ROC was prolonged by more than factor 2. It effectively oxygenated hypoxic cells for at least 4 hours, without increasing harmful reactive oxygen species (ROS) production or affecting cellular viability (measured by release of troponin, LDH and lactate).

Conclusions

E-ROC provides gradual oxygen supply after one-time charge without harming cells. This opens new possibilities for use of artificial oxygen carriers in organ preservation and surgical procedures without active blood flow support.

C 04-04

Impact of albumin-derived artificial oxygen carriers on monocytes

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The author has objected to a publication of the abstract.

C 04-05

The Role of the Hypoxia-Inducible Factors in Urinary Bladder Carcinoma – Project Outline

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Content

The adaption of cells to oxygen deficiency (hypoxia) is dependent on the hypoxia-inducible factor (HIF). Thus, the HIF transcription factor complex activates specific genes that enable the cells to adjust their metabolism to the available oxygen conditions. Hypoxia and inflammatory processes, like bacterial urinary tract infections, lead to an activation of HIFs. Further, hypoxia plays an important role in carcinomas. Urinary bladder carcinoma is one of the most common cancer types with about 30,000 new cases in Germany each year. 90% of bladder carcinoma arise from urothelial cells. HIF-1 appears to play an important role in the urothelium and is a potential prognostic marker for poor outcome in bladder cancer. It is discussed if chronic bladder infections predispose the development of bladder cancer.
My dissertation will investigate how altered HIF-1α expression in urothelial cells affects bladder cancer development and how previous, repeated bladder infections influence bladder cancer formation. To this purpose, we will combine a repeated infection with uropathogenic *E. coli* with the administration of the carcinogen N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN). *Hif-1a*^{fl} x K14-Cre mice will be used to study how non-functional HIF-1α in urothelial cells affects the formation of urinary bladder carcinoma. Additionally, murine bladder organoids and the human uroepithelial cell line HBLAK will be cultivated to analyze underlying signaling pathways in more detail. Further, experiments to investigate the permeability of differentiated HBLAK cell layers under inflammatory conditions are planned.
These data will help to deepen our understanding of the interplay between HIF-1, urinary bladder infections and bladder cancer formation.

C 04-06

Effect of hypoxic priming in apoptotic cell clearance by bone marrow-derived murine macrophages

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Content

Dead cell clearance is an essential mechanism to maintain the homeostasis in organs. A defect in the apoptotic cell clearance can play a major role in the etiology of several autoimmune diseases, such as rheumatoid arthritis (RA).

During RA and other recurrent inflammatory diseases, organs are exposed to recurring and chronic tissue hypoxia due to excessive immune cell recruitment in the joints and tissue swelling.
It is known that phagocytosis is enhanced under prolonged chronic physiological hypoxia, nevertheless the effect of a recurring hypoxia on the apoptotic clearance capacity and on the metabolic consequences are not studied so far.
In this context, we study the efficiency of in vitro apoptotic cells clearance by bone marrow-derived macrophages under hypoxic conditions (1% O2 for 24h after differentiation) and under hypoxic priming (1% O2 for 24h at day 3 of differentiation) using CFSE-labelled apoptotic Jurkat cells for phagocytosis assay. Confocal laser scanning microscopy will be used to assess the localization and engulfment of apoptotic cells as well as analysis of the mitochondria dynamic under hypoxia and hypoxia priming. Mitochondria staining will be performed using immunofluorescence staining against TOM20 (Translocase Of Outer Mitochondrial Membrane 20) to produce high resolution mitochondria staining images and consequent analysis of mitochondrial morphology, dynamics, and function will be performed using semi-automated image processing pipelines.
Flow cytometry measurements will provide quantitative assays on the number of engulfed dead cells for each condition and Seahorse assay will be used to compare the metabolic status of macrophages under hypoxia and hypoxia priming.

C 04-07

Deciphering the role of hypoxia-inducible factors in ferroptotic cell death in a cell culture model of age-related macular degeneration

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Content

Age-related macular degeneration (AMD) is the most common blinding disease in the elderly population, which primarily affects central vision because the photoreceptors in the macula degenerate due to a dysfunction and atrophy of the retinal pigment epithelium (RPE). Dry AMD is the most common type of advanced AMD, with no promising treatment available to date. Major risk factors for AMD are oxidative stress and hypoxia in RPE cells. Both are associated with accumulation of hypoxia-inducible factors (HIFs), dimeric transcription factors with an oxygen-labile α-subunit and a constitutively expressed β-subunit.
In a previous study, we have established a cell culture model of dry AMD in a human RPE cell line (ARPE-19) and demonstrated that HIF stabilization exacerbates oxidative damage leading to cell death by ferroptosis, an iron-dependent cell death mode. Based on these findings, we performed siRNA-mediated knockdown of *HIF1A* and *HIF2A* to investigate their respective roles in the regulation of ferroptosis. We found cell death to be aggravated in cells with *HIF2A* knockdown, while knockdown of *HIF1A* resulted in decreased cell death. Furthermore, we discovered that transferrin receptor 1 (TFR1), which is responsible for iron import, and heme oxygenase-1 (HO-1), which was recently

described to promote ferroptosis, was regulated in a HIF-dependent manner. In line, siRNA-mediated knockdown of *TFR1* and *HO1* decreased cell death. Our results show that selective HIF inhibition could be a promising treatment approach against dry AMD and using our dry AMD model, we have already identified promising inhibitors that protected RPE cells from cell death.

C 04-08

Investigation of artificial perfluorocarbon-based oxygen carriers under shear forces in a vessel-like perfusion system

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Content

Artificial oxygen carriers (AOCs) offer a promising alternative to erythrocyte concentrates for oxygen transport. This study aimed to assess perfluorocarbon-based AOCs' stability and functionality under physiological blood flow conditions.

Two types of 8 % AOC emulsion in 0.9 % NaCl or DMEM cell-culture medium were synthesized and perfused for up to 24 hours under normothermic conditions using a peristaltic pump at 25 rpm. Samples were collected at 0, 1, 3, 6, and 24-hour intervals and analyzed using dynamic light scattering, rheometry, and respirometry. A control solution of AOCs incubated statically at 37 °C and 5 % CO_2 underwent similar characterization.

Dynamic light scattering revealed a slight increase in AOCs mean particle diameter from 186 to 226 nm after 24 hours of perfusion for NaCl-medium, while the statically-stored control solution showed a larger increase to 243 nm. AOCs in DMEM showed larger particle diameters overall, starting at 216 nm, but with a similar increase when incubated statically (up to 227 nm). Viscosity remained constant during perfusion and incubation for both, NaCl and DMEM AOCs with values of 1 mPas (37 °C, shear rate: 333 s^{-1}). AOCs exhibited consistent oxygen capacity throughout the study (2.1-2.5 µmol/mL), higher than the NaCl control solution with values of 1.1-1.4 µmol/mL.

