



DPG

DEUTSCHE
PHYSIOLOGISCHE
GESELLSCHAFT



12th Annual Meeting

of the Young Physiologists

30-31 March 2023

Kiel, Germany



Abstract Booklet



Dear Young Physiologists,

We are very happy to welcome you in Kiel for the 12th Symposium of the Young Physiologists. This year, we have the record number of 78 registered participants, who contribute abstracts for 28 talks and 44 posters from all fields of physiology.

We hope that the broad field of subjects does inspire you and lets you learn about topics that seem not to be related to your personal research. Please join the discussions to make the symposium successful.

More chances to make contacts will be the Social Dinner in the Lille Brauerei on Thursday evening.

We wish you a fruitful symposium and a nice stay in Kiel.

Yours,

Ann-Sophie, Bisher, Catrin, Inga, Lea & Marcus

Special thanks go to our sponsors:

- Kiel Life Science (KLS)
- Nanion Technologies GmbH
- Kiel Marine Science (KMS)
- Alumni und Freunde der CAU e.V.
- Deutsche Physiologische Gesellschaft (DPG)
- B.Braun-Stiftung
- Dekanat der Medizinischen Fakultät der CAU

This symposium would not have been possible without their help.

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PROGRAM OVERVIEW

date	beginning	
30.03.2023	8:00	Start Registration
	8:45	Welcome Event
	9:00	Talk Session - Neuroscience
	10:15	Coffee Break
	10:30	Talk Session - Ion Channels in context
	11:45	Lunch
	12:45	Poster Session 1
	13:45	Talk Session - Open Animal
	14:45	Coffee Break
	15:00	Workshops
	18:00	Social Event (Start: Jacks Kitchen)

date	beginning	
31.03.2023		
	9:00	Talk Session - Tissue Physiology
	10:15	Coffee Break
	10:30	Talk Session - Channels and Biophysics
	11:45	Lunch
	12:45	Keynote Speech
	13:30	Poster Session 2
	14:30	Coffee Break
	14:45	Talk Session - Pathophysiology
	16:00	Voting/Coffee Break
	16:30	Prize Awarding/ Closing Event

GENERAL INFORMATION

KIEL AND THE UNIVERSITY

The city of Kiel is the capital of the most northern German state, Schleswig-Holstein, with approximately 250,000 inhabitants. Kiel's immediate proximity to the Baltic Sea (Kiel Fjord) makes it very interesting for water sport fans and as a holiday destination. Furthermore, with the Kiel Canal (Nord-Ostsee-Kanal) Kiel has the busiest artificial waterway in the world accommodating even more ships than the Suez and Panama Canals combined.

Kiel's university Christian-Albrechts-Universität zu Kiel (CAU) was founded in 1665 by its name giver duke Christian Albrecht von Schleswig-Holstein-Gottorf comprising the theological, the law, the philosophical and the medical faculty. With currently roughly 27.000 matriculated students the CAU is Schleswig-Holsteins only full university and the biggest and best-known university in Germany between the North and the Baltic Sea.

The research at the Institute of Physiology at Kiel University presently focuses on the Physiology of Membrane Transport, Ion Channels, Systems Neuroscience, Sensorimotor Control, Molecular Neurophysiology and Autonomic Nervous System and Pain.

VENUE

The address of the venue is:

Institut für Physiologie, Christian-Albrechts-Universität zu Kiel
Hermann-Rodewald-Str. 5 in 24118 Kiel

As this year's venue is not far from the city center, most ways are quite short. The closest bus-stop is "Universität" and can be reached by the lines: 60 S, 6, 50, 61/62, 81, 91/92).

You enter the Institute of Physiology through the Foyer where posters will be presented, and catering is offered during breaks. We will also offer a

wardrobe on the ground floor for you to store your bags and poster rolls. If you wish to keep your belongings in a locker, please remember to bring your own padlock.

SOCIAL PROGRAM AND DINNER

After the workshops on Thursday, we will start a guided city tour in our famous red double-decker bus around the city and to the Baltic Sea. The bus will pick us up at the University (bus station at Jack's Kitchen) and the drop off will be directly at the Lille Brauerei (here also non-alcoholic beer and soft drinks can be purchased). Here a food Truck (John's Burger) will offer authentic American fast food, vegetarian options are also available.

None of these activities are obligatory, however we highly encourage you to take part in both events to strengthen your networks and make this meeting a full success. For a smooth process and good organization, we need an estimate of the amount of people joining either activity, so if you are willing to participate, please sign up via this link:

<https://terminplaner6.dfn.de/de/b/0ee7c9e37b481707ee70c45d5caaeeb7-134206>

ORAL PRESENTATIONS

The oral presentations are scheduled for 10 minutes talk + 5 minutes discussion. Please send us your PowerPoint presentation until Wednesday, March 29th, 4 pm via e-mail: jp-symposium@physiologie.uni-kiel.de, so that we can save your file to our presentation laptop. As it was handled in the last years, we recommend using our computer, though, if necessary, you may use your own notebook, but we will not be able to offer technical assistance in that case.

POSTER SESSIONS

Please prepare your posters in A0, portrait format. The posters will be hanging in the Foyer of the institute during the whole two days. Authors of even poster numbers are expected to present their poster on Thursday 30th, odd numbers are to be presented on Friday 31st. Please remove your poster at the end of the conference.

CERTIFICATE OF ATTENDANCE

Attendance certificates will be sent to you via e-mail after the conference. Please sign the attendance list we will pass during the sessions on both days.

AWARDS FOR BEST TALKS AND POSTERS

In both categories, Poster and Talk, you have the chance to elect your favourite presentation. The prizes will be awarded on Friday at the end of the conference.

TRAVEL GRANTS

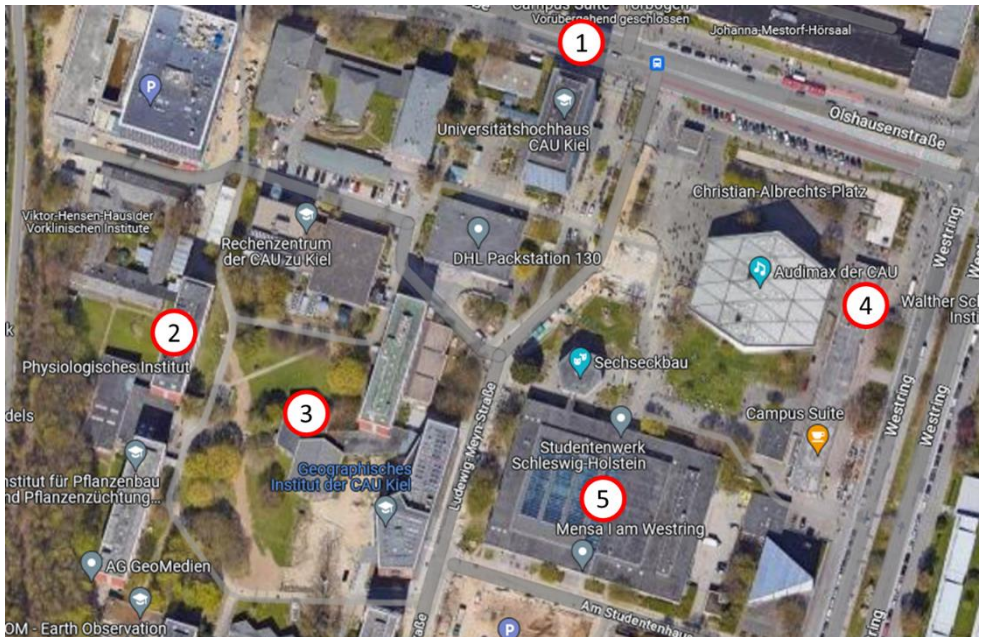
As in the last years, the DPG offers travel grants for all active participants, up to 250 €/person upon proving real travel costs. To apply for the travel grant, please keep the certificate of attendance and fill the form we send you via e-mail after the Symposium.

Usually, when you do your travel cost settlement with your employing institute, you will then state that you received third-party-funding.

BREAKS

For the short breaks and the poster sessions, we serve hot and cold beverages, cakes, cookies and some fruit in the Foyer of the Institute of Physiology. For lunchtime, we offer you warm, vegan dishes.

University map



1. Bus stop "Universität"
2. Institute of Physiology
3. Lecture Halls
4. Jacks Kitchen (City-Tour location)
5. Mensa I

WORKSHOPS

This year ADInstruments and Nanion Technologies offer 3 interesting workshops for you on Thursday 30th of March to get hands on experience on different topics. We encourage you to take this opportunity and sign up for one of the workshops via:

<https://terminplaner6.dfn.de/de/b/8ca159ece841eee48d213499633097d2-134243>

The workshops are as follows:

- 1) Live Experiments by Nanion Technologies
“Measure More Membrane – Using automated patch clamp for academic and industrial Ion Channel Research”
- 2) Rapid Research by ADInstruments
“Get a hands-on opportunity to design and run your own experiment”
- 3) Modern Teaching by ADInstruments
“Teaching 4.0 – Teaching and Education in Times of Tablet and Smartphone!”

For more information please refer to the workshop flyers sent out to all “in-time registration” at the end of February.

DETAILED PROGRAM

Thursday, 30.03.2023

08:00 Registration

08:45 Welcome Event

09:00 Oral Session I: Neuroscience

Chairs: Aytug K. Kiper, (Marburg), Catrin Przibylla-Diop (Kiel)

09:00 Johannes Seiler, Mainz

O-01: Modeling forgetting in an auditory cued go/no-go task with spontaneous synaptic remodeling

09:15 Constance Badali, Cologne

O-02: SpaceBike – A First Glimpse into Weightless Neuromechanics

09:30 Jan Freiberg, Kiel

O-03: Tripedalia cystophora - exploring system level information processing using a simple nervous system

09:45 Niels Röhrdanz, Kiel

O-04: Contact-free measurement of respiratory rate in Anolis carolinensis as a behavioral readout of arousal

10:00 Meilan Zuo, Berlin

O-05: Circadian clock function in primary HUVECs of human neonates

10:15 Coffee Break

10:30 Oral Session II: Ion Channels in context

Chairs: Dominik Lenz (Marburg), Lea Christine Neelsen (Kiel)

10:30 Ammar Hawashin, Aachen

O-06: Tannic acid and green tea interact with the membrane to modulate acid-sensing ion channels

10:45 Merten Prüser, Heidelberg

O-07: A Systematic Approach to Studying Heteromerisation in K2P-Channels

11:00 Hoang An Dinh, Berlin

O-08: T- and L-type calcium channels maintain calcium oscillations in the murine zona glomerulosa

11:15 Markus Rapedius, Munich

O-09: Characterization of cardiac electrophysiology from iPSC derived as well as cardiomyocytes directly isolated from native myocardium

11:30 Lunch Break

12:45 Poster Session I: even Poster-numbers

13:45 Oral Session III: Open Animal

Chairs: Marianne Musinszki (Kiel), Ann-Sophie Matt (Kiel)

13:45 Jasper Hildebrand, Kiel

O-10: Digestive processes in sea urchin larvae

14:00 Mirjam Knop, Kiel

O-11: The mitochondrial Sirtuin Sirt4 regulates the intestinal expression of lysozymes thereby controlling the composition of the microbiota

14:15 Christine Blurton, Kiel

*O-12: Functional characterization of prosaposins in the nematode *Caenorhabditis elegans**

14:30 Inga Petersen, Kiel

O-13: pH and [Na⁺] maintenance in the alkaline midgut of the sea urchin larva: the role of Na⁺/H⁺ exchange mechanisms

14:45 Coffee Break

15:00 Workshops (till 17:45)

18:00 Social Event City-Tour (registration required) start at Jack's Kitchen

20:00 Social Evening (Lille Brauerei)

Friday, 31.03.2023

Oral Session IV: Tissue Physiology

Chairs: Benedikt Fels (Lübeck), Inga Petersen (Kiel)

09:00 Catarina Quintanova, Kiel

O-14: Unrecognized role of claudin-10b in the basolateral infoldings of the thick ascending limb

09:15 Christina Windhaber, Vienna

O-15 Three-dimensional equine colon enteroids – one step closer to mimicking the intact intestinal epithelium

09:30 Johannes Jäger, Aarhus

O-16: Metabolic flux analysis in porcine cortical kidney tubules in a model of mild and severe ischemia reperfusion

09:45 Emrulla Spahiu, Hannover

O-17: Investigation of Ca²⁺ regulation of thin filament motility driven by myosin motors from cardiac atrial and ventricular tissues

10:00 Judith Bossen, Kiel

O-18: Transcriptomic insights into the physiology of the terminal tracheal cells in Drosophila

10:15 Coffee Break

10:30 Oral Session V: Channels and Biophysics

Chairs: Marcus Schewe (Kiel), Bisher Eymsh (Kiel)

10:30 Luca Matteo Todesca, Münster

O-19: Non-small cell lung cancer (NSCLC) migration is impaired by KCa3.1 inhibition via increased β 1-integrin expression

10:45 Sven Kuspiel, Aachen

O-20: Electrophysiological characterization of the interaction between secreted protein 1 (SEP1) and acid-sensing ion channel 1a (ASIC1a)

11:00 Aytug K. Kiper, Marburg

O-21: KCNQ1, an unexpected mediator of cold temperature perception

11:15 Marianne Musinszki, Kiel

O-22: TWIK-1 and TWIK-2: Elusive Two-Pore Domain Potassium (K2P) Channels with Common Pharmacology and Differential Regulation

11:30 Bisher Eymsh, Kiel

O-23: Novel insights into the inhibition of the THIK1 channels (KCNK13) by general anesthetics