The study demonstrated the AOCs stability and sustained oxygen transport capacity under physiological flow conditions. Integrating a 3D-printed blood vessel into the perfusion system will advance the future research on perfluorocarbon nanoemulsions for clinical use.

C 04-09

Abstract has been withdrawn.

C 04-10

The effect of isoleucine preconditioning on neuroprotection via HIF-1α signaling after oxygen-glucose deprivation insult

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Content

Background:

Isoleucine is an essential branched-chain amino acid, but our knowledge of its role in neuronal cells after ischemic stroke is limited. One study has shown that isoleucine activates mammalian target of rapamycin (mTOR) in neuroblastoma cells. Importantly, its target, P70S6K, regulates hypoxia-inducible factor-1α (HIF-1α).

Plans:

Our study investigates the effect of isoleucine on OGD-induced cell death and whether it affects HIF-1α at RNA and protein levels after OGD. Moreover, it is necessary to assess whether isoleucine pretreatment causes any changes in HIF-1α target genes and isoleucine-related genes. Additionally, we plan to examine the involvement of mTOR signaling in potential isoleucine-HIF-1α interaction.

Methods:

In our study, we use oxygen-glucose-deprived (OGD) *SH-SY5Y* cell, a neuroblastoma cell line, to simulate cerebral ischemic stroke *in vitro*. Cytotoxicity was assessed by LDH release. Protein levels of HIF-1α, mTOR signaling, and the RNA level of *HIF-1A*, canonical HIF-1α target genes and isoleucine-related genes will be analyzed.

Results and Conclusions:

Our current results suggest that isoleucine pretreatment does not exacerbate OGD-induced cytotoxicity in *SH-SY5Y* cells. *HIF1A* RNA level is not significantly affected by isoleucine. Interestingly, we observed that isoleucine reduced the significant induction of certain HIF-1α target genes. *I.e.,VEGFA, GLUT1*. However, a larger sample size and further investigation are needed to better understand the relationship between isoleucine, neuroprotection and HIF-1α signaling.

C 04-11

Insoluble HIFa protein aggregates by cadmium disrupt hypoxia-PHD-HIFa-EPO signaling in renal EPO-producing epithelial (NRK-52E) and interstitial (FAIK3-5) cells

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Content

The kidney is the main organ that senses changes in systemic O_2 pressure by hypoxia-PHD-HIFa-EPO (HPHE) signaling, resulting in adaptive target gene activation. The non-essential transition metal cadmium (Cd^{2+}) is nephrotoxic and disrupts the renal HPHE pathway, which may promote Cd^{2+}-associated chronic renal disease (CKD). A deeper molecular understanding of Cd interference with renal HPHE signaling is missing, and no data with renal cell lines are available. In rat kidney NRK-52E cells, which model the proximal tubule, and murine fibroblastoid atypical interstitial kidney (FAIK3-5) cells, which mimic renal EPO-producing cells, the chemical hypoxia mimetic dimethyloxalylglycine (DMOG; 1 mM) or hypoxia (1% O_2) activated HPHE signaling. Cd^{2+} (2.5-20 µM for ≤ 24 h) preferentially induced necrosis (trypan blue uptake) of FAIK3-5 cells at high Cd^{2+} whereas NRK-52E cells specially developed apoptosis (PARP-1 cleavage) at all Cd^{2+} concentrations. Cd^{2+} (12.5 µM) abolished HIFa stabilization and prevented upregulation of target genes (quantitative real-time polymerase chain reaction and immunoblotting) induced by DMOG or hypoxia in both cell lines, which was caused by the formation of insoluble HIFa aggregates. Strikingly, hypoxic preconditioning (1% O_2 for 18 h) reduced apoptosis of FAIK3-5 and NRK-52E cells at low Cd^{2+} concentrations and decreased insoluble HIFa proteins. Hence, drugs mimicking hypoxic preconditioning could reduce CKD induced by chronic low Cd^{2+} exposure.

C 05 | Pulmonary system and respiration

C 05-01

Abstract has been withdrawn.

C 05-02

Serum calcification propensity is increased in systemic sclerosis and associates with lung diffusion capacity

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Content

Question

Systemic sclerosis (SSc) is an auto-immune disease associated with inflammation, fibrosis and calcinosis. Pulmonary fibrosis is the major cause of mortality in these patients. Systemic inflammation induces dysregulation of the mineral buffering system, which induces increased formation of calciprotein particles (CPPs). Therefore, indicators of mineral buffering were investigated in patients with SSc and correlated to disease activity.

Methods

Serum calcification propensity (T50) and hydrodynamic radius of secondary CPPs (CPP2) were measured in 78 SSc patients and 44 controls without SSc, and correlated with disease activity markers of SSc.

Results

A lower T50 and increased radius of CPP2 was observed in SSc patients as compared to controls. Concomitant, phosphate concentrations and parathyroid hormone (PTH) were slightly elevated, while fibroblast growth factor 23 (FGF23) was not significantly different in SSc patients as compared to controls. In a longitudinal assessment of SSc patients, no difference in T50, CPP2 radius, phosphate and PTH was observed during the observational period, but FGF23 levels increased. A correlation of modified Rodnan skin score with CPP2 radius was observed, but this association was not independent of other factors in a multivariate linear regression model. In contrast, age and T50 emerged as significant modifiers of lung diffusion capacity in SSc.

Conclusions

These observations indicate a deranged mineral homeostasis in SSc and a correlation of T50 with pulmonary function. Further studies are required to investigate a possible functional relevance in disease progression.

C 05-03

Exploring the impact of the A2B receptor in the pulmonary vasculature

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Content

The G protein-coupled A2B receptor (A2BR) and its endogenous ligand adenosine are known to affect vascular tone regulation and smooth muscle cell growth in systemic arteries. Receptor and ligand show elevated levels in pulmonary hypertension and under hypoxic conditions. We therefore wanted to investigate the role of the receptor in pulmonary arteries (PAs).

We analyzed A2BR expression by qPCR, Western Blotting and immunohistochemistry. Pulmonary arterial tone regulation by A2BR stimulation was examined using different *ex vivo* techniques (wire-myography, functional lung slices and isolated perfused lung (IPL)). Growth and proliferation assays were performed in human pulmonary artery smooth muscle cells (hPASMCs).