11:45 Lunch Break

12:45 Keynote Lecture: Marian Y. Hu & Meike Stumpp, University of Kiel, "Life after the PhD: Road trip of a science couple"

13:30 Poster session II: odd Poster-numbers

14:30 Coffee Break

14:45 Oral Session VI: Pathophysiology

Chairs: Luca Matteo Todesca (Münster), Johannes Jäger (Aarhus)

14:45 Kieu Nhi Tran Vo, Berlin

O-24: Human induced pluripotent stem cell derived Adrenocortical Cell Model Reveals the Pathophysiology of KCNJ5 Mutation in Primary Aldosteronism

15:00 Jan-Philip Kühle, Kiel

O-25: Airway specific inhibition of glutathione peroxidase as well as activation of Nrf2 increases lifespan in a Drosophila COPD model

15:15 Yves Schild, Essen

O-26: Metabolism of peripheral blood mononuclear cells of patients suffering from hereditary haemorrhagic telangiectasia

15:30 Thorsten Loeck, Münster

O-27: The role of the Na⁺/Ca²⁺-exchanger in the migration of pancreatic stellate cells

15:45 Akram Hamidi, Nordrhein-Westfalen

O-28: HIF-1 α regulates the metabolism and response of murine hepatocytes derived cells to radiation therapy

16:00 Voting / Farewell Coffee

16:30 Award Ceremony for Poster and Talk Prizes and Concluding Remarks

ABSTRACTSORAL SESSIONS – THURSDAY MARCH 30TH

ORAL SESSION I: NEUROSCIENCE

O-01: Modeling forgetting in an auditory cued go/no-go task with spontaneous synaptic remodelingJohannes P.-H. Seiler¹, Simon Rumpel¹

¹ *Institute of Physiology, University Medical Center of the Johannes Gutenberg University Mainz, Hanns-Dieter-Hüsch-Weg 19, 55131 Mainz, Germany*

The well-balanced storage of new information in long-term memory and its eventual erasure via forgetting strongly affects an individual's cognitive capabilities and enables flexible, goal-oriented behavior in environments with unsteady sensory cues. It is believed that forgetting occurs when the formation of new memories leads to the corruption of old memories by overwriting. However, recent studies showed spontaneous remodeling of synaptic connections and stimulus representations over the time course of days, even under constant environmental conditions. This raises the question in how far, apart from active overwriting, also intrinsic neural dynamics lead to spontaneous forgetting.

Here, we aim to address the relationship between forgetting, adaptive behavior and its neural mechanisms as well as to establish a behavioral paradigm allowing its operationalization. We present a multi-step auditory-cued learning paradigm in which mice (i) initially learn to discriminate two stimuli in a go/no-go task, (ii) forget over a pause interval of several days, (iii) are tested again for memory retention and (iv) finally undergo a reversal learning. We find that the learned behavior undergoes a substantial time-dependent decay, indicating a link to spontaneous remodeling of the brain. Furthermore, subsequent reversal learning is impaired in individuals with higher forgetting rates.

We use a modified reinforcement learning model (Bathellier et al., 2013) to gain insight in the dynamics of synaptic remodeling and their link to initial learning, forgetting and reversal learning. Here, forgetting acts as a natural 'washout' of memory traces in sensory and decision-related neurons. We observe that multiple

types of synaptic remodeling, which continuously redistribute synaptic weight within and across neural circuits, are required to accurately simulate behavior. Together, our findings offer a starting point to understand forgetting as a natural phenomenon that emerges from intrinsic plasticity of synaptic connections and may be altered by manipulations of these dynamics.

O-02: SpaceBike – A First Glimpse into Weightless Neuromechanics

Constance Badali¹, Petra Wollseiffen¹, Stefan Schneider¹

¹*German Sport University, Institute of Movement and Neuroscience, Cologne, Germany*

Movement is a fundamental capacity of human life and essential for life on earth. Walking, running and jumping are considered natural forms of movement that are important for locomotion and everyday activities [1]. Regarding the plans of national and international space agencies with longer stays in weightlessness, it is vital to investigate the influence of weightlessness on neuromuscular performance. It has long been known that weightlessness affects all parts of the human body individually, but the interaction of the whole system should also be studied. A lab-based study on Earth have already shown that it is possible to record and localise cortical activity of the brain during cycling with an electroencephalogram (EEG) and combine this with electromyography (EMG) [2]. The EEG and EMG activity showed comparatively stable oscillations over different load intensities and showed a strong correlation at higher watt loads.

The project “SpaceBike” presented here seeks to deepen the knowledge of the relationship between mechanical components of muscle physiology and their neuromuscular control in reduced gravity environments. A first measurement campaign took place in 2022 in a parabolic flight. This is a suitable analogue on Earth to weightlessness in space. One parabola consists of four phases that begin at 1G, enter hypergravity at 1.8G and peak at 22 seconds of weightlessness before ending at 1.8G and 1G again.

During this manoeuvre, participants were on a bicycle ergometer and cycled at an individual cadence in three load levels at 1G and 0G. Cortical and muscular activity was measured simultaneously with a 64-electrode EEG cap and 14 EMG sensors attached to the legs. The knowledge gained here contributes to the safety of the astronauts and can be transferred to clinical application in the long term and to the

further development of neurorehabilitation strategies for patients with traumatic or degenerative brain diseases.

References:

- [1] Kramer, A., et al., *Leg stiffness can be maintained during reactive hopping despite modified acceleration conditions*. J Biomech, 2012. **45**(10): p. 1816-22.
- [2] Schneider, S., et al., *Cortical current density oscillations in the motor cortex are correlated with muscular activity during pedaling exercise*. Neuroscience, 2013. **228**: p. 309-14.

O-03: *Tripedalia cystophora* - exploring system level information processing using a simple nervous system

Jan-Frederik Freiberg¹, Lisa Knoff², Wilhelm Braun², Claus C Hilgetag², Jan Bielecki¹

¹Institute of Physiology, Kiel University, Kiel 24118, Germany. ²Department of Computational Neuroscience, University Medical Center Eppendorf, Hamburg 20246, Germany.

Visual information processing has historically been explored by the means of several model organisms, but none are more appropriate for systems level investigation than the box jellyfish *Tripedalia cystophora*. The box jellyfish possesses four distinct structures called Rhopalia, which each bear six eyes of four different types. The visual system enables the box jellyfish to perform several visual behaviors like long distance navigation, light shaft detection and obstacle avoidance. All the neurons involved in the visual information processing are situated in the rhopalia and form the rhopalian nervous system (RNS). Combined with a small number of neurons involved in the visual stimulus processing, *Tripedalia cystophora* possesses many topological advantages for exploring systems level visual information processing. *T. cystophora* utilizes the lower lens eye (LLE) to actively avoid obstacles like mangrove roots in its natural habitat. The visual input to the LLE is processed within the RNS and produces the swim pacemaker signal output which controls the bell contractions in a 1:1 manner, and thereby the behavior of the animal. We presented resected rhopalia with vertical bars of varying contrasts and recorded the activity of the pacemaker clusters using extracellular electrophysiology. By applying conventional calcium dyes, we were able to monitor simultaneous cellular activity from multiple cells involved in processing a visual stimulus in the RNS. We furthermore generated a model of the wiring of the neuronal network in the Rhopalium. The overall aim is to integrate biological information on sensory information processing in electronic hardware

circuitry to ultimately acquire pattern recognition functionality in hardware circuits.

O-04: Contact-free measurement of respiratory rate in *Anolis carolinensis* as a behavioral readout of arousal

Niels Röhrdanz, Anil S Menon, Peer Wulff

Department of Physiology, Hermann-Rodewald-Straße 5, 24118 Kiel, Germany

In cognitive animal research, it is important to find readouts that allow conclusions on mental processes or states.

In this study, we aimed to establish a contact-free method to measure respiratory rate in *Anolis carolinensis*. Respiratory rate has previously been shown to be a sensitive measure of fear in reptiles. To our knowledge, there is no method for extracting the breathing frequency in lizards without using sensors that are mounted on the lizard's body and therefore induce additional stress.

However, since breathing in *Anolis* is purely thoracic, changes in chest width provide a trackable readout.

We used DeepLabCut software to track thorax width over time in different experimental settings.

In the present study, we show that *Anolis* (n=4) react to visual and auditory predator (red-tailed hawk) stimuli with a change in the respiratory rate.

In conclusion, we present a method for reliable contact-free measurements of the respiratory rate in reptiles. We anticipate this method to prove useful in the analysis of fear and stress also in other species.

O-05: Circadian clock function in primary HUVECs of human neonates

Meilan Zuo¹, Achim Kramer², Christof Dame^{1*} and Lina K. Sciesielski^{1*}

¹ *Department of Neonatology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany*

² *Institute of Medical Immunology, Laboratory of Chronobiology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany*

**equally contributing authors*

Question: During human gestation, maturation and synchronization of the fetal circadian clock probably mainly rely on maternal signals transmitted via the placenta. After birth, the clock molecular network of the neonate adapts to environmental cues, and ultradian patterns develop into circadian rhythms as the child matures. However, it is unknown whether the infant expresses its own endogenous rhythms at birth. As preterm neonates are prematurely deprived of maternal zeitgebers and often exposed to non-rhythmic environmental conditions due to neonatal intensive care, knowledge about the onset of endogenous rhythms in neonates becomes clinically relevant. In our explorative study, we investigated if circadian clock function already exists in term vs. preterm neonates at the time of birth and if yes, whether it qualitatively differed.

Methods: We studied the molecular clock of human neonates in primary human umbilical vein endothelial cells (HUVECs) obtained from (a) term (>37 weeks gestation), (b) preterm (<37 to 28 weeks gestation) and (c) extremely preterm (<28 weeks gestation) neonates. Clock function in cultured cells was analyzed by live bioluminescence recording of a luciferase reporter gene under circadian control and by quantitative PCR of typical clock genes in 48h time series.

Results: We obtained valid recordings and detected circadian oscillations for all gestational ages. However, it seemed like the percentage of HUVECs capable of circadian luciferase expression was higher in the group of term neonates than in the group of preterm and extremely preterm neonates. We detected circadian clock gene expression in all groups.

Conclusion: A functional peripheral clock exists in HUVECs of most of the term and some of the preterm neonates at their respective times of birth. Nevertheless, further replicates are required to assess potential qualitative differences between rhythms of term and preterm neonates.

ORAL SESSION II: ION CHANNELS IN CONTEXT

O-06: Tannic acid and green tea interact with the membrane to modulate acid-sensing ion channels

Ammar Hawashin¹, Audrey M. Ortega-Ramírez¹, Stefan Gründer¹

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Acid-sensing ion channels (ASICs) belong to the DEG/ENaC family and can be activated by extracellular acidification. ASICs give rise to transient and sustained currents and are involved in neurotransmission and pain sensation. Polyphenols and green tea can interact with a variety of ion channels. However, the effect of the large polyphenol tannic acid (TA) and green tea on ASICs and their mechanism of action remains unknown. Here we show that TA inhibited the transient current of rat ASIC3 expressed in HEK cells with an apparent IC₅₀ of 2.2 mM. Furthermore, it potentiated the sustained current, induced a second transient current and a leak current. Sustained currents induced by TA were amiloride-sensitive and increased with decreasing pH. Applied during the sustained current, TA concentration-dependently either inhibited or potentiated the sustained current. TA shifted the activation curve of ASIC3 to more acidic pH values, while the SSD curve remained unchanged. Other ASIC subunits were also inhibited by TA. Pentagalloylglucose, which corresponds chemically to the central part of the structure of TA, as well as a green tea extract produced changes of the ASIC current which were comparable to TA. TA and the green tea extract both inhibited inward currents generated by gramicidin channels which indicates interaction with the membrane as a potential common mechanism of action. These data show that TA and green tea change membrane properties to modulate ASICs in a complex manner.

O-07: A Systematic Approach to Studying Heteromerisation in K2P-Channels

Merten Prüser^{1,2}, Marianne Muszinski¹, Sönke Cordeiro¹, Thomas Baukrowitz¹

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² *Universitätsklinikum Heidelberg, Innere Medizin III: Kardiologie, Angiologie und Pneumologie, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany*

Heteromerisation, i.e. the formation of active channels by different subunits, is a frequently employed method with which cells increase ion channel diversity. It has been studied extensively in the tetrameric Kv- and Kir-potassium channel families. In recent years, several examples of heteromerisation in the K2P-channel family, in which two subunits form functional channels, have also been identified. In the present study, we used a combination of electrophysiological and imaging methods to systematically investigate which K2P-subunits are able to form functional heterodimeric channels with one another.

First, our group used a dominant-negative (DN-) assay to systematically screen the K2P-channel family for electrophysiological evidence of heterodimerisation. To achieve this, we initially created dominant negative pore mutants, which, when coexpressed with their corresponding WT-channels in *Xenopus oocytes*, significantly depressed the WT-current in a concentration-dependent manner. Next, we coexpressed the DN-pore mutants with the WT-subunits of other K2P-channels, allowing us to compare the current reduction of many different combinations of heterodimeric K2P-channels. One pairing of K2P-subunits that displayed particularly strong current inhibition in our DN-assay is TREK-1/TALK-2.

Second, in order to verify the findings of the DN-assay, we used two additional methods to obtain evidence of protein-protein interaction for combinations of K2P-channels which showed strong inhibition in the coexpression studies: Co-Immunoprecipitation and Fluorescence Resonance Energy Transfer (FRET) microscopy. Both methods indicate protein-protein interaction for TREK-1/TALK-2.

Third, one of the main reasons for why heteromerisation of ion channels occurs *in vivo* is because it is an easy way for cells to express channels with novel or intermediate properties and, hence, increase ion channel diversity. Concatenation is a well-established method to electrophysiologically characterise heteromeric channels. In concatenated heterodimeric TREK-1/TALK-2 channels, we identified temperature sensitivity as one intermediate characteristic.