Using qPCR, Western Blotting and immunohistochemistry, we found A2BR expression in lung, PAs, aorta and bladder and located the A2BR particularly in the smooth muscle layer of PAs. In wire-myography, preincubation of mouse PAs with adenosine or the A2BR specific agonist BAY60-6583 (BAY) induced a right-shift of dose-response curves of different constrictors (5-HT and U46619). Single dose application of BAY after preconstriction with these constrictors caused a pronounced vasorelaxation in PAs. The effect was attenuated by additional preincubation with the A2BR specific antagonist PSB-603. Similar effects were observed using functional lung slices and IPL. *In vitro*, BAY strongly diminished cell growth and proliferation, again partly reversible by PSB-603.

A2BR activation results in pulmonary vasorelaxation and reduces hPASMC proliferation. Further experiments are required to investigate the underlying signaling pathway and the role of A2BR in disease.

C 05-04

High-magnitude cyclic stretch differentially regulates protein and RNA sialylation in the alveolar epithelial glycocalyx

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Content

Sialic acids are integral components of cell surface glycoconjugates and modulate barrier integrity, mechanosensation, infection and immunity. These functions are specifically relevant at the blood-gas barrier of the lung, yet the abundance, regulation and functions of sialic acids at the alveolar epithelial surface remain unknown. Here, we investigated the effects of mechanical stretch on sialic acid abundance in alveolar epithelial cell surface proteins and RNA.

Human primary alveolar epithelial cells (hPAEpC) were exposed to high magnitude cyclic stretch at 0.25 Hz for 48 hours to mimic biomechanical forces at the alveolar epithelium during high tidal volume ventilation. Total and *de novo* formed cell surface sialic acids were labeled with biotin and detected with streptavidin in protein and RNA extracts by Western and RNA blots, respectively. Stretch-dependent changes in the genes regulating sialic acid biosynthesis were assessed by RNA sequencing and confirmed by Western blot.

Exposure of hPAEpC to high magnitude cyclic stretch increased sialic acid abundance and *de novo* formation in proteins, but decreased sialic acid abundance with no alteration in *de novo* synthesis in RNA. RNA sequencing and Western blot analysis revealed a cyclic stretch- dependent upregulation of the *GNE* gene and its encoding enzyme, UDP-GlcNAc 2-epimerase/ManNAc kinase, which is critical for sialic acid synthesis.

Here, we demonstrate cyclic stretch-induced protein hypersialylation and RNA hyposialylation at the alveolar epithelial glycocalyx. Given that sialic acids promote the adhesion of both infectious pathogens and immune cells, these effects may contribute to the pathogenesis of ventilator-associated pneumonia and ventilator-induced lung injury.

C 05-05

Beta arrestin 1 in asthma bronchiale

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Content

Chronic obstructive lung diseases like asthma are a leading cause of morbidity and mortality in western countries. Current treatment regimens focus on the modulation of G protein-coupled receptors (GPCRs) in order to lower airway tone. Beta arrestins are ubiquitously expressed cytosolic proteins that modulate GPCR signaling. Thereby, beta arrestins are also involved in the pathophysiology of numerous diseases but their role in the (patho)physiology of asthma is still unclear.

Acute asthma was induced by Ovalbumin (OVA) in WT and beta arrestin1 -/- mice. Airway resistance in response to methacholine was determined by Flexivent measurements. Leukocyte counts were assessed by DiffQuick staining and counting in bronchoalveolar lavage fluid (BALF). Cell invasion around the airways was determined by H&E stainings of lung sections. In addition, goblet cell hyperplasia and collagen deposition were analyzed by PAS and Sirius red stainings, respectively.

Flexivent measurements revealed a pronounced airway hyperresponsiveness (AHR) in beta arrestin1 -/- OVA mice when compared with WT OVA animals. Also, total cell counts and the number of eosinophils in BALF were strongly increased in beta arrestin1 -/- OVA vs WT OVA mice. PAS stainings demonstrated a comparable increase of goblet cells in OVA mice of both genotypes. Evaluation of collagen deposition revealed an elevation of collagen selectively in beta arrestin1 -/- mice.

These results indicate that beta arrestin 1 may play an important role in the pathophysiology of asthma. Further analyses will have to reveal the underlying mechanism.

C 05-06

Fibroblast-specific actions of C-Type Natriuretic Peptide/cyclic GMP signaling in Pulmonary Fibrosis

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Question

Pulmonary fibrosis (PF) is a progressive lung disease characterized by excessive fibroblast activity and collagen deposition, which can be idiopathic (IPF) or secondary to a severe inflammation. This study explores the impact and therapeutic potential of C-type natriuretic peptide (CNP).

Methods and Results

Our *in vitro* studies showed that human lung fibroblasts express the cGMP-synthesizing Guanylyl cyclase-B (GC-B) receptor for CNP. Treatment with synthetic CNP increased cGMP levels and significantly inhibited the profibrotic (Collagen 1 expression), proliferative (BrdU incorporation), and migratory (Wound closure) effects of growth factors. Notably, GC-B expression/activity and the antifibrotic effects of CNP were preserved in fibroblasts from patients with IPF. To dissect whether endogenously formed endothelial CNP exerts protective paracrine effects, we developed a genetic mouse model with fibroblast-specific GC-B deletion. Notably one week after intratracheal bleomycin administration such fibroblast GC-B knockout mice showed increased pulmonary plasma leakage and elevated albumin and Galectin-3 levels in bronchoalveolar lavage fluid, indicating augmented inflammation. However, the extent of subsequent interstitial fibrosis and the decline in lung compliance did not significantly differ between GC-B knockout and control mice.

Conclusions

Exogenous CNP exerts antifibrotic effects in cultured lung fibroblasts from healthy individuals and IPF patients. Mice with fibroblast-specific GC-B deletion exhibit increased inflammation but unaltered fibrosis in response to bleomycin. This suggests that endogenous CNP can inhibit the proinflammatory properties of fibroblasts but not their transition to profibrotic myofibroblasts. We are currently investigating the involved mechanisms and the therapeutic potential of exogenously administered long-acting CNP in experimental PF.

C 05-07

Pharmacological Gq -protein inhibition improves right heart function in hypoxia-induced pulmonary hypertension

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Content

Pulmonary hypertension (PH) is a progressive disease characterized by chronic pulmonary vasoconstriction and vascular remodeling leading to right heart hypertrophy and dysfunction. Gq-proteins are involved in both, vascular and cardiac (patho)physiology. Therefore, we analyzed the effect of the pan-Gq protein inhibitor FR900359 (FR) on pulmonary vascular and right heart remodeling and function in a murine PH model in vivo.

To induce PH in mice the Sugen 5416/Hypoxia (SuHx) model was used. During disease development, mice received intraperitoneal applications of FR or the solvent DMSO. Pulmonary vascular remodeling was examined by H&E stainings of lung sections. Right ventricular hypertrophy and cardiac function were analyzed by assessment of the Fulton index and pressure-volume catheter measurements, respectively.