In conclusion, in recent years, strong evidence has emerged that many K2P-channels are able to form functional heterodimeric channels. Consequently, there is a need to find a systematic approach to studying these heteromers. In our lab, we used a combination of electrophysiological and imaging methods to a) identify which K2P-subunits are able to heterodimerise with each other and b) determine the characteristics of the heteromers we found.

O-08: T- and L-type calcium channels maintain calcium oscillations in the murine zona glomerulosa

Hoang An Dinh, Ute Scholl, Gabriel Stölting

*Berlin Institute of Health, Hypertension and Molecular Biology of Endocrine Tumors
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The hormone aldosterone is produced in the zona glomerulosa of the adrenal gland. Aldosterone plays a crucial role in maintaining serum electrolytes and blood pressure. The two major stimuli of aldosterone synthesis are angiotensin II and hyperkalemia. Stimulation leads to membrane potential depolarizations which activate voltage-gated calcium channels. This influx of Ca^{2+} provides the signal for aldosterone production. It was previously suggested that the T-type calcium channel $\text{Ca}_v3.2$ is required for voltage oscillations and calcium influx in response to these stimuli.

However, observations from $\text{Ca}_v3.2$ knock-out (KO) mice could not corroborate these findings, as aldosterone levels were unchanged between KO and wildtype (WT).

In our study, we investigated alternative calcium entry pathways in murine ZG cells by using calcium imaging, RNA-seq and patch clamp.

We found that intracellular Ca^{2+} oscillations in ZG KO cells resembled those of WT despite the loss of $\text{Ca}_v3.2$. ZG KO cells showed similar calcium levels in response to stimulation as WT.

RNA-Seq showed that no calcium channels or transporters were upregulated to compensate for the loss of Ca_v3.2.

Application of T- and L-type Ca²⁺ channel antagonists revealed that the Ca²⁺ spikes observed in the KO cells are not maintained by any T-type but were instead dependent on L-type Ca²⁺ channels.

We found these channels also in WT ZG cells where they are vital components for physiological calcium signaling in addition to Ca_v3.2. Blockade of both, T- and L-type calcium channels completely abolished oscillatory calcium signals and increase of intracellular calcium evoked by angiotensin II.

Our results indicate that both, T- and L-type calcium channels play a crucial role in the response of the ZG to external stimuli. Our data suggests that blockade of T- or L-type channels alone will not lower aldosterone levels but a combined inhibition may provide a promising strategy to investigate.

O-09: Characterization of cardiac electrophysiology from iPSC derived as well as cardiomyocytes directly isolated from native myocardium.

Authors: Markus Rapedius¹, Fitzwilliam Seibert^{2,3,4}, Funsho E. Fakuade^{2,3,4}, Philipp Tomsits^{5,6,7,8}, Aiste Liutkute^{2,3,4}, Nadine Becker¹, Lukas Cyganek^{2,3,4,9}, Sebastian Clauß^{5,6,7,8}, Niels Voigt^{2,3,4}, Niels Fertig¹.

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³ DZHK (German Center for Cardiovascular Research) partner site Göttingen, Göttingen, Germany.

⁴ Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), Georg-August University Göttingen, Göttingen, Germany.

⁵ Department of Medicine I, University Hospital, LMU Munich, Munich, Germany.

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and is often associated with characteristic tachycardia-induced alterations of cellular electrophysiology which promotes AF chronification and treatment resistance. 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, human models or low throughput methods. Much progress has been made in the development of automated patch-clamp (APC) systems that allows for high throughput electrophysiological measurements. APC could therefore be a useful tool for increasing the throughput of electrical investigations into AF mechanisms. We have investigated key currents and action potentials (AP) in human atrial-specific iPSC-derived cardiomyocytes (iPSC-CM) and in atrial cardiomyocytes directly isolated from native swine myocardium using an APC system (Nanion SyncroPatch 384). We observed typical subtype-specific electrophysiological characteristics including a shorter AP and smaller L-type calcium current ($I_{Ca,L}$). For primary cardiomyocytes we observed smaller inward rectifier potassium current (I_{K1}) in atrial cells compared to ventricular cells. 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 cells following application of the M-receptor agonist carbachol. Taken together, the recording of atrial APs, ionic currents in iPSC-CM and freshly isolated mammalian cardiomyocytes implies that APC can accelerate the throughput of electrophysiological measurements of mammalian cardiac tissue. This could facilitate robust studies of AF mechanisms and substantially impact AF-related drug development programs and might allow for drug screening using native cardiomyocyte material.

ORAL SESSION III: OPEN ANIMAL

O-10: Characterization of digestive proteases in sea urchin larvae

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A basic concept of life is that in order to maintain the disequilibrium between itself and its environment, an organism must constantly expend energy. For

heterotrophic organisms, digestion is the only way to obtain energy and to provide building blocks for body structures.

Protein is an integral part of animal nutrition because most heterotrophic organisms cannot synthesize essential amino acids. An efficient exploitation of protein from the diet is therefore a basal requirement for any digestive system including marine larvae. By combining genetic information and protease inhibition we were able to characterize parts of the digestive machinery in larvae of the sea urchin *Strongylocentrotus purpuratus* in response to different feeding regimes. We found four candidate genes, e.g. *trypsin-2*, *aminopeptidase N*, an *enteropeptidase* and *carboxypeptidase B* in single cell transcriptome data and proved that *trypsin-2* plays a major role in protein digestion demonstrating a food-induced response of transcript abundance using qPCR. Furthermore, using different protease inhibitors, we were able to show how digestive proteases are classified both *in vitro* and *in vivo*. The results we found could be of use as a basis for the investigation of evolutionary relationships both at the level of proteins and organisms and to better predict organisms fate under near future ocean changes.

O-11: The mitochondrial Sirtuin Sirt4 regulates the intestinal expression of lysozymes thereby controlling the composition of the microbiota

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Sirtuins (Sirts) are evolutionarily conserved NAD⁺-dependent protein deacetylases that regulate several cellular processes such as metabolism, stress response, genomic stability, and longevity. In mammals, three of the seven members of the Sirtuin family are located in the mitochondria. One of these mitochondrial Sirtuins, Sirt5, was associated with an alteration of the microbiota. We used the model organism *Drosophila melanogaster* to further analyze its importance. Here, Sirt4 is the sole mitochondrial Sirtuin, and therefore a candidate gene for controlling the microbial composition. Using proteomic analyses and an activity assay, we were able to show that Sirt4 deficient animals exhibit a massively increased expression and activity of different lysozymes. This is accompanied by an altered microbiota, which shows a shift towards gram-negative Acetobacteraceae. In addition, we demonstrated that *Wolbachia* infection exerts a comparable, albeit attenuated, effect on the microbiota via reduction of Sirt4 expression.

Since Sirt4 has already been associated with metabolic regulation, we also investigated the impact of enterocyte specific manipulation of Sirt4 in the intestine

on the metabolism and body composition. Additionally, we could show a reduced survival in flies with downregulated Sirt4 expression in enterocytes.

O-12: Functional characterization of prosaposins in the nematode *Caenorhabditis elegans*

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Keywords: *C. elegans*, antimicrobial proteins, prosaposin, SAPLIP

Prosaposin is a highly conserved glycoprotein, which encodes in humans for four saposins. The saposins A, B, C and D are derived after proteolytic cleavage and activate several lysosomal hydrolases. Members of the saposin-like protein (SAPLIP) family share common structural features like six cysteines, that form strong disulfide bonds, and four to five alpha helices. Although SAPLIPs share common structural features, the functions in eukaryotes are diverse and they have been traced down to the most primitive levels of eukaryotes.

Unsurprisingly, the nematode *Caenorhabditis elegans* owns 28 genes, which give rise to 34 SAPLIPs and are called saposin-like proteins (*spp*). Functional characterization of SPPs in *C. elegans* revealed an antimicrobial activity which is achieved by an interaction of the antimicrobial protein (AMP) with the negatively charged membrane of bacteria. This interaction initiates the pore-forming activity of AMPs and leads to the lysis of the bacteria. The antimicrobial activity has already been shown for several SPPs, but up to now the functional analysis is limited to SPPs with a single SAPLIP domain.

Consequently, the functional analysis of prosaposins from *C. elegans* *in vivo* and *in vitro* might reveal new insights into the multifunctional family of the SAPLIPs in the worm. The genes *spp-8* and *spp-10* from *C. elegans* are predicted to encode for four and three SAPLIP domains, respectively. The first step in the functional characterization of prosaposin was the estimation of the lifespan and the reproduction rate of *C. elegans* mutant strains lacking specific SAPLIP domains. The knock-out mutant strains showed alterations of the reproduction rate and the lifespan. Interestingly, the mutant strains *spp-8(ok3758)* and *spp-10(ok1585)* had a higher risk to suffer matricidal hatching. Besides internal hatched larvae, the larval development itself was delayed in these two mutant strains. These findings point out an important role of prosaposins in the physiology of *C. elegans*.

O-13: pH and [Na⁺] maintenance in the alkaline midgut of the sea urchin larva: the role of Na⁺/H⁺ exchange mechanisms

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Analogous to the situation in several lepidopteran and dipteran insects also the larval stages of some echinoderms and hemichordates (ambulacraria superphylum) evolved highly alkaline midguts up to pH 10.5. Despite its pivotal role in species' sensitivity to changes in seawater pH, the underlying epithelial transport mechanisms are largely unknown. Using ion-selective microelectrodes we found that pluteus larva of the purple sea urchin [*Strongylocentrotus purpuratus*] not only have highly alkaline midgut fluids (pH ~9) but also a substantial reduced sodium concentration (150 mM) compared to the surrounding sea water (450 mM). We pharmacologically investigated the role of Na⁺/H⁺-exchangers in intracellular pH (pH_i) regulation and midgut proton and sodium maintenance using the NHE inhibitor 5-(n-ethyl-n-isopropyl)amiloride (EIPA). While life cell imaging demonstrated the importance of NHEs in pH_i regulation of midgut epithelia cells, basolateral EIPA application decreased midgut pH whereas luminal application, via micro-injections, increased midgut [Na⁺], without affecting pH. Gene expression analyses identified putative NHE candidates for midgut pH and Na⁺ homeostasis. Based on this information an antibody was generated against the sea urchin Slc9a2, which showed that the protein is localized in luminal membranes of the midgut. Additionally the use of specific *vivo morpholino* knock down of *spslc9a2* provoked an increase in midgut [Na⁺] without affecting pH underlining the role of this transporter in midgut Na⁺ maintenance.

This work provides new insights of NHEs being involved in ion regulatory mechanisms, especially the maintenance of pH and [Na⁺] in larval midgut fluids and thereby show conserved features to insect and vertebrate digestive systems, which may contribute to the ability of sea urchin larvae to cope with changes in seawater pH.

ORAL SESSION IV: TISSUE PHYSIOLOGY

O-14: Unrecognized role of claudin-10b in the basolateral infoldings of the thick ascending limb

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The thick ascending limb (TAL) of the loop of Henle is part of the nephron and reabsorbs around 30% of the filtered NaCl while also being water tight. The paracellular pathway is determined by claudin expression in the tight junctions (TJ). In the TAL, TJs show a mosaic expression of either claudin-10b or claudin-3/-16/-19. Claudin-10b TJ confers paracellular Na⁺ permeability. Absence of Claudin-10b leads to a re-arrangement of the TJ architecture and the take-over of the other claudins, increasing divalent cation- and decreasing Na⁺ permeability. Additionally, claudin-10b is present outside of the TJ in basolateral membrane of the TAL, where its function is not yet understood. We isolated murine TAL segments and performed triple staining with claudin-10, NKA and the chloride channel subunit Barttin. The localization of all proteins co-localized in the infoldings of the TAL and NKA and Barttin expression were not changed in IF or WB by cKO. We then measured the relative speed of ouabain inhibition and fluorescein diffusion into the infoldings. The relative speed of inhibition was higher in the cKO and fluorescein diffusion was facilitated. These results suggest a role of claudin-10b in the stabilization of the infoldings by forming a complex between neighboring invaginated membranes

Keywords: TAL, claudin-10b, infoldings

O-15: Three-dimensional equine colon enteroids – one step closer to mimicking the intact intestinal epithelium

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Epithelial damage due to gastrointestinal disorders is a leading cause of mortality in horses. An improved understanding of epithelial adaptation and repair mechanisms might help to develop new therapeutic approaches. However, current cell culture models do not reflect the complex architecture of the intestinal epithelium *in vivo* and might neglect species-specific differences. Therefore, we aimed to establish an *in vitro* model of the equine colon epithelium mimicking the *in vivo* condition.

Intestinal stem cells (crypts) were isolated from intestinally healthy horses euthanized for unrelated reasons. After propagation, the equine colon enteroids (eqCE) were cultivated with either proliferation medium (PM) or one of four differentiation media (DM1-4). We characterised the enteroids histomorphologically and analyzed mRNA expression levels of specific cell markers in comparison to the native epithelium of the same horses.

EqCE showed both spherical growth and budding in all conditions (PM, DM1,2 and 4) except for DM3 where the enteroids had a mainly spherical morphology. The mRNA expression of the stem cell marker olfactomedin 4 was not significantly different irrespective of the medium, but enterocyte markers, e.g., villin, EPCAM and Na⁺/K⁺-ATPase, indicated a higher similarity to the native epithelium in DM3. The goblet cell marker mucin 2 was increased at gene expression level in DM4 which is supported by higher goblet cell prevalence in H&E stainings.

In conclusion, we succeeded in isolation, propagation and differentiation of eqCE consisting of multiple cell types that resemble the equine colon epithelium *in vivo*. In line with the 3R principles, the eqCE will serve as a suitable and applicable model to investigate the pathomechanisms underlying equine gastrointestinal disorders.

O-16: Metabolic flux analysis in porcine cortical kidney tubules in a model of mild and severe ischemia reperfusion

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Background and Aims:

Cortical tubules (CTs) are responsible for reabsorption of essential metabolites. CT-disfunction leads to kidney injury and may contribute to the transition from acute

kidney injury to chronic kidney disease. Cationic amino acids can improve tubular function and salt induced hypertension.¹

The glucose metabolism in CT-cells is well investigated, but the metabolism of amino acids only to a far lesser extent.²

Method:

We quantified the metabolic flux of glucose and amino acids in a porcine model of unilateral ischemia reperfusion injury. The left kidney underwent either 45 minutes or 2 hours of ischemia followed by 7 days of reperfusion. We dissected CTs of both kidneys to incubate them with isotopic labelled glucose, L-lysine, and L-arginine.

The formed isotopic labelled metabolites were extracted and analyzed with UHPLC/QQQ-based mass spectroscopy.

Results:

We observed a shift from aerobic respiration to anaerobic glycolysis, and loss of gluconeogenesis in injured CTs and control CTs in the 2h group.

Arginine uptake into injured CTs and its metabolization via the urea cycle was decreased in both groups. NO-synthesis was decreased in injured CTs of the 2h group.

Lysine metabolization was significantly increased in injured CTs of the 45 min group but not of the 2h group.

Conclusion:

We investigated the CTs' metabolism in a model of ischemia reperfusion without interference of other organs or interstitial kidney cells.

After prolonged ischemia, the kidney experiences a comprehensive alteration of the tubular metabolism that persists for at least 7 days. Injured CTs exploit the faster but less efficient anaerobic glycolysis, causing possible changes in the glucose supply in the whole body. The decrease in ammonia handling and NO-synthesis may affect neural cells and systemic blood pressure control.

The tracing of lysine, and the observation of increased lysine metabolization in injured CTs may identify lysine metabolism as key player in the progression of kidney diseases.

O-17: Investigation of Ca²⁺ regulation of thin filament motility driven by myosin motors from cardiac atrial and ventricular tissues

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Ca^{2+} is a crucial regulator of striated muscle contraction, which is initiated by Ca^{2+} binding to troponin C on thin filaments (TFs; comprising actin-tropomyosin-troponin complex), triggering a conformational change and exposing myosin binding sites on actin. In earlier studies, muscle tissue-purified native or reconstituted thin filaments were used to study the Ca^{2+} regulation. However, the myosin motors and the TFs were from diverse species or different tissue sources. Here, we compared TFs and motors derived from rabbit atrial or ventricular tissues for their Ca^{2+} sensitivity, under native conditions.

We developed a novel protocol whereby both the TFs and the motors can be extracted from small quantities of myocardial tissue. We achieved extracting sufficient proteins for functional assays from 50 mg tissues, which is >50x less tissue material compared to earlier studies. TF composition and stoichiometry determined by the densitometry analysis was 7:1:1 for the actin:tropomyosin:troponin complex. The fluorescently-marked TFs were studied using *in vitro* thin filament motility assay at varied Ca^{2+} concentrations. The velocity-pCa relationship followed a sigmoidal curve and yielded a maximum speed of 2.6 $\mu\text{m/s}$ for atrial (n=3) and 1.5 $\mu\text{m/s}$ for ventricular myosin (n=3), respectively. Interestingly, the velocities were more than 2-fold higher than the bare actin filaments, i.e., 0.9 and 0.8 $\mu\text{m/s}$ for ventricular and atrial myosin, respectively. Also, the ventricular myosin and TFs showed higher Ca^{2+} sensitivity compared to the atrial ones. When the TFs were swapped, the ventricular TFs improved the gliding speed of atrial myosin while the atrial TFs reduced it.

These studies suggest that the native TF and myosin combination is critical to understanding the Ca^{2+} sensitivity of the velocity or force generation by respective motors. Our approach can be extended to studying precious human cardiac samples, i.e., small-size myectomy, to address the alteration of Ca^{2+} sensitivity in cardiomyopathies.

O-18: Transcriptomic insights into the physiology of the terminal tracheal cells in *Drosophila*

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The *Drosophila* tracheal system with its dorsal trunks and primary, secondary, and terminal branches shares comprehensive structural and functional similarities with

the mammalian lung on one hand and blood vessels on the other. Here, the so-called terminal tracheal cells (TTCs) are responsible for the most important task of respiratory organs, the gas exchange. The TTCs are tightly connected to all tissues and organs to ensure oxygen supply. These cells show structural plasticity in response to nutritive cues and hypoxia similar to endothelial tip cells. Beyond that, an inter-organ communication of these cells with intestinal cells was reported. Due to their branched structure and their distribution throughout the whole body, dissection and detailed investigations on a molecular level are challenging. We developed a protocol for dissection and RNA sequencing and were able to get deep insights into the transcriptomic profile of these specialized cells. Based on our results, we were able to substantiate previous results and got new insights underlying the molecular mechanisms regarding different physiological aspects like the hypoxia response. Hypoxia response in the TTCs is characterized by a modified branching structure to cover a larger area to ensure oxygen supply of the tissue. The main responsible signaling pathway that regulates this cellular process is the conserved FGF/FGFR pathway. Our RNA-Seq results and follow-up experiments indicate an unexpected role of the *Drosophila* VEGF/VEGFR homolog in the hypoxia response. With this, we enhanced the understanding of these cells that are of special interest in endothelial cell research and associated pathogenesis.

Oral Sessions – Friday March 31st

ORAL SESSION V: CHANNELS AND BIOPHYSICS

O-19: Non-small cell lung cancer (NSCLC) migration is impaired by K_{Ca}3.1 inhibition via increased β 1-integrin expression

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K_{Ca}3.1 channels play an important pathophysiological role in non-small cell lung cancer (NSCLC) by regulating some cancer cell functions like migration and invasiveness. EGF receptor (EGFR) mutations are frequent in this type of cancer. In this case EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib are commonly used therapeutics. Almost all patients initially responding to erlotinib develop acquired resistance promoting cancer aggressiveness and progression. Moreover, K_{Ca}3.1

channels are also involved in the EGFR-TKIs resistance mechanism. The aim of this study is to unravel mechanisms by which blocking K_{Ca}3.1 channels with senicapoc contributes to overcome erlotinib-resistance. We first performed a meta-analysis on microarray data sets that revealed a differential expression of K_{Ca}3.1 channels between erlotinib-resistant and sensitive NSCLC cells. This differential expression was highly correlated with genes involved in integrin signaling. Therefore, using single-cell force spectroscopy, 3D migration and Western blot techniques we focused on the impact of K_{Ca}3.1 inhibition on cell-matrix adhesion and migration, and the role that β 1-integrins have in these processes. Our results indicate that K_{Ca}3.1 channel inhibition in erlotinib-resistant NSCLC cells increases cell adhesion by an elevated β 1-integrin expression. This expression depends on activation of pathways related to a mitochondrial ROS release caused by senicapoc inhibition of the channel (JNK/JUN pathway). As shown with migration experiments, this mechanism can contribute to overcome erlotinib resistance by decreasing motility and subsequently the invasiveness of the NSCLC cells, thus inhibiting the spreading of the cancer.

O-20: Electrophysiological characterization of the interaction between secreted protein 1 (SEP1) and acid-sensing ion channel 1a (ASIC1a)

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Acid-sensing ion channel 1a (ASIC1a) is a trimeric ion channel directly activated by a fast drop of extracellular pH. It is a member of the Deg/ENaC family of ion channels and has a high abundance in the CNS. ASIC1a plays a role in various physiological and pathophysiological processes e.g. synaptic transmission, long-term potentiation and stroke. For many ion channels e.g. AMPA receptors, highly specific interaction partners were described. For ASIC1a however, the existence of such binding partners is unknown. A knock-out (KO)-controlled proteomic screen revealed secreted protein 1 (SEP1) as possible interaction partner for ASIC1a. Co-immunoprecipitation verified direct interaction between SEP1 and ASIC1a. Current density of ASIC1a was strongly potentiated (3-5 fold, $p < 0.001$) in heterologous expression systems while biophysical properties remained unaltered. We were able to show SEP1 secretion and therefore conclude, that SEP1 binds to the extracellular site of ASIC1a. A possible enzymatic function and the exact interaction site of SEP1 is still unclear. Current research focuses on the role of SEP1 in long-term potentiation (LTP). We expect the same degree of importance of SEP1 in LTP as

ASIC1a, since cortical neurons of SEP1^{-/-}-mice exhibit no detectable ASIC1a currents.

O-21: KCNQ1, an unexpected mediator of cold temperature perception

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Low temperatures and cooling agents, like menthol, evoke a cold sensation by activating the peripheral cold receptors TRPM8 and TRPA1, cation channels belonging to the family of transient receptor potential channels. However, reduction of potassium currents during cold perception might be an additional or synergistic mechanism of cold sensation which was previously discussed for stretch-activated K_{2P} potassium channels. However, polymodal potassium channels that sense cold temperatures and are blocked by cooling drugs were not yet identified. Thus, we searched for menthol-sensitive potassium channels and surprisingly identified the voltage-gated potassium channel KCNQ1, which is defective in the cardiac Romano-Ward or Jervell-Lange-Nielsen syndromes, as equally menthol-sensitive as TRPM8. Unexpectedly, as previously not studied or reported, we found KCNQ1 to be expressed in the skin and dorsal root ganglion neurons. In addition, to the strong menthol-sensitivity of KCNQ1, the channel is highly sensitive to mildly cold temperatures, in the range of 25 to 35°C. Strikingly, male *KCNQ1* knock-out mice display strong deficits in cold avoidance to moderately cool temperatures, similar as observed for TRPM8 knock-out animals. These effects are supported by C-fiber recordings of *KCNQ1* knock-out mice, displaying altered action potential firing properties. Due to their unexpected functional expression in the peripheral nervous system and strong temperature- and menthol-sensitivity,

we propose a novel extracardiac function for KCNQ1 channels which is the perception of moderate cold temperatures.

Key words: menthol, KCNQ1 knock out mice, cold sensation, C-fiber nociceptors, temperature behaviour assay

O-22: TWIK-1 and TWIK-2: Elusive Two-Pore Domain Potassium (K2P) Channels with Common Pharmacology and Differential Regulation

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Two-pore domain potassium (K2P) channels regulate cellular excitability and are involved in many (patho)physiological processes, e.g. secretion, anesthesia, inflammation, and pain. They are modulated by many intra- and extracellular stimuli as pH, phosphorylation, cellular lipids and, as known lately, heterodimerization with other K2P subunits. The TWIK subfamily of K2P channels have non-canonical selectivity filter sequences and have long been considered nonfunctional, leaving them poorly characterized. Unlike other family members, TWIK-1 and TWIK-2 channels are primarily found in intracellular compartments, hampering their investigation by electrophysiological means.

TWIK-1 is ubiquitously expressed throughout the body, with high expression levels in brain, heart and kidney, but its function remains elusive. TWIK-2 has recently been found to reside in lysosomes and it is involved in NLRP3 inflammasome activation in immune cells like macrophages. TWIK-3 has not been recorded to date. Moreover, although TWIK-1 was the first K2P channel which was crystallized, the location of the gate(s) and its mode of gating are still under debate.

To gain insight into the regulation properties and gating mechanisms of TWIK channels, we investigated wildtype and mutant channels in cultured HEK293 cells and *Xenopus* oocytes. TWIK channels contain a retrieval motif in the C-terminal domain, which can be mutated to enhance their stability at the plasma membrane, making them accessible to electrophysiological characterization. Furthermore,

exchange of potassium for rubidium ions activates TWIK-1 currents in excised patches. We here show that also TWIK-2 currents are enhanced by rubidium, which enabled us to investigate the regulation of TWIK-1 and TWIK-2 by several pharmacological modulators of K2P channels, cellular lipids, and kinase activation. We found that TWIK-1 and TWIK-2 channels share a common pharmacology and we identified small molecules which allowed us to identify residues involved in the transduction of stimuli to the gating relevant structure of TWIK-1 and TWIK-2 channels.

O-23: Novel insights into the inhibition of the THIK1 channels (KCNK13) by general anesthetics

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The two-pore domain potassium channels (K2P) are the most recently discovered subgroup of potassium channels consisting of 15 mammalian members are composed of two subunits each containing 4 transmembrane domains with 2 pore forming domains. In previous studies we and others have shown that K2P channels are gated by a multitude of stimuli including physical (temperature, tension), chemical (lipids, H⁺, small molecule drugs) and intracellular signaling pathways. THIK-1 is a member of the K2P family of K⁺ channels and widely expressed in ZNS but its physiological functions are large unknown with the exception of microglia cells where THIK-1 activation was shown to be involved in microglial immune surveillance und inflammatory cytokine release. The mechanisms that regulate THIK-1 channels, however, are poorly understood.

In this study we focused on name giving property of the THIK-1 channel (Tandem Pore Domain Halothane-Inhibited K⁺ channel) being the rapid inhibition by the general anesthetic halothane that we studied in inside-out patches expressing THIK1.

Utilizing docking-software and cysteine screening mutagenesis, we identified residues, which increase as well as decrease the sensitivity of halothane inhibition. These results are discussed in the context of a THIK-1 homology model and provide first mechanistic insights into the regulation of these channels by general anesthetics.