While pulmonary vascular wall thickness and right heart hypertrophy were elevated in SuHx compared to normoxia (Nx), these increases were prevented by FR. Furthermore, FR reduced right ventricular systolic pressure increase induced by PH (DMSO-Nx: 23.14 ± 0.45 mmHg, n=3 vs. DMSO-SuHx: 33.80 ± 3.03 mmHg, n=7 vs. FR-SuHx: 22.91 ± 2.91 mmHg, n=4, $p<0.001$) and decreased the slope of the end-systolic- and end-diastolic-pressure-volume-relationships to normal values for Nx. No differences in stroke volume, ejection fraction and end-diastolic volume were observed. These data suggest that pharmacological Gq inhibition by FR improves heart remodeling and function in PH. To evaluate if inhibition of Gq-proteins directly affects heart function, experiments will be performed in cardiomyocyte specific Gq/G11-knockout animals. In addition, we will use the model of pulmonary artery banding to examine Gq inhibition in a hypoxia-independent model.

C 05-08

Heterozygous titin deletion causes altered right ventricular remodelling in a mouse model of pulmonary hypertension

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Content

Patients with pulmonary arterial hypertension show changes in titin phosphorylation and right ventricular (RV) stiffness. To assess the role of titin in pulmonary hypertension (PH) in detail, we induced the disease in a heterozygous titin knockout mouse model ($Ttn^{tm1a/+}$, termination of translation after exon 3) and examined the effects on RV structure and function.

$Ttn^{tm1a/+}$ mice appeared phenotypically normal. To induce PH in WT and $Ttn^{tm1a/+}$ animals, mice were exposed to Sugen 5416/hypoxia (SuHX) for 3 weeks. Cardiac function was assessed by pressure-volume catheter measurements. For morphological analysis, heart sections were stained with wheat germ agglutinin. The expression of cardiac proteins was analysed by western blot. Lung sections were stained with H&E.

Measurement of pulmonary arterial wall thickness showed an increase in WT and $Ttn^{tm1a/+}$ SuHX mice compared to normoxia (NX) control groups. Hemodynamic analysis demonstrated the expected increase in RV systolic pressure in SuHX-WT and SuHX-$Ttn^{tm1a/+}$ mice when compared to NX. There was no difference in the volume parameters, but the slope of end systolic pressure volume relationship (ESPVR) was increased in SuHX in both genotypes, whereas the end diastolic pressure volume relationship (EDPVR) was only elevated in WT SuHX. Morphometry revealed that elevated afterload in SuHX-induced PH resulted in RV hypertrophy in WT but not in $Ttn^{tm1a/+}$ animals. Interestingly, western blot analysis demonstrated an upregulation of Cronos in the left and right ventricle, in $Ttn^{tm1a/+}$ compared to WT mice in NX.

These data suggest that loss of titin alters pressure-induced right ventricular remodelling in $Ttn^{tm1a/+}$ mice.

C 06 | Endothelial cells: angiogenesis

C 06-01

Identification and functional characterization of endothelial cell microproteins encoded by non-canonical open reading frames.

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Content

The relevance of non-canonical small open reading frames (smORFs)-encoded microproteins (miPs; <100 amino acids) in endothelial cells is mostly unknown. We combined deep RiboTag-RNA sequencing and bioinformatic pipelines optimized for downstream proteomic studies to identify miPs in endothelial cells by mass spectrometry. This approach led to the identification of 1,577 intracellular and 686 released endothelial cell miPs. Vascular inflammation *in vitro* (interleukin-1β) and *in vivo* (partial carotid artery ligation) profoundly altered smORF and miP expression. Moreover, we identified 362 miPs in human serum, 27 of which were significantly decreased and 34 significantly increased following transcoronary ablation of septal hypertrophy, a procedure that involves a short period of myocardial ischemia. The functional importance of 256 miPs for endothelial cell growth and viability was demonstrated by a high-throughput CRISPR/Cas9 screen. The detailed functional characterization of selected human endothelial cell miPs was performed combining gain- and loss-of-function approaches with multi-omic analyses. These studies revealed the ability of miPs to form complexes with other proteins to affect endothelial cell activation, cell cycle progression, intracellular transport, and transcriptional regulation, in line with their diverse cellular localization, including caveolae, the cytosol, the cytoskeleton, paraspeckles or nucleoplasm. Altogether, endothelial cell miPs represent an emerging class of key molecular players involved in a plethora of biological processes. When secreted, they may exert autocrine or paracrine functions, potentially relevant for human cardiovascular disease.

C 06-02

Beyond silencing: The HUSH complex maintains endothelial angiogenic activity through novel interactions

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Content

The human silencing hub complex (HUSH) represses retroelements, thereby contributing to innate immunity against genome invadors. We have previously observed that the lncRNA *HIF1α-AS1* facilitates partial repression of genes in endothelial cells (EC) through HUSH recruitment. We therefore hypothesize that in EC, HUSH has a function beyond silencing (viral) products of reverse transcription.

FANTOM5 data revealed that EC highly express the known HUSH components MPP8, TASOR and Periphilin. MPP8, the central subunit, interacted with H3K9me3 and dsDNA. It was induced by laminar flow and interleukin-1β but repressed in EC isolated from glioblastoma. *In vitro* binding experiments with MPP8 deletion mutants showed that its C-terminal domain binds the lncRNA *HIF1α-AS1*, while its N-terminal domain -including the chromodomain- was important for dsDNA binding. Protein interaction studies revealed that MPP8 binds all complex members, the effector histone methyltransferase SETDB1, but not -as previously reported- to NP220. Interestingly, additional interactors of MPP8 were identified, such as TASOR2, TAF15, RBMX and NUMA1. Knockdown of MPP8 and SETDB1, but not NP220, decreased angiogenic capacity of EC. In line with this, MPP8 and SETDB1, but not NP220 was needed for *HIF1α-AS1* binding to its triplex targets. RNA- and ATAC-seq after knockdown of MPP8 and SETDB1 revealed a strong overlap in target genes, which were mostly downregulated.

In EC, the HUSH complex has a function beyond silencing, and supports gene expression which is potentially mediated by novel interaction partners. Thus this, the HUSH complex support the endothelial angiogenic function.

C 06-03

Abstract has been withdrawn.