ORAL SESSION VI: PATHOPHYSIOLOGY

O-24: Human induced pluripotent stem cell derived Adrenocortical Cell Model Reveals the Pathophysiology of *KCNJ5* Mutation in Primary Aldosteronism

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Adrenocortical research has long suffered from a lack of adequate disease models, which has hampered advances in understanding disease mechanisms and developing therapies for adrenocortical disorders. Criteria for adequate human-derived adrenocortical disease models include ease of genomic manipulation and near-physiological hormone production, which are currently unmet. Here, we developed an improved human induced pluripotent stem cell (hiPSC)-based differentiation system from intermediate mesoderm toward adrenocortical cells. Heterologous expression of steroidogenic factor-1 and the addition of niche factors resulted in cells that produced a wide range of adrenal steroids, including aldosterone and cortisol. These cells maintained their proliferative capacity during extended cultivation. Using CRISPR/Cas9, we introduced a *KCNJ5* mutation found in aldosterone-producing adenomas and familial hyperaldosteronism. Besides elevated aldosterone production, mutant cells showed increased proliferation, a hallmark of tumor development that was missing in prior models. We conclude that our system is a valid tool to study the pathophysiology of adrenocortical diseases.

O-25: Airway specific inhibition of glutathione peroxidase as well as activation of Nrf2 increases lifespan in a *Drosophila* COPD model

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Chronic obstructive pulmonary disease (COPD) is a syndrome encompassing different chronic lung diseases and it is the third leading cause of death worldwide. To model COPD in *Drosophila*, animals were chronically exposed to cigarette smoke, which induced major hallmarks of the disease including structural changes of the airways and a reduced lifespan. Glutathione metabolism and the Nrf2 signaling pathway are known to mitigate the negative effects of cigarette smoke and thus counteract early COPD pathogenesis. The transcription factor Nrf2, which is negatively regulated by Keap1, controls expression of major enzymatic antioxidants including those of the glutathione metabolism. Here, Nrf2 was ectopically activated via tissue-specific CRISPR/Cas9 of *keap1* silencing exclusively in the airway system. Smoke exposed flies died significantly faster than control flies, but activation of Nrf2 exclusively in the trachea rescued most of this reduced lifespan phenotype. Moreover, CS exposure caused higher mortality rates after low oxygen treatment, while ectopic Nrf2 activation also rescued this phenotype partially. Interference with the Nrf2 signaling pathway also affects downstream targets including the glutathione metabolism. Glutathione peroxidases (gpx) catalyze the conjugation of reduced glutathione (gsh) to hydrogenperoxide (H₂O₂), leading to the reduction of H₂O₂ at the cost of reducing the pool of reduced glutathione. Silencing of gpx in the airways leads to higher cellular GSH/GSSG ratios and, more importantly, increases lifespan of CS treated flies.

Taken together, activation of Nrf2 signaling and inhibition of glutathione peroxidase exclusively in the airway epithelium could be an effective treatment strategy for COPD patients that should be explored in future studies.

O-26: Metabolism of peripheral blood mononuclear cells of patients suffering from hereditary haemorrhagic telangiectasia

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Hereditary haemorrhagic telangiectasia (HHT) is a rare disease (~1:5000), which is caused by mutations of genes within the transforming growth factor beta (TGF- β) pathway. The altered TGF- β signalling impacts angiogenesis, resulting in malformations of blood vessels, which can lead to life threatening bleedings ^{1,2}. Furthermore, an impaired TGF- β signalling alters development and function of the immune system ³. Even though, sepsis is the leading cause of death for HHT patients, this branch of the disease is poorly understood and there is no disease specific treatment ⁴.

As the hypoxia inducible factor-1alpha (HIF-1 α) is also involved in angiogenic and inflammatory processes and our group showed previously significant reduction of gene expression of HIF-1 α mRNA and protein abundance in peripheral blood mononuclear cells (PBMCs) of HHT patients, we speculated that the induction of HIF-1 α might modulate the immune response of HHT patients ⁵.

Hence, we investigated the metabolic profiles of PBMCs in HHT patients, using the *Seahorse extracellular flux analyser*. We showed significantly reduced glycolysis and oxidative phosphorylation in resting PBMCs of HHT patients. However, the glycolysis rate could be restored by HIF-1 α stabilisation via Roxadustat administration to a level comparable to non-HHT controls, whilst the oxidative phosphorylation was only mildly reduced. The loss of HIF-1 α in HHT-PBMCs causes metabolic impairment and thereby may explain the altered immune response of HHT patients. To substantiate this thesis, we will link the obtained metabolic data with the activity of various T-cell subpopulations by monitoring the Cysteine and Glucose metabolism through flow cytometry. The exact observation of various T-cell subpopulations may help us to understand the immune deficiency of HHT patients and to find a personalized treatment for these patients.

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O-27: The role of the Na⁺/Ca²⁺-exchanger in the migration of pancreatic stellate cells

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Introduction

The pancreatic ductal adenocarcinoma (PDAC) is the most frequent pancreatic cancer. PDAC cells activate pancreatic stellate cells (PSCs). Once activated PSCs are remodeling of the tumor microenvironment (TME) by producing large amounts of extracellular matrix. This leads to physico-chemical properties of the TME that are characterized by hypoxia, acidity as well as increased stiffness and pressure. Moreover, PSCs can co-metastasis with pancreatic cancer cells and form a tumor niche.

Purpose

The $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) is an important Ca^{2+} regulatory transport protein. It removes Ca^{2+} (forward mode) or transports Ca^{2+} in the cells (reverse mode). This study aimed to understand whether the migration of PSCs is regulated by the NCX in the different physico-chemical properties of the TME.

Methods

Migration of murine and human PSCs was monitored with 2D live-cell imaging experiments that recapitulated some of the conditions of the TME. The NCX of murine PSCs was blocked with ORM-10103. The expression of NCX in human PSCs was knocked down with RNAi. The driving forces of the NCX were analyzed by ionic imaging of Ca^{2+} , Na^+ and the membrane potential.

Results

The blockade of the NCX in the conditions of the tumor microenvironment resulted in a differential impact on the migration behavior of PSCs. Inhibiting NCX under control conditions accelerates PSC migration. Migration increases upon NCX inhibition when PSCs are pressurized or treated with PDGF. The inhibition of the NCX has no effect under hypoxia. Under acidic conditions NCX inhibition leads to reduced migration.

Conclusion

The migration of PSCs depends on cues from the TME. The $\text{Na}^+/\text{Ca}^{2+}$ exchanger plays a differential role in translating these cues into an altered migratory behavior. When NCX operating in the forward mode is inhibited, PSCs migrate faster in most

conditions. This indicates that the NCX-mediated extrusion of Ca^{2+} contributes to a slow mode of migration of PSCs.

O-28: HIF-1 α regulates the metabolism and response of murine hepatocytes derived cells to radiation therapy

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Hepatocellular Carcinoma is common cancer affecting approximately half a million people worldwide. Improving the effectiveness of radiotherapy as a crucial treatment option for HCC can have a significant impact on patient outcomes. Hypoxia-inducible factor (HIF) is the main oxygen sensor that regulates adaptation to cellular hypoxia and is involved in the ionizing radiation (IR) induced increase of radioresistance. To fully understand the role of HIF-1 α , we have established transduced culture of murine hepatocyte derived cells (mHDC) as an in vitro model, which bears a complete knockout for HIF-1 α . The next objective was to examine the role of HIF-1 α in DNA damage repair and sensitivity to IR in vitro. The third goal was to investigate the correlation between hypoxia and hepcidin expression, and the final aim was to define the effect of HIF-1 α deletion on cellular respiratory metabolism.

HIF-1 α depleted cells displayed increased IR-induced DNA damage as indicated by an increase in γH2AX foci. HIF-1 α deficient cells showed delayed DNA repair after IR under hypoxia, demonstrating that non-homologous end joining repair capacity was reduced. Furthermore, our experiments showed no major difference in ROS levels in all treatment groups, so it is implausible that ROS are involved in the effects caused by HIF-1 α deficiency. Data showed that in HIF-1 α knockout cells the oxygen consumption rate (OCR) significantly increased while the extracellular acidification rate (ECAR) was reduced compared to WT cells. In addition, the number of mitochondria decreased after hypoxia but increased in knockout cells in comparison with wildtype cells.

Overall, our findings suggest that HIF-1 inactivation increases mHDC cell radiation sensitivity as well as OCR, which is associated with reduced glycolysis.

Keywords: Hypoxia, HIF-1 α , DNA damage repair

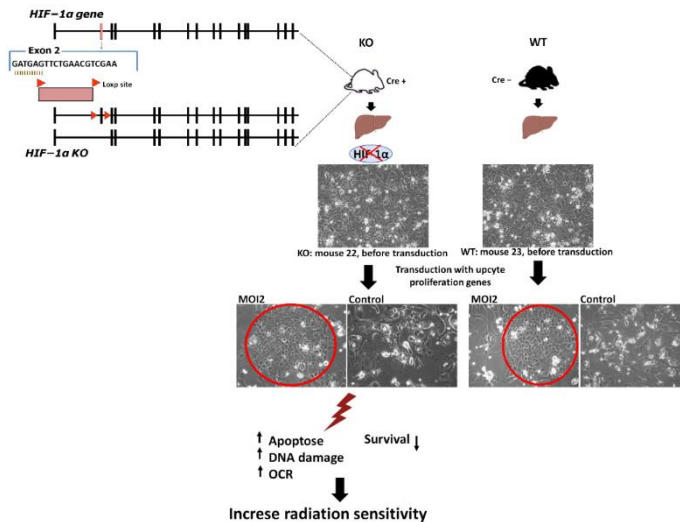


Figure 1: HIF-1 α knockout cells displayed increased IR-induced DNA damage as demonstrated by increased generation and persistence of H2AX foci. HIF-1 α deficient cells showed delayed DNA repair. Our data suggest that HIF-1 α inactivation increases OCR and radiation sensitivity of mHDC cells.

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POSTER SESSIONS

P-01: The Functional relationship between the Hypoxia inducible factor 1alpha and primary cilia in neuronal cells

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Primary cilia, also called "The cells antenna", are centriole based sensory cellular organelles, which are present on almost all mammalian cells and became importance during the last decades. They perform a variety of functions, including maintaining neuroplasticity and the adaption to hypoxic conditions via a variety of signalling pathways, including the sonic hedgehog signalling, the Wnt-signalling or the PDGFR β -signalling pathway. Based on these observations, the work pursued the goal to investigate the functional relationship between the hypoxia inducible factor 1alpha and primary cilia in neuronal cells. The establishment of neuronal HIF1alpha-knockout cells, based on the use of a specific tamoxifen induced Cre/LoxP-system, served as the base for this work. Thereby the knockout efficiency was determined by Western-Blotting and especially quantitative PCR analysis. Using these cells, the influence of HIF1alpha on the primary cilia length and diverse signalling pathways was examined. Thereby significant effects on the Notch signalling-pathway could be observed. In addition to the expression of Gata3, this also includes the expression of the Notch receptors Notch3 and Notch4. The relationship between a possible HIF-associated regulation of the Notch signaling pathway and an adaptation to hypoxic conditions must be determined by further experiments.

P-02: The hypoxia-inducible factor-2alpha affects the MEK/ERK signaling pathway via primary cilia

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The ability of cells to communicate is a prerequisite for essential processes such as proliferation, apoptosis, migration and differentiation. These processes are partly realized by primary cilia, which are antennae-like structures on the surface of most mammalian cell types. Primary cilia allow cellular signaling via Hedgehog, Wnt, TGF-

β and much more. Disruption of this signal transmission leads to a multitude of deadly human diseases, like the Joubert syndrome (JBTS), the Meckel–Gruber syndrome (MKS), or the Bardet–Biedl syndrome (BBS). This often involves deformations of the central nervous system, such as forebrain disorders, hydrocephalus or exencephaly. Cilia length, in part controlled by the activity of intraflagellar transport (IFT), is a parameter for adequate function of primary cilia. Especially in situations without suitable oxygen supply, it is necessary to provide relevant signals for counter-regulations quickly. One of the most important factors in such critical situations are hypoxia-inducible factors (HIFs). Especially HIF-2 is promoting neurogenesis and has a protective effect on neuronal stem cells. Here we show, in murine neuronal cells, that intraflagellar transport protein 88 homolog (IFT88) directly interacts with the hypoxia-inducible factor-2 α (HIF-2 α). Furthermore, HIF-2 α accumulates in the ciliary axoneme and promotes ciliary elongation under hypoxia. Loss of HIF-2 α affected ciliary signaling in neuronal cells by decreasing transcription of Mek1/2 and Erk1/2. Targets of the MEK/ERK signaling pathway, such as Fos and Jun, were significantly decreased. Our results suggest that HIF-2 α influences ciliary signaling by interacting with IFT88 under hypoxic conditions. This implies an unexpected and far more extensive function of HIF-2 α than described before.

P-03: The temperature-weight illusion

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Background: Hand-held cold objects hand seems to be heavier than warm objects of the same mass and shape. We examined the conditions that lead to such a temperature-weight-illusion (TWI).

Methods: Twenty volunteers grasped cold (16°C), cool (24°C) and lukewarm (32°C) objects with the dominant hand and lifted them from the palm of the non-dominant hand. All objects had the same shape, material and weight (700g). We measured the forces of the grasping dominant fingers. Since these forces are scaled predictively, they reflect the perceived weight, i.e. TWI.

In a “touch only” - condition the suspended objects were only touched lightly from below feel their temperature with the palm of the non-dominant hand. In a “pressure and touch” - condition the objects were placed onto the palm, so that temperature and pressure on the skin were sensed before the grip-lift.

Results: In the “touch only” condition, the objects’ temperature had no significant effect on the fingertip forces (no TWI). In contrast, a TWI occurred in the “pressure and touch” condition. Cold (16 °C) objects were grasped more vigorously than warm objects (32 °C): The peak grip force and the peak grip and load force rates of the initial grip-lift trials were significantly increased.