C 06-04

The Heart's Capillary Network: A Highly Modular Structure with Anisotropic and Elongated Submodules

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Content

The coronary capillary network is vital for supplying oxygenated blood to the myocardium, supporting sustained cardiac performance and overall cardiovascular health. Given the critical role of the capillary system's architecture in its effectiveness, it is imperative to provide a morphometric description of its structure. To address this issue, we combined 3D vascular imaging with network analyses. Experimentation was done on anesthetized 7 female and 7 male 8-week-old C5BL/6J mice. The analysis of the cardiac microvascular architecture was quantified on the fluorostained capillary networks of the left and right ventricle and septum on 3D light-sheet microscopy images of optically cleared hearts. Centerline voxels of capillaries were used to construct networks, representing bifurcations as nodes and establishing connections between them if directly connected by the centerline. Our results indicate that the capillary networks exhibit a high degree of segregation and clearly defined submodules. Within these submodules, there is an elevated level of local integration, while interactions between different modules can be interpreted as bottlenecks. Moreover, individual submodules were found to be highly elongated and oriented in a preferential direction, which is attributed to the anisotropic nature of local connections. These characteristics are independent of the gender of mice or the anatomical region of the heart. Importantly, obtaining these novel insights into the capillary network's structure would not be possible without the interdisciplinary integration of advanced microscopic techniques with modern computational network-based methods. The proposed methodology also provides a robust framework for analyzing various types of vascular networks in health and disease

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C 06-05

Identification of endothelial long non-coding RNAs to promote regenerative angiogenesis in the damaged myocardium

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Content

Long non-coding RNAs (lncRNAs) are RNAs, which are longer than 200nt have been found to exhibit a wide range of important functions in the cardiovascular system. Their contribution to endothelial function including differentiation and proliferation has been well documented. Targeting lncRNAs that are involved in injury-induced angiogenesis could offer therapeutic opportunities in cardiovascular diseases (CVD). The aim of this study is to unravel and characterize lncRNAs that dynamically control damage-induced regeneration of endothelial cells (ECs).

To induce a regenerative phenotype of ECs damage-associated molecular patterns (DAMPs) models were developed. This included EC treatment with activated platelet-derived products (PDP) or supernatants from necrotic tissues (NMC). NMC treatment significantly increased EC proliferation. PDP treatment caused an increase in apelin (APLN) expression, an endothelial tip cell marker. To specifically target regenerating ECs, an APLN eGFP-destabilized system was developed. With the aid of these models, we aim to perform RNA-sequencing of regenerating ECs in order to identify differentially expressed lncRNAs during this process.

Alongside, three novel lncRNA candidates were chosen to be analyzed upon their role in endothelial regeneration. The first candidate Lnc1 was 80-fold increased upon quiescence. Therefore, knockdown of Lnc1 could be an approach to increase proliferation. Analysis of a hypoxia-induced angiogenesis RNA-Sequencing data identified the other candidates (Lnc2 and Lnc3). Hypoxia but also DAMP treatment elevated Lnc2 and Lnc3 expression levels, suggesting that their knockdown could attenuate endothelial regeneration.

We have identified promising candidates to modulate angiogenic responses and proceed with further characterization.

C 06-06

The Gap Junctional Communication (GJC) between endothelial progenitor cells (EPC) and endothelial cells (EC) promotes the formation of angiogenic networks

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Question

EPC have a strong angiogenic potential and support neovascularization and endothelial repair. Connexins (Cx) are transmembranous proteins which form gap junction (GJ) channels between adjacent cells. EPC specifically express Cx43. Here, we investigated the role of Cx43 in the formation of angiogenic networks in co-cultures of EPC and EC *in vitro*.

Methods

Mouse embryonic EPC (E7.5) were co-cultured with EC (HUVEC, HMEC or PAEC) for 3-6 days. Cx43 expression was downregulated with a specific siRNA against Cx43. The GJC was inhibited pharmacologically using different GJ blockers (heptanol combined with meclofenamic acid (hep/mec), carbenoxolone (CBX)). The inhibiting effect of the

siRNA and the GJ blockers was analysed in western blots and by scrape-loading/dye transfer assays (SLDT). Cx43 localisation in co-cultures was investigated by immunofluorescence staining.

Results

Capillary-like networks were formed spontaneously in co-cultures of EC with EPC (CTL) after 3-5 days (HUVEC n=7, HMEC n=6, PAEC n=6). This angiogenic network formation was significantly reduced by a specific Cx43 siRNA as well as by blocking the GJC (mean±SEM; length of branches: HMEC: CTL: 368±21µm, CRsi: 360±19µm, si43: 299±7µm, n=1, $p<0.01$, hep/mec: 285±7µm, n=6, $p<0.01$). SLDT confirmed the GJ inhibiting effect (mean±SEM; diffusion-length of dye: PAEC: CTL:324±3µm, hep/mec:254±15µm, n=4, $p<0.01$).

Conclusions

Our results suggest that the GJC of EC and EPC in co-cultures is necessary for the spontaneous formation of angiogenic networks. Further analyses are required to clarify whether Cx43 might additionally contribute to the formation of capillary-like networks in a channel-independent manner via regulating endothelial cell migration.

C 06-07

The inflammation-induced microprotein miP-PSTPIP2 modulates endothelial cell activation

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Microproteins (miPs) of fewer than 100 amino acids, encoded by non-canonical small open reading frames (smORF) are expressed in human endothelial cells. The purpose of this study was to characterize the function of miP-PSTPIP2, a novel 46 amino acid endothelial cell miP that is encoded by a smORF within the coding sequence of the proline-serine-threonine phosphatase interacting protein 2 (PSTPIP2) transcript, albeit in a different reading frame. The expression of smORF-PSTPIP2 was increased in ligated carotid arteries from atherosclerosis-prone apolipoprotein E-deficient mice and IL-1b-treated endothelial cells. The miP localized in actin-rich domains in the cell membrane (caveolae) and the cytoskeleton, along with cytosolic vesicles and the nucleus. Immunoprecipitation combined with mass spectrometry revealed that miP-PSTPIP2 associated with proteins involved in cytoskeletal regulation, intracellular transport, integrin signalling, clathrin adaptor activity and the nucleosome. Using a proximity ligation assay we confirmed the interaction between miP-PSTPIP2 and CAV-1 and clathrin heavy chain. Overexpression of miP-PSTPIP2 led to a downregulation of genes associated with cell cycle progression and DNA repair, resulting in a significant reduction in endothelial cell proliferation. The siRNA-mediated knockdown of PSTPIP2 and miP-PSTPIP2 i.e., the host gene and the smORF, significantly decreased endothelial cell activation as demonstrated by reduced monocyte adhesion to IL-1β-stimulated endothelial cells. Collectively, miP-PSTPIP2 is an IL-1b-regulated miP that interacts with key factors involved in intracellular transport and cell adhesion to modulate endothelial cell function.