Conclusion: Cold objects appear to be heavier and are therefore initially grasped and lifted with more force than otherwise identical warm objects. This effect is only present when a cold object (weight) puts pressure on the skin. Moderate cold may potentiate the conversion of mechanical force into excitatory current in mechanoreceptors that respond primarily to pressure on the skin. Cold sensitivity of mechanosensitive Piezo ion channels might be an underlying mechanism.

P-04: Roxadustat treatment affects oxygen sensing in peripheral human leukocytes.

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The Hypoxia-inducible factor (HIF) prolyl hydroxylase inhibitor (PHI) Roxadustat is the first drug of its class that is approved and clinically used for the treatment of patients with chronic kidney disease-related anemia since 2021 in Europe. Roxadustat specifically inhibits prolyl hydroxylases (PHDs) and prevents HIF- α degradation, thus enabling HIF transcriptional activity and stimulation of erythropoietin (EPO) synthesis. Besides the beneficial effects of EPO synthesis on a systemic level, the effects of Roxadustat on distinct cell types are poorly understood. Precise regulation of the Hypoxia-inducible factor pathway is also especially important to ensure adequate immune response and metabolic adaption of leukocytes. Therefor we addressed the question of how clinically relevant concentrations of the prolyl hydroxylase inhibitor Roxadustat affect human peripheral blood-derived mononuclear cells (PBMC). We assessed the in vitro effects of different Roxadustat concentrations on human PBMCs regarding its potency to activate the HIF pathway by protein and gene expression analysis (Immunoblotting, qRT-PCR), tolerability by studying the cell viability (flow cytometry) and metabolic function by measuring oxygen consumption rate and extracellular acidification rate of living cells in a time-dependent manner (Seahorse

Extracellular Flux Analysis). Further, we investigated the influence of Roxadustat treatment on immune stimulation using lipopolysaccharide treatment. We could show, that Roxadustat treatment did not affect the overall viability of PBMCs in the tested concentrations. However, we observed strong HIF-1 α protein accumulation, HIF target gene expression and hence the induction of glycolytic activity upon Roxadustat treatment. Additionally, Roxadustat seemed to boost the inflammatory response of PBMCs when combined with lipopolysaccharide treatment. Further studies are needed to ensure the safety and usability of the drug and to reveal possible immune modulatory effects of Roxadustat.

P-05: The influence of hypoxia-inducible factor-2 α on synaptic architecture and function

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Sufficient oxygen supply in the brain is fundamental to avoid hypoxia and disturbances in synaptic transmission. A major role in the hypoxic response is attributed to the transcription factor Hypoxia-inducible factor-2 alpha (HIF-2 α). By using brain-specific *Hif-2 α* knockout mice, we showed that synaptogenesis is highly oxygen-dependent and a *Hif-2 α* knockout led to an altered expression of synapse-associated genes (1). However, the exact role of HIF-2 α at the synapse remains unknown. Thus, we analyzed the synaptic ultrastructure of the hippocampal CA1 region of wild-typic and *Hif-2 α* knockout mice by electron microscopy. We found an overall intact synaptic morphology of *Hif-2 α* KO animals with only minor changes in the length of the synaptic cleft. Morphometrically analyses of the hippocampal CA1, CA3 and Dentate Gyrus regions furthermore showed no differences between the two genotypes. We next addressed the regulation of synaptic transmission by HIF-2 α in vitro by use of a co-culture system with combinations of wildtypic and/or *Hif-2 α* KO neurons and astrocytes, where we can directly challenge the cells with hypoxia. Both cell types showed a good response to hypoxia through up-regulation of HIF target genes like *Vegf* and *Phd3*, with an overall stronger induction in astrocytes compared to neurons. We furthermore saw a HIF-2 α -dependency on *Vegf* and *Phd3* expression in astrocytes, but not in neurons. Besides, wildtypic astrocytes containing functional HIF-2 α seem to have an indirect, inhibitory effect on the neuronal expression of synapse-associated genes like *Neurogranin* and *Bdnf*

even under normoxic conditions. How exactly and why HIF-2 α , a transcription factor normally active under low oxygen levels, influences synapses even under normoxia is still under investigation so far.

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P-06: Loss of LPP increases weakening of the vessel wall in hypertensive mice

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Arterial hypertension is a high prevalence disease with complications such as stroke, hypertensive nephropathy, and aortic dissections. Lipoma preferred partner (LPP), a LIM domain protein, is a mechanotransducer expressed exclusively in vascular smooth muscle cells, which may play a role in the cellular and vascular response to hypertension, but its exact role remains unclear.

6- and 12-month-old LPP knockout (KO) mice, as well as age-matched wildtype litter mates, were made hypertensive using the DOCA-salt treatment (without nephrectomy). Arterial blood pressure was then monitored weekly using a non-invasive tail-cuff method, and cardiac morphology and function as well as the aorta were examined using ultrasound imaging. After 21 days, mice were euthanized, and descending aortae and hearts were collected and further analyzed by (immuno)histochemistry.

Without hypertension, KO mice showed no phenotypic anomalies, and echocardiography did not reveal any differences in cardiac function in hypertensive mice. Remarkably, 75% of the hypertensive 12-month-old male LPP-KO mice died from aortic dissection between day 10 and 17. Dissections occurred distal to the aortic arch, and in some cases even reached the renal bifurcation. Mice with dissections had lower systolic blood pressure compared to animals without dissections. Hypertensive female LPP-KO mice, however, were significantly less likely to develop vascular pathologies, and their survival rate was 100%.

These results indicate a gender- and age-dependent protective effect of LPP. The phenotype in males is reminiscent of Marfan syndrome, in which males die earlier than females from ascending aorta aneurysms that occur because of instability of the vessel wall due to a fibrillin-1 mutation. Hence, LPP may similarly be implicated in maintaining stability of the vascular wall; however, further experiments are necessary to elucidate the signaling pathways and mechanisms by which LPP can modulate the occurrence of aortic aneurysms and dissections in the face of hypertension.

P-07: Inhibitory effects of Galanin on Cortical Spreading Depolarization are conserved in rodents

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The neuropeptide Galanin (Gal) has pleiotropic functions ranging from influencing the release of hormones in hypothalamus, control of the release of neurotransmitters in hippocampus, control of food intake, sleep and stress resilience. Here, we investigated its effects on cortical spreading depolarization (CSD) and neuronal excitability represented in parameters of the electroencephalographic activity in rats and mice. In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) we recorded the electrocorticogram with arrays of glass microelectrodes in two areas (treated and untreated) at cortical depths of 400 and 1200 μm that were separated by a wall made from dental acrylic. In case of mice, we recorded only in one area (control phase: ACSF; treatment phase: Gal) at cortical depth of 250 μm . CSD were induced by KCl microinjection. CSD-related direct current (DC) potential shifts, changes in extracellular potassium concentration and in regional cerebral blood flow (only in rats) were continuously monitored. We applied Gal at doses of 10^{-6} to 10^{-10} M for 3h in rats and Gal at 10^{-7} M for 1h in mice. Control cortical slices were stained for localization of Gal in rats and mice.

After topical application of Gal on cortex we found a decrease of brain excitability (decrease of CSD amplitude and propagation velocity and increase of the

application time of KCl to ignite a CSD). In rats, this effect was observed as all-or-none, with responding and non-responding animals. However, the effect in mice was more consistent, with a general effect in the described CSD parameters. Gal has been identified in all cortical neurons in rodents.

We conclude that Gal has the potential to decrease the generation of CSDs in both rat and mouse cortex. Thus, protection against potentially damaging cortical mass depolarization by Gal is conserved in both rodent species.

P-08: Role of SLC26A2 in the kidney

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Chondrodysplasias are hereditary disorders, some of which are caused by mutations in a transmembrane protein, called SLC26A2. In cartilage, SLC26A2 acts as a sulfate transporter, and clinical severity of patients with diastrophic chondrodysplasia, is related to the residual activity of SLC26A2 and the sulfation state of proteoglycans in the extracellular matrix (Rossi *et al.*, 1998). So far, SLC26A2 research has focused on the investigation of its role in bone and cartilage, although it is expressed in various organs. Interestingly, SLC26A2 is found in the proximal tubule of the kidney, a site of sulfate reabsorption (Chapman and Karniski, 2010). The aim of this study is to investigate the role of SLC26A2 in the kidney, its regulation and function.

Opossum kidney cells (OK cells) are a common *in vitro* model of the proximal tubule. Using CRISPR/Cas9-based genome editing, we generated *SLC26A2* knock-out OK cell lines. Preliminary data showed decreased uptake of radioactive sulfate by knock-out cells compared to the wildtype controls.

We also aim at generation of an *in vivo* mouse model of renal *Slc26a2* deficiency through conditional knock out of *Slc26a2* in the proximal tubule employing the Cre/lox-system. After the generation of the model, we will isolate brush border membrane vesicles and perform sulfate transport assays.

For quantitative analysis of membrane targeting, we inserted an AviTag in one of an extracellular loop. Further, we generated constructs with depleted glycosylation sites and deleted C-terminal domains using site-directed mutagenesis. Protein with mutated glycosylation sites successfully trafficked to the cell membrane. Contrarily, deletion of an intracellular C-terminal domain that contributes to dimer formation, led to intracellular retention of the protein.

Understanding the role of SLC26A2 in the kidney could provide better insight on the pathology of chondrodysplasias and help characterize mechanisms of sulfate homeostasis in the body.

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P-09: The Effect of isoleucine preconditioning on neuroprotection via HIF-1 α signaling after oxygen-glucose deprivation insult

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INTRODUCTION: Isoleucine is one of the glucose-lowering essential branched-chain amino acids. Its role in regulation of neuronal cells after ischemic stroke is rarely studied. Most acute stroke patients exhibit symptoms of hyperglycemia. Furthermore, hyperglycemia impairs hypoxia-dependent protection of hypoxia - inducible factor-1 α (HIF-1 α). Downstream of HIF-1 α , the relocation of P53 to the nucleus after cellular stress is promoting the inhibition of malignant cells growth. In addition, relocalization of mitochondria is dependent on HIF-1 α during long term hypoxia in tumor cells.

In this study, the neuroprotection of proper isoleucine preconditioning should be validated and connected to the stabilization of the HIF-1 α protein. Besides, the relation between the evidence of increased level of HIF-1 α and the promotion of nuclear P53 should be investigated. Moreover, it is important to investigate whether pretreatment with isoleucine contributes to the amount and redistribution of mitochondria.

RESEARCH DESIGN: First, different concentrations of isoleucine will be given to cells for different time periods before oxygen-glucose deprivation (OGD) insult. Cell viability will be measured by cell counting kit-8 assay and LDH release assay. Next, HIF-1 α protein and its target genes will be measured after isoleucine preconditioning by western blotting and qPCR. By generating a HIF-1 α knockout cell line constructed by a lentivirus- based CRISPR/Cas9 system, nuclear P53 and

Somatic mitochondria will be measured by western blotting and immunofluorescence staining. To evaluate the effect of nuclear P53 and mitochondria on injured cells, MDM2, P53 nuclear transporter, and CHCHD4, a regulator of mitochondria localization, will be detected by western blotting. As increased somatic mitochondria are accompanied by the accumulation of nuclear reactive oxygen species (ROS), ROS will be detected by immunofluorescence staining.

P-10: Pharmacologically hypoxia inducible factor stabilization restores metabolism in hereditary hemorrhagic telangiectasia

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Objective: TGF- β is a cytokine that regulates both angiogenesis, and the immune response. An impairment of the TGF- β pathway leads to immune deficiency as indicated in HHT patients.^{1,2} The interplay of hypoxia inducible factors (HIFs) and TGF- β is crucial for both inflammatory, and angiogenic processes.^{3, 4} Our group could show that HIF-1 α is downregulated in HHT-patients.⁵ The factor accumulates in situations with increased energy demand, e.g. during an infection, and ensures the proper functionality of leukocytes via adjustment of the cell-metabolism.⁴ After stimulation, several leukocytes undergo a HIF-1 α dependent switch from aerobic mitochondrial respiration to anaerobic glycolysis (glycolytic switch). Therefore, we analyzed HHT-leukocytes metabolism with and without pharmacologically stabilization of HIF-1 α .

Method: We investigated the metabolism of freshly isolated HHT-peripheral blood mononuclear cells (PBMCs), using the Agilent Seahorse XF extracellular flux analyzer. Mitochondrial respiration of the cells was determined by measuring oxygen consumption rate and glycolysis by measuring the lactate-induced acidification.

Results: Resting PBMCs showed significantly reduced glycolysis and oxidative phosphorylation in HHT patients. However, pharmacological HIF-1 α stabilization restored the glycolysis to an extent comparable to non-HHT controls, whilst mildly reducing the oxidative phosphorylation.

Conclusion: The loss of HIF-1 α in HHT-PBMCs causes metabolic impairment and thereby may explain the altered immune response of HHT patients. Further studies will link the obtained metabolic data with the activity of various T-cell subpopulations by monitoring the cysteine and glucose uptake via flow cytometry. HIF-stabilization may be a potential therapeutic target in HHT patients suffering from infections.

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P-11: An Endothelial Nitric Oxide Synthase Polymorphism - Its Compensation and Implication in Chronic Inflammatory Diseases

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The T-786C single nucleotide polymorphism (SNP) in the promoter of the endothelial nitric oxide synthase (*NOS3*) gene leads to a decreased bioavailability of nitric oxide (NO), thus facilitating endothelial dysfunction. The homozygous -786C-variant is more prevalent in patients suffering from coronary heart disease (CHD) and rheumatoid arthritis (RA) but neither disease is more severe. We suspect enhanced release of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) as compensating mechanism. Increased serum concentrations of 15d-PGJ₂ in CHD patients are published. This study investigates genotype frequency for psoriatic arthritis (PsA) and 15d-PGJ₂ levels for PsA and RA.