C 06-08

Quantifying the influence of cell-cell interactions on the dynamics of confluent endothelial cells

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Question

Experimentally observable movements of confluent cells emerge from the combination of active motion, interactions with other cells and adhesion to the extracellular matrix. As these processes may depend on many cellular characteristics, it is the aim of this study to quantify the temporal and spatial development of cell-cell interactions directly from the observation of cell positions.

Methods

We followed several 10.000 endothelial cells from human umbilical veins over a period of 48 hours. Cell tracks were automatically determined by analyzing the labeled cell nuclei (Hoechst 33342). Model-based quantification and model selection regarding the cell-cell interactions were performed with Bayesian data analysis.

Results

The calculation of the pair-correlation function from the cell positions gives an estimation of cell-cell interactions. In addition, we derived model-based parameterizations of the interactions. It is noteworthy that cells show only repulsive forces despite the confluent cell arrangement. Thereby, forces strongly increase for short distances of approximately 15 µm, but quickly decrease to zero at intermediate distances of 30 µm. Individual cells display a generalized Ornstein-Uhlenbeck-like characteristic and high heterogeneities. These observations suggest that cells within the confluent layer perform active, correlated movements within a shallow, repulsive force field of their neighbors, which mainly acts as guardrail at short distances.

Conclusions

The application of mathematical models allows the estimation of mechanistic effects like cell-cell interactions, avoiding direct measurements that could also trigger artificial changes. The observed, complex dynamics might influence the recovery of confluent cells after injury.

C 07 | Renal: Tubular function and transport

C 07-01

Development of a Predictive Equation for Estimating 24-Hour Urine Phosphate Excretion from Spot Urine Collection

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24-hour urinary phosphate excretion is indicative of intestinal phosphate absorption in steady-state conditions. Nevertheless, 24-h urine collections are cumbersome and error-prone. Previous studies have indicated that spot urine phosphate could serve as a practical and effective substitute to predict 24-hour urine phosphate mostly in chronic kidney disease patients. Here, we investigated the precision and reliability of spot urine phosphate measurements in comparison to the collection of 24-hour urine phosphate in a cohort including 761 stone kidney patients and 207 controls, the Swiss Kidney Stone Cohort (SKSC).

Using data from the SKSC Pearson correlation coefficients and Bland–Altman plots were used to assess the relationship between spot urine phosphate and creatinine and 24-h urinary phosphate excretion (24hUrP). Additionally, backward multivariate analysis was performed to predict urinary phosphate excretion.

Log-transformed 24hUrP with the spot urine phosphate and creatinine yielded the best model fit. In addition to spot urine phosphate and creatinine concentrations, inclusion of age, sex, and BMI significantly improved prediction of 24hUrP. Compared with spot urine phosphate and creatinine alone (r^2 =0.0561, $p < 0.001$) the new equation predicted 24hUrP (r^2 =0.182, $p < 0.001$) more accurately.

Here, presenting a new formula for predicting 24-hour urinary phosphate excretion by incorporating spot urine phosphate and creatinine, along with age, sex, and BMI.

C 07-02

The murine TMEM16J variant T595A modulates intracellular calcium signaling and cytokine release in mouse tubular epithelial cells and T- lymphocytes.

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Content

TMEM16J (T16J, ANO9) is a phospholipid scramblase that, along with SIGIRR and PKP3, plays a crucial role in immune response regulation and inflammation control. Variants in the SIGIRR/PKP3/T16J genes have been associated with severe inflammatory diseases. Notably, a T16J variant (T595A in mice) has been identified through GWAS as a strong promoter for chronic kidney disease (CKD). However, the precise disease mechanism and function of T16J remain to be fully elucidated.

In murine kidneys T16J is expressed in the apical membrane of proximal tubular epithelial cells. In T16J T595A knock-in mice tubular epithelial cells exhibit elevated expression of the T595A variant. Additionally, there is peritubular interstitial expansion and increased expression of TGF-β1 in tubular epithelial cells, suggesting kidney inflammation. Furthermore, T16J T595A is also expressed in murine CD3-positive lymphocytes. When lymphocytes isolated from lymph nodes of T16J T595A knock-in and wild-type mice are cultured for 24 hours, activation by CD3/CD28 antibodies leads to increased whole-cell current, Ca^{2+} signaling and IL-2 release compared to wild-type lymphocytes. Experiments conducted in Jurkat T-lymphocytes reveal that T16J controls RO2959 sensitive store operated calcium influx (SOCE) through ORAI1 by activating plasma membrane Ca^{2+} ATPases ATP2B1 and ATP2B4 via the PDZ protein DLG1.

These findings suggest that T16J modulates immune responses in tubular epithelial cells and T-lymphocytes, which is enhanced in cells expressing the T16J variant T595A. Further investigations are necessary to understand the activating effect of the T16J T595A variant on Ca^{2+} signaling, its role in immune responses and its implications in chronic kidney disease.

C 07-03

Influence of age and sex on renal phosphate homeostasis

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Content

Aging is associated with significant alterations in mineral metabolism, leading to enhanced susceptibility to renal and cardiovascular disorders. Sex disparities in renal and cardiovascular diseases, and kidney stones are observed in humans. Despite recognized associations, the interplay between aging, renal function, and altered mineral metabolism remains elusive. With aging societies, understanding this relationship is essential for healthy aging. This study investigates how age and/or sex influence hormones and metabolic factors regulating phosphate homeostasis.

In male and female C57BL/6N adult mice, aged (18.5 - 23.5 months) and young (4.5 months old), electrolytes, hormones regulating phosphate homeostasis, and biochemical parameters in plasma and urine were measured. Renal histological and senescence markers were investigated. Additionally, kidney and plasma metabolomic analysis was conducted.

Notably, fibroblast growth factor 23, crucial in phosphate homeostasis, was increased with aging independently of sex, leading to decreased plasma phosphate in females. Increased calcitriol possibly accounted for maintaining phosphate levels in males. Aged males exhibited renal senescent cell accumulation. They were also more affected by inflammatory changes observed in aged animals' kidneys and displayed greater accumulation of amyloids in the kidneys compared to females. Furthermore, age and sex differences in renal and plasma metabolome were discovered.

Phosphate homeostasis differs between aged animals and young adults, with aged mice showing varying severity levels of renal lesions and accumulation of renal senescent cells. Our findings suggest discernible sex differences in phosphate metabolism and renal function during aging. Additional analyses are planned, including assessment of renal phosphate transporter expression and activity, kidney proteomics.

C 07-04

Aging uncovers the critical role of WDR72 for kidney function in female mice

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Content

WDR72 is a member of the WD-40-repeat protein family, which is involved in complex multiprotein assembly. In the kidney, WDR72 is enriched in intercalated cells. However, its precise localization in tubules is not yet defined. WDR72 is hypothesized to play a role in acidification through vesicular trafficking and assembly of H+ATPase subunits. Although barely studied in the kidney, *WDR72* has been associated with several renal phenotypes in GWAS, including chronic kidney disease and distal renal tubular acidosis (dRTA).

Preliminary data on young *Wdr72*-KO mice demonstrated the development of dRTA in acid-challenged females. Wdr72-KO males seemed partially protected, exhibiting only inappropriately alkaline urine.

In this study, we used 18-months-old *Wdr72*-KO mice on a control diet, to determine the role of *Wdr72* in kidney aging. Female *Wdr72*-KO showed alkaline urinary pH and reduced blood bicarbonate. Moreover, *Wdr72*-KO females lost weight, had polyuria, and consumed more water and food. Increased blood urea suggested impaired kidney function, whereas increased lactate combined with decreased glycemia could reflect mitochondrial stress or dysregulated gluconeogenesis, two metabolic functions regulated in the proximal tubules. *Wdr72*-KO males displayed a lighter phenotype.

Our data reproduced in basal conditions the dRTA phenotype observed in young acid-loaded females. This emphasizes the role of WDR72 in renal aging, where the aging kidney may lose the ability to compensate for dRTA and blood urea levels increase. Additionally, our data suggest a potential involvement of WDR72 in proximal tubule metabolism and subsequent kidney function.

C 07-05

Contribution of the YER motif to the function of organic cation transporter 1, with focus on the substrate specificity of Y361

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Content

OCT1 is a polyspecific organic cation transporter, which is located in the sinusoidal membrane of human hepatocytes. It facilitates the hepatic uptake of drugs like morphine and metformin and endogenous substrates like thiamine and

serotonin. Recently, cryo-electron microscopy (cryo-EM) structures proposed Y361, E386, and R439 (the YER motif) as key amino acids involved in substrate interaction. However, the precise mechanism governing the broad, but still specific substrate recognition of OCT1 remains to be resolved. This study aimed to complement existing cryo-EM data with functional analyses of the YER motif.

We used site-directed mutagenesis, mutating Y361, E386, and R439 to alanine and other selected amino acids, transfected the mutants into HEK293 cells, and assessed their transport capabilities across a panel of fourteen OCT1 substrates.

The E386A mutation led to a complete loss of transport for all substrates, which was not restored by aspartate, another negatively charged amino acid. The R439A mutation also led to a complete loss of function. However, this could be restored by lysine, another positively charged amino acid. The Y361A mutation abolished uptake for all substrates except fenoterol. Using ligand structure walking followed by docking and MD simulations, we pinpointed the second aromatic ring as responsible for the substrate-specific interactions with Y361.

Combining cryo-EM data with functional analyses, we confirmed the role of the YER motif in the OCT1-mediated transport and identified a substrate-specific involvement of Y361. This underlines the necessity of functional verification alongside structural predictions to understand the substrate-specific interactions of polyspecific membrane transporters.

C 07-06

Effect of cellular polarization and asymmetric medium osmolality on gene expression profile in murine collecting duct cells

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Content

Introduction

Renal epithelial cells exhibit a polarized distribution of proteins between the apical and basolateral membranes, which plays a crucial role in the reabsorption of ions, fluids, and solutes from the ultrafiltrate into the bloodstream. In the collecting duct, there can be an asymmetry in osmolality between the luminal and the interstitial side. The cultivation of most epithelial cells is performed on plastic dishes, resulting in incomplete polarization. This potentially influence gene expression and subsequently modulate cellular responses to specific treatments or stimuli in cell culture experiments.

Question

Here we investigated the impact of cellular polarization and symmetric and asymmetric hypertonic challenges on the gene expression profile of renal epithelial cells.

Methods

Next-generation RNA sequencing (NGS) was used to analyze the gene expression profile of murine collecting duct cells (mpkCCD) cultured on transwell inserts (TW), which support cell polarization, or on plastic dishes under both isosmotic (300 mOsm) and hyperosmotic (600 mOsm) conditions. Cells cultured on TWs were also treated with hyperosmotic medium on the apical and isosmotic medium on the basal side and vice versa.

Results

Culturing cells on TW led to massive alterations in gene expression compared to cells cultured on plastic dishes. Interestingly, treatment with hyperosmolar medium in the apical chamber elicited a more pronounced gene expression response than in the basal chamber.

Conclusions

This study highlights the significance of proper cell polarization in epithelial cells and its profound effects on gene expression, underscoring the necessity for further research to elucidate the functional implications of these observations.

C 07-07

Sex differences in renal acid handling in *Wdr72*-deficient mice

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Content

Question

Biallelic inactivating genetic variants in *WDR72* are described in families with distal renal tubular acidosis. Single-cell transcriptomics showed that *WDR72* is enriched in renal intercalated cells. WDR72 is a member of the WD-40-repeat protein family and is hypothesized to play a role in acid-base handling by the kidney through vesicular trafficking and H^+ATPase subunits assembly. Our aim is to investigate the role of Wdr72 in renal acid handling in mice.

Methods

We studied 50-to-70-day-old male and female *Wdr72* wildtype (WT) and knockout (KO) mice under a standard diet or a 4-days acid challenge.

Results

On standard diet, female and male *Wdr72*-KO mice showed a more alkaline urine than WT mice. During an acid challenge, only female *Wdr72*-KO exhibited a more pronounced hyperchloremic metabolic acidosis. Additionally, they showed inappropriate alkaline urine and low ammoniuria. A drastic decrease in urea clearance and urine excretion of potassium, sodium, and chloride was also observed. Kidney Wdr72 protein expression increased with the acid load in

females but not in males. In female *Wdr72*-KO kidneys, the a4, A, B1, B2 and G3 H-ATPase subunits were not regulated by the acid load, but their plasma membrane expression was mostly preserved.

Conclusions

Although Wdr72 is expressed in male kidneys, its expression is not regulated by acid load, and males can cope with acid load. In females, Wdr72 expression is increased by acid load, leading to metabolic acidosis when Wdr72 is absent. Our results emphasize the role of Wdr72 in intercalated cells function and final renal acid excretion.