We drew blood samples from 349 patients with RA, 235 patients with PsA and 267 healthy controls. Genotype data of about 1,000 newborns were used as secondary control group. We performed PCR-based polymorphism analysis. 15d-PGJ₂ was detected via liquid chromatography-tandem mass spectrometry. Additional medical data were retrieved from patients' medical records.

Patients with RA had the highest CC-genotype frequency (19.3%) followed by PsA (15.8%), CHD (15.3%) and healthy controls (12.0%). Comparison between patients and newborns but not control patients was significant. Serum 15d-PGJ₂ was highest in patients with CHD ($M:61.5 \pm SEM:2.3$ pg/ml, $P<0.0001$, $n=386$) followed by PsA (50.1 ± 3.1 pg/ml, $P<0.0001$, $n=199$) and RA (34.9 ± 1.6 pg/ml, $P=0.0323$, $n=282$) as compared to healthy controls (24.8 ± 1.1 pg/ml, $n=265$). Genotype comparison (TT, CT, CC) was not significant.

The T-786C SNP of the *NOS3* gene may influence different chronic inflammatory diseases. Genetically determined NO shortage promotes the endothelial inflammatory phenotype and is the first step in atherosclerosis/CHD. NO shortage may also be the starting point of RA and PsA. Serum levels of 15d-PGJ₂ are significantly elevated in all patient cohorts but no genotype dependance could be shown. This may be due to unspecific release of 15d-PGJ₂ upon NO shortage. Secondly, inflammatory activity in patients with RA or PsA is altered by treatment with anti-inflammatory drugs.

P-12: Outlook: Investigation of the molecular background of hypoxia inducible-factor reduction in patients with hereditary hemorrhagic telangiectasia

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About 85,000 people in Europe suffer from hereditary hemorrhagic telangiectasia (HHT). HHT is caused by various mutations in genes within the transforming growth factor- β (TGF- β)/ bone morphogenetic protein 9 (BMP9) signaling pathway. These mutations lead to pathological widening of blood vessels (telangiectasia) potentially causing life-threatening hemorrhages in liver, lungs, intestines and brain. Beside the regulation of angiogenesis TGF- β modulates the immune response as regulatory cytokine. Therefore, HHT patients suffer from an impaired immune response. Apart from the well characterized interplay between TGF- β and the hypoxia inducible factors (HIF) in inflammatory and angiogenic processes our

group could recently show a reduced HIF-1 α gene expression and protein abundance in leucocytes from HHT patients.

Within this study we want to elucidate the molecular background of HIF reduction in HHT leucocytes. For this purpose, we will isolate human peripheral blood mononuclear cells (PBMCs) from volunteers which are not affected by HHT. We will knock down the different members of the TGF- β /BMP9 pathway by using small interfering RNA (siRNA) against the similar genes. After successful verification of the respective knockdown, we will investigate the effect on abundance of HIF-1 α protein via Western blot.

Within these experiments we hopefully identify the responsible protein for HIF-1 α reduction in HHT patients, and thereby could find new insights into HIF-1 α regulation and new treatment opportunities for HHT patients.

P-13: Receptor-specific regulation of G protein-activated K⁺ channels

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G protein-activated inwardly rectifying K⁺ (GIRK) channel signaling is an important mechanism of the parasympathetic regulation of the heart rate and cardiac excitability. GIRK channels are inhibited during stimulation of G_q coupled receptors (G_qPCRs) by depletion of phosphatidylinositol 4,5-bisphosphate (PIP₂) and/or channel phosphorylation by protein kinase C (PKC). Different efficiencies of G_qPCRs to inhibit GIRK currents are attributed to receptor-dependent changes in the PIP₂ concentration and/or receptor-specific coupling to distinct PKC isoforms. α 1B-AR and ET-R-induced GIRK current inhibition is mediated by the Ca²⁺-dependent PKC isoforms PKC α and PKC β , respectively (1). The contribution of different PKC isoforms to angiotensin receptor (AT₁-R)-induced GIRK channel inhibition was analyzed in voltage-clamp experiments in rat atrial myocytes and HEK293 cells. By using selective pharmacological inhibitors of different PKC isoforms, we demonstrate that AT₁-R-induced GIRK inhibition is independent of cPKC activation. In contrast, GIRK current recordings in the presence of the PKC ϵ inhibitory peptide eV1-2 showed abrogation of the AT₁-R mediated channel inhibition, suggesting involvement of this novel PKC isoform in GIRK regulation. Furthermore, expression of the dominant-negative PKC Isoform DNPKC ϵ significantly reduced AT₁-receptor desensitization and attenuated the onset of GIRK inhibition, indicating that PKC ϵ regulates both GIRK channel activity and the strength of the receptor signal via a negative feedback mechanism.

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P-14: The transforming growth factor beta leads to hypoxia-inducible factor-1-alpha degradation in human leukocytes

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The transforming growth factor beta (TGF- β) is a regulatory cytokine involved in angiogenesis and the immune response. Beside TGF- β , hypoxia inducible factors (HIFs) play a critical role in the regulation of the immune response. Studies have shown that immune cells lacking HIF-1 α are dysfunctional. Several publications indicate an interaction of TGF- β with HIFs.

Hereditary hemorrhagic telangiectasia (HHT) patients suffer from mutations affecting genes from TGF- β pathway, therefore dilated abnormal vessel structures and an impaired immune response is reported. We recently showed that HHT patients have a decreased *HIF1A* gene and HIF-1 α protein expression. Detailed information's how TGF-beta influences HIF expression in human leukocytes is still missing.

We isolated human peripheral blood mononuclear cells (PBMCs) from buffy coats and treated them with TGF- β (5, 25, 50, 100 ng/ml) for 4 and 24 hours. Our results indicate that TGF- β leads to HIF-1 α protein degradation in human leukocytes. *HIF1A* gene expression seems to be not affected. We suggest that TGF- β has an influence on the activity of HIF-1 α degrading prolyl hydroxylase 2 (PHD2), which we want to investigate in further experiments.

The findings could help to understand the impaired immune response in HHT and therefore reveal new options for therapies.

P-15: The impact of hypoxia-inducible factors on ferroptotic cell death in the retinal pigment epithelium

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Age-related macular degeneration (AMD) is the most common blinding disease in the elderly population. This disease mainly affects the central vision because the photoreceptors in the macula degenerate due to a dysfunction and atrophy of cells of the retinal pigment epithelium (RPE). Advanced AMD is divided into two types, wet and dry AMD. Dry AMD is the most common type, but unlike the wet AMD, there is no promising treatment available against this type to date. Major risk factors for AMD are oxidative stress and hypoxia. 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 established a cell culture model for dry AMD. Using this model, we demonstrated that HIF stabilization along with induction of oxidative stress exacerbates oxidative damage in RPE cells. Furthermore, it was identified that ferroptosis, an iron-dependent cell death mode, is mainly responsible for these effects. In the present study, we are investigating the respective roles of HIF-1 and HIF-2 in AMD pathophysiology with strong focus on ferroptosis. Initial knock-down experiments showed increased cell mortality in cells with *HIF2A* mRNA knock-down, but no difference in mortality in cells with *HIF1A* mRNA knock-down compared to control cells, suggesting that HIF-2 has a protective effect. Currently, we are investigating the underlying mechanisms at the molecular level.

P-16: Characterisation of human Neuropsin and development of new mutants for optogenetic stimulation of the G_q signaling cascade

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G_q signaling plays a pivotal role in many cellular processes but is also involved in the pathogenesis of many diseases including cancer, autoimmunity, cardiac hypertrophy and heart failure. The transition between physiological to pathological signaling, however, is not well understood since current pharmacologic and/or genetic approaches lack spatiotemporal resolution. In clear contrast, optogenetic GPCR stimulation offers an unprecedented advantage due to high temporal and spatial precision and cell specific-expression.

Neuropsin, a UV-sensitive bistable GPCR was characterized to prove its selective activation of the G_q pathway. We show UV light-induced IP₃ production in transgenic HEK hOPN5 cells as well as the activation of TRPC6 channels by DAG. In Ca²⁺-imaging with X-Rhod (1.5 μ M), the light sensitivity (eLi50) was 3.26 ± 0.17 μ W/mm² (n=17) and the most efficient wavelength for activation 388 ± 2.7 nm

(n=14). We compared light with pharmacological stimulation and also proved that light stimulated Ca^{2+} transients were abolished in $\text{G}_{q/11}$ -KO cells while it was preserved in G_i -KO cells.

By combining structural modelling and sequence alignments, few mutations were predicted around the 8Å region of the retinal chromophore to alter the photo-excited state thereby to have a fine-tuned activation spectrum and altered kinetics. The expression rate and expression strength of the transgenic hOPN5 mutant cells along with cOPN5 cells were analyzed. They were further characterized using Ca^{2+} -imaging. The light sensitivities were ranging from (eLi50 in $\mu\text{W}/\text{mm}^2$) 71.35 to 1.97 (n=10). The most efficient wavelength for activation was shifted maximally by 10 nm to 386 ± 4.6 , n=10, and in case of cOPN5 (406 ± 7.9 , n=5). We further investigated the difference of retinal dependence which is of critical importance for translational approaches.

Thus, we created new hOPN5 mutants which are expected to have altered spectral properties and are under investigation, to create a highly efficient optogenetic tool to regulate G_q signaling in cells to control organ function.

Abbreviations: Ca^{2+} , Calcium ions; cOPN5, chicken Neuropsin/Opsin5; DAG, Diacylglycerol; eLi50, half maximal effective light intensity; GPCR, G protein-coupled receptor; G_q , G_q -type heterotrimeric G proteins; G_i , G_i -type heterotrimeric G proteins; HEK, human embryonic kidney 293 cells; hOPN5, human Neuropsin/Opsin5; IP3, Inositol 1,4,5 trisphosphate; KO, knock out; n, number of experiments; TRPC6, canonical transient receptor potential cation channel 6; UV, ultra-violet light; X-Rhod, red shifted Xanthene based rhodamine like Ca^{2+} fluorescent indicator-1; WT, wild-type.

P-17: Dietary Protein intake influences cell proliferation and survival in *Drosophila* intestinal tumor model

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Intestinal tumors are among the most common cancers worldwide, and like many other tumor entities, show an unsatisfactory prognosis necessitating the development of safe and highly effective therapies. Different nutritional regimens have been found to have a strong impact on tumor cells. Therapeutically relevant

nutritional interventions include caloric restriction and dietary restriction. Caloric restriction is characterized by a moderate reduction in daily caloric intake, while dietary restriction usually refers to a specific reduction in the protein content of the diet. Both interventions can increase lifespan in various organisms. In the present study, we established an intestinal tumor model by targeting ectopic expression of an oncogene (constitutively active *EGFR* allele) to intestinal stem cells (ISC) of the fruit fly *Drosophila melanogaster*. In this project, we used this intestinal tumor model to assess the effects of different concentrations of dietary protein on cell proliferation, gut phenotype, and, most importantly, on lifespan. Here, this intervention resulted in a substantially reduced lifespan and structural changes in the midgut. Reducing dietary protein intake in different concentrations reduced tumor proliferation dose-dependently. Another effect of reduced protein intake was a mostly restored gut structure and functionality, as well as a normalized ISC phenotype. Reducing the dietary protein intake was also able to increase the lifespan of animals but depended on the amount of protein intake. In conclusion, reduced protein administration reduces the growth of intestinal tumors effectively. Dietary protein restriction can therefore be a very promising form of adjuvant cancer therapy.

P-18: Do albumin-derived perfluorocarbon-based artificial oxygen carriers (A –AOCs) interact with murine J774 macrophages?

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Although albumin-derived perfluorocarbon-based artificial oxygen carriers (A –AOCs) are well established in our research group¹, interactions with immune cells have not been investigated so far. To explore whether A –AOCs activate immune cells, we used the murine J774 macrophages, for a better comparability of *in vitro* results with the subsequent experiments with primary macrophages isolated from mice.

To analyse the influence of A –AOCs on cytokine release, we measured the level of IL-1 β and TNF- α in J774 macrophages by ELISA. J774 macrophages were treated with medium, 10 –2 % of A–AOCs, LPS or medium with 5 % BSA for 24. To confirm the non-activation of J774 macrophages by A –AOCs, we quantified the mRNA expression of selected immunologically relevant genes by qPCR after treatment with medium, LPS (1 μ g/ml) or 10-2% of A-AOCs at 21% O₂ for 24 h. To investigate

the uptake of A –AOCs. J774 macrophages (5×10^5 cells/ml) were labelled with TRITC-Concanavalin A (100 µg/ml) for 30 min and were incubated with 4% of FITC-labelled A –AOCs for 30 min and 2 h & visualized at 488 nm and at 643 nm with confocal microscopy.

ELISA measurements revealed activation only for J774 macrophages treated with LPS, a potent activator of macrophages. In addition, the results of qPCR showed low RNA expression of the selected immunologically relevant genes after 24 h incubation with A –AOCs. These results demonstrate that A –AOCs up to 10 % do not activate the murine J774 macrophages. Furthermore, confocal microscopy showed uptake of A –AOCs by J774 macrophages already after 30 min, which further increased after 2 h of incubation.

Taken together, our results demonstrate that, although J774 macrophages engulf A –AOCs, J774 macrophages are not activated in the presence of A –AOCs.