C 07-08

Separating basolateral from apical potassium currents in individual distal nephron cells

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Content

Distal nephron cells possess tertiapin Q (TPNQ)-inhibitable apical Kir1.1 K^+ channels and TPNQ-insensitive basolateral K^+ channels. Ba^{2+} inhibits apical and basolateral K^+ channels. Using microdissected mouse renal tubular fragments, we previously determined the TPNQ-sensitive (ΔI_{TPNQ}) and the remaining Ba^{++}-sensitive whole-cell current (ΔI_{Ba}) components in distal nephron cells patched from the apical side. Each component contributed ~50 % of the total K^+ current. In contrast, in excised apical outside-out patches TPNQ inhibited >90% of K^+ channel activity. We hypothesized that TPNQ-insensitive ΔI_{Ba} in apical whole-cell recordings originated from K^+ channels present in the basolateral membrane which in cut-open tubules is likely to be reached by apically applied Ba^{++}. To test this hypothesis and to verify that TPNQ selectively inhibits apical Kir1.1 channels but not basolateral K^+ channels, we performed whole-cell and outside-out patch clamp recordings from distal nephron cells approached from the basolateral membrane without opening the tubular fragments. In whole-cell recordings ΔI_{Ba} measured from the basolateral side was similar to that measured from the apical side. In contrast, ΔI_{TPNQ} was not detected in the majority of whole-cell recordings from the basolateral side with a few exceptions possibly due to TPNQ diffusion into the lumen. Importantly, in outside-out patches from the basolateral membrane no inhibitory effect of TPNQ was observed, while Ba^{++} completely inhibited K^+ channel activity. We conclude that in whole-cell recordings from the apical side of distal nephron cells ΔI_{TPNQ} solely represents apical Kir1.1 channel activity, while determining ΔI_{Ba} can be used to assess basolateral K^+ channel activity.

C 07-09

Genetic deletion of Claudin-3 does not alter intestinal paracellular phosphate absorption

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Content

Question

Intestinal absorption of phosphate is bimodal, consisting of an extensively studied transcellular pathway and a poorly characterized paracellular mode, even though the latter one likely contributes to the bulk of absorption under normal dietary conditions. Claudin-3 (*Cldn3*), a tight junction protein present along the whole intestine in mice, has been proposed to tighten the paracellular pathway for phosphate. The aim of this work was to characterize the phosphate-related phenotype of *Cldn3*-deficient mice.

Methods

Cldn3-deficient mice and wildtype littermates were fed standard diet or challenged for 3 days with high dietary phosphate. Faeces, urine, blood, intestinal segments and kidneys were collected. Measurements included faecal, urinary and plasma concentrations of phosphate and calcium, plasma levels of phosphateregulating hormones, evaluation of trans- and paracellular phosphate transport across jejunum and ileum, and analysis of intestinal phosphate and calcium permeabilities.

Results

Faecal and urinary excretion of phosphate as well as the plasma concentration of phosphate were comparable in both genotypes, under standard and high phosphate diet. However, *Cldn3*-deficient mice challenged with high dietary phosphate showed reduced urinary calcium excretion and increased plasma levels of calcitriol. Intact FGF23 concentration was also comparable in both groups, regardless of the dietary conditions. We found no differences either in intestinal phosphate transport (trans- or paracellular) and phosphate and calcium permeabilities between genotypes.

Conclusions

Our data do not provide evidence for a decisive role of *Cldn3* for intestinal phosphate absorption and phosphate homeostasis. However, upon challenge with high dietary phosphate, calcium-handling is disturbed in the absence of *Cldn3*.

C 07-10

ABCB1 along with AK1 mediates nucleotide-gated channel regulation of ROMK2

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The author has objected to a publication of the abstract.

C 07-11

Involvement of Xenotropic and Polytropic retroviral receptor 1 in phosphate transport

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Content

Phosphate (Pi) is absorbed from the diet in the intestine via paracellular and transcellular pathways. The latter involves Pi uptake at the brush border membrane (BBM) of epithelial cells followed by efflux to the blood stream at the basolateral membrane (BLM). Pi uptake is mediated by the SLC20 and SLC34 transporter gene families. Maintaining cellular homeostasis and completing transepithelial transport requires an efflux transporter located in the BLM. However, no such basolateral Pi efflux transporter has been successfully identified in renal and intestinal epithelial cells.

Some reports have proposed xenotropic and polytropic murine leukemia virus receptor (XPR1) to be a Pi efflux protein. However, neither its presence nor its activity at the BLM has been demonstrated. Here, we aim to investigate XPR1's potential role in Pi efflux in renal epithelial cells. The role of XPR1 is tested in several renal and intestinal epithelial cell lines (WT and XPR1 knockout clones prepared using the CRISPR-Cas9 methodology) grown on filters. Followed by measuring radioactive 32P apical uptake and basolateral efflux in the cell models.

Our preliminary results show that Xpr1 KO in renal opossum kidney (OK) cells leads to a significant decrease in Pi uptake with a concomitant change in Pi efflux, indicating that XPR1 potentially modulates Pi uptake and adapts Pi efflux rather than mediating efflux. Next, XPR1 subcellular localization will be assessed by transfecting OK and LLCPK cells with SNAP-tag-hXPR1 constructs. Also, the role of predicted XPR1 splice variants in Pi transport is examined.

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Targeting subunit of the myosin-light-chain-phosphatase, MYPT1 at threonine 696 prevents development of hypercontractile phenotype of aged mouse interlobar arteries

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Content

Question

Vascular tone of pre-glomerular arteries plays a pivotal role the in maintenance of constant glomerular filtration and long-term blood pressure regulation. In the present study, we explored the regulation of vascular tone of mouse interlobar arteries (IAs) by phosphorylation of the targeting subunit of the myosin-light-chain-phosphatase, MYPT1 at threonine 696.

Methods

Expression of MYPT1-T696 in IAs from wild type (WT) mice and animals carrying a point mutation with replaced MYPT1-threonine 696 by non phosphorylatable alanine was determined by Western blot. Isometric tone of IAs from wild types (WT) and heterozygote animals (MYPT1-T696A/+) carrying the mutation was measured by wire myography.

Results

The expression of MYPT1-T696 in IAs from MYPT1-T696A/+ mice was reduced by~50%. In young IAs the mutation did not affected reactivity to alpha1-agonist, phenylephrine or endothelial responsiveness to acetylcholine. In line with this the responsiveness of young IAs to direct activation of soluble guanylate cyclase by cinaciguat or RhoA-kinase inhibition by Y27632 was not affected in IAs from MYPT1-T696A/+ mice. Aging augmented phenylephrine responsiveness and attenuated those to cinaciguat and Y27632 in IAs from WTs. In contrast, IAs from aged MYPT1-T696A/+ animals showed similar responsiveness to Y27632 as IAs from young WT and MYPT1-T696A/+ mice.

Conclusions

The present study provides evidences that the myosin phosphorylation plays a minor role for IAs-tone regulation in young age. The study also supports the view that MYPT1-T696 alanine mutation prevents development of hyperreactive phenotype of IAs in aging and introduces a novel therapeutic concept for targeting of pathological pre-glomerular tone.

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