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P-19: Analysis of the transport mechanism of SLC26A3 and SLC26A6

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Solute Carrier Family 26 (SLC26) is a conserved anion transporter family with 10 members in humans. The SLC26 genes (except for SLC26A5) encode multifunctional anion exchangers and anion channels that transport a broad range of substrates with important roles in electrolyte transport and homeostasis throughout the body ^[1]. Among the ten human SLC26 paralogs, SLC26A3 and A6 are important in normal function and disease states of the intestine. They are $\text{Cl}^-/\text{HCO}_3^-$ exchangers but SLC26A6 can also mediate the transport of divalent anions such as sulfate and oxalate. Transport mode appears to be electrogenic in some studies, with a stoichiometry monovalent: monovalent of 1:2 ^{[3][4]} and divalent: monovalent of 1:1 ^{[4][6]}. In other studies, the transport mode appears to be electroneutral ^{[2][5]}, so both transport mode and stoichiometry are still controversial. In this study, we aim, at first, to solve this controversy.

To do so we used two kind of approaches: confocal microscopy to be sure of the membrane localization of our constructs and patch clamp transport current analysis in presence of different anions and record of reversal potential (V_{rev}) to have a qualitative view of the type of transports.

Our imaging data show a good membrane localization of *mSLC26A6* in both CHO and HEK293T cells but *hSLC26A3* seems to be membrane localized only in HEK293T cells. Our patch clamp data performed on *SLC26A6* show the presence of a $\text{Cl}^-/\text{oxalate}$ and $\text{Cl}^-/\text{sulfate}$ and Cl^-/SCN^- electrogenic exchange with a higher selectivity for oxalate and a lower for SCN^- . Very minimal V_{rev} left-shift is recorded in both cell-type in presence of extracellular HCO_3^- , this could indicate an electroneutral $\text{Cl}^-/\text{HCO}_3^-$ exchange or a low selectivity for bicarbonate leading to a very small and not detectable current. We also provide data of a possible oxalate/ SCN^- exchange. Same analysis will be performed on *SLC26A3* transfected cells and recordings of reversal potential will be used to define both electrogenicity or –neutrality and the quantitative stoichiometry of the transports.

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P-20: Mouse models of familial hyperaldosteronism with *CLC-2* chloride channel mutation

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Primary aldosteronism (PA) is the most common cause of secondary hypertension and is associated with cardiovascular and kidney disease. The overproduction of the hormone aldosterone in the adrenal glands causes an increased salt resorption in the kidneys, which in turn leads to the hypertension. The majority of PA cases occur sporadically and are caused by, e.g., aldosterone-producing adenomas. However, the disease can also be inherited in the form of familial hyperaldosteronism (FH) as a Mendelian disease. Previous studies of FH patients enabled the identification of various disease genes, that encode for, e.g., several ion channels. It was recently shown that gain-of-function mutations in the *CLCN2* gene, which encodes the *CLC-2* chloride channel, cause FH and aldosterone-producing adenomas. To expand the knowledge about the genetics of these FH cases, their pathophysiology is examined with the help of mouse models. A previously characterized mouse model with a heterozygous *Clcn2*^{R180Q/+} knock-in (KI) mutation analogous to the most common mutation in humans already showed

moderately elevated aldosterone levels but no apparent signs of end-organ damages. Since this heterozygous mouse model only shows a mild form of PA we are currently characterizing homozygous *Clcn2*^{R180Q/R180Q} KI mice with a C57BL/6 background. Next to this, we investigate whether the severity of the disease phenotype is dependent on the strain of mice. Therefore, studies are currently carried out with a mouse model with the same *Clcn2* KI mutation but on a different genetic 129/Sv background. The examinations of the models include aldosterone- and renin concentration measurements in blood; determination of albumin, creatinine and overall protein in urine; as well as histological examinations of possible end-organ damages. Preliminary results are shown on this poster.

Keywords: Primary aldosteronism; Familial hyperaldosteronism; ClC-2 chloride channel; *CLCN2*; Knock-in mouse models

P-21: Potential of urinary extracellular vesicles in the display of podocyte injury

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Background: Upregulation of the ubiquitin-proteasome system (UPS) within podocytes occurs upon injury and correlates with disease progression in rodent models. During progressive podocyte injury, the proteasome system becomes impaired resulting in the accumulation of non-degraded proteins. Here we tested whether the abundance of urinary extracellular vesicles (EVs) and their content on UPS proteins displays the proteostatic condition of glomerular podocytes.

Methods: EVs were isolated from mouse and human urine by using a combination of differential ultracentrifugation and liquid size exclusion chromatography (SEC). The abundance and proteostatic content of EVs and glomeruli were analyzed in three mouse models of podocyte injury. THSD7A-associated membranous nephropathy (MN), adriamycin nephritis (AN) and diabetic nephropathy (DN) were induced in mT/mG, balb/c and ob/ob mice. Different doses of THSD7A antibody were used. Mouse urine was collected prior to and on different days after disease induction. The abundance and proteostatic content of EVs was analysed by

immunoblotting and compared to the proteostatic situation in podocytes. The pulldown of podocyte-derived EVs was established from human urine via immunoprecipitation with podocyte-specific antibodies and analyzed by electron microscopy. EVs from human nephrotic patient urine were investigated for differences in EV amount and podocyte specific origin.

Results: The amount of podocyte-derived EVs increases differentially in murine podocyte injury models. The abundance increases in a dose- and time-dependent manner in THSD7A MN, but not in AN und DN. Both human and murine urinary EVs contain UPS components. The proteostatic content changes in a disease-dependent manner, mirroring the proteostatic situation of podocytes. Human podocyte-specific EVs differentiate in size from total urinary EVs. EV amount in patient urine differs depending on the nephrotic syndrome disease.

Conclusion: Further analysis of podocyte-specific EVs have the potential to give insight into the proteostatic status of podocytes, possibly reflecting prognosis of underlying podocyte injury.

P-22: Inducing bladder cancer in mice by administration of BBN via drinking water

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Bladder cancer is a common but often only in late stages discovered form of cancer due to its non-symptomatic progress. Our group is working on hypoxia inducible factors (HIF) which are responsible for the adaptation of cells to low oxygen concentrations. It is known that HIFs play an important role in hypoxic regions in tumors but also in the function of the immune system. To better understand the role of the hypoxia inducible factor 1 (HIF1) in myeloid derived immune cells (monocytes, macrophages, dendritic cells) in the context of bladder cancer we set up a murine experimental model.

Mice with and without functional HIF1alpha in myeloid derived immune cells were treated with the cancerogenic substance N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN) via drinking water for 20 weeks. This led to tumor growth in primarily the urinary bladder. Also, a control group of mice received normal drinking water. Generating bladder cancer by administering BBN via drinking water has many advantages. Developing cancer over a long time period is well comparable to the development of bladder cancer in humans. Also due to the metabolization of BBN

it is very unlikely to develop cancer in other organs than the bladder influencing the immune system. The form of administration also minimizes distress of the mice.

Over the time of BBN administration, the weight of the mice and the volume of drunk water were observed. After preparation of the mice their blood and urinary bladders were examined. BBN intake had an influence on size, weight and color (blood supply) of the urinary bladders. Tumor evolving will be verified with further histology and immune histochemistry of the bladder tissue.

P-23: Survey of the protein secretome of *Drosophila Melanogaster* in trachea and nephrocytes

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Although *Drosophila melanogaster* is a well-established model organism, we still lack an understanding of inter-organ communication and especially protein trafficking. Here we focused on different parts of the trachea and nephrocytes. To tackle this problem experimentally we used a GAL4- UAS system to target the expression of a biotin ligase to label secreted proteins originating from different tissues. Through that, it was possible to trace the path of proteins within and between tissues. With immunohistochemical analysis, we are looking for indications of potential cross-tissue interactions and pathways. In addition, we employed a pulldown assay for all biotin-labeled proteins, which will be combined with a proteomic analysis to identify secreted proteins. This approach serves a more precise understanding of pathways and interactions in the model organism *Drosophila melanogaster*. What our results indicate is that there is an autonomous production of different types of secretory proteins in all tissues tested, and that the identification of these proteins is still pending. Here, we expect different types of transport of secreted proteins from the organs of choice. Tracheal cell-derived proteins should act locally, which fits our observations. In contrast, nephrocytes secrete these proteins into the circulation. This study will provide insight into this important aspect of interorgan communication.

P-24: Renal kidney slices as an *ex vivo* model to study the regulation of pendrin (SLC26A4)

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The kidneys play a crucial role in systemic acid base homeostasis. Central for this task are the intercalated cells (ICs) in the distal nephron, which secrete acid (H^+) or base (HCO_3^-) into the urine. Upon alkalosis the HCO_3^-/Cl^- exchanger pendrin (SLC26A4) is activated leading to increased urinary base excretion. However, the precise mechanism underlying pendrin activation is not fully understood. Here we examine whether precision-cut kidney slices (PCKS) are a suitable *ex vivo* model to study regulation of pendrin.

Kidneys of C57BL/6 mice were harvested, decapsulated, bisected along the longitudinal axis, and stored in ice-cold, oxygen-enriched Hanks' Balanced Salt Solution (HBSS/O₂). Each half was cut with a tissue chopper (McIlwain) into 300 μ m slices, which were stored in ice-cold HBSS/O₂. Afterwards the slices were incubated for up to 96 hours on Millicells with Williams Medium E (supplemented with 2.7 g/L glucose) at 37 °C and 5 % CO₂. We analysed pendrin regulation upon secretin stimulation and viability of the slices by qPCR, western blotting, and PAS staining.

We observed an increase of pendrin protein levels after secretin treatment. Furthermore, using the *ex vivo* slice culture we were able to separate membrane localized pendrin from intracellular pendrin by biotinylation and succeeding immunoprecipitation. However, PAS stainings, mRNA and protein expression analysis of the cultured slices showed a severe reduction of tubular structures and transporters, which was strongest within the first 24 h.

Our data show, that kidney slice cultures can be used as an *ex vivo* model for study e.g. secretin dependent pendrin regulation. The next step is to improve slice viability by adding further supplements to the medium and by increasing the O₂ supply.

P-25: Establishing a Cellular Model of Oxaliplatin-Induced Cold Hyperalgesia through Physical and Chemical Cooling

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Oxaliplatin-induced cold hyperalgesia (OICH) is a prevalent painful side effect of the widely used chemotherapy, with an incidence of up to 90% in treated patients. OICH often limits the dose and length of treatments (Park et al., 2013). The mechanisms behind it remain largely unclear. We developed a new in-vitro model to investigate cellular mechanisms of OICH in primary sensory neurons. Moreover, we aimed to study the responses of cold-sensitive neurons and identify their characteristics.

Native DRG neurons from Sprague-Dawley rats were stimulated in-vitro with L-menthol (130 μ M) and icilin (30 μ M) as TRPM8/TRPA1 agonists, followed by a physical cooling ramp using Peltier elements (Warner instruments) to 12°C and back to 20°C. Heat sensitivity was tested with a heating ramp from 24°C to 40°C. Nociceptive quality and general depolarization were tested with Capsaicin (1 μ M) and KCl (140 mM). During stimulations, intracellular Ca²⁺ transients were measured using fluorometric dye (Fura-2 AM).

130 neurons were analyzed (Xcellence rt, Olympus), 27% of which were defined as cold-sensitive. Some cells were active at temperatures of 15 – 21°C, whereas others became active at 12 – 15°C. 76% of the cold-sensitive neurons reacted to heat stimulation and capsaicin application as well. 20 cells responded to icilin, which is a mixed TRPM8/TRPA1 agonist, considered responsible for cold and pain sensation.

We established a reproducible, although technically difficult in-vitro model of cooling as method of studying cold transduction in primary sensory neurons. After analyzing results from native DRG neurons and improving the model, oxaliplatin-treated neurons will be next used to investigate the impact of oxaliplatin on DRG neurons, regarding threshold activation as a response to physical cooling, thereby making a step towards developing potential treatments for cold hyperalgesia and allodynia.

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P-26: Novel Candidate Disease Gene for Familial Hyperaldosteronism

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Primary aldosteronism (PA) is one of the main causes of secondary hypertension, commonly occurring sporadically caused by aldosterone producing adenomas or bilateral aldosteronism. It can also be inherited as a Mendelian disorder (familial hyperaldosteronism, FH). There are different types of FH, caused by several disease genes. In two families of our cohort with FH without mutations in the known disease genes, two mutations in a new gene previously not associated with FH or hypertension were discovered. These mutations occur in close proximity to each other and at a very conserved region which is likely involved in the catalytic activity of the resulting protein. We explore and discuss the potential structure and function of the candidate gene as well as its product and investigate the effect of wild type and mutant proteins on aldosterone synthase expression. Furthermore, we investigate the underlying regulatory mechanisms by evaluating RNA-Seq data of a knock down in human adrenocortical cell line as well as using mouse ssRNA-Seq data to estimate a gene regulatory network.

P-27: Morphology, excretion and homeostasis of the alkaline larval sea urchin midgut under changing oceanic conditions

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Decreases in seawater pH lead to a developmental delay associated with increased metabolic rates in sea urchin larvae (Stumpp et al. 2011). Furthermore, reductions in seawater pH also lead to changes in midgut physiology including decreased midgut pH and digestion efficiencies (Stumpp et al. 2013). To date the physiological responses including morphology, excretion and homeostatic regulation of the alkaline larval midgut under different pH and feeding regimes remain unknown.

This work could already demonstrate that despite reductions in body size under decreased seawater pH conditions the size and the morphology of the midgut is not compromised (Stumpp et al. 2020). Preliminary results suggest that feeding activity has a strong impact on midgut physiology, including a stimulation of luminal alkalization and NH_4^+ excretion rates. In following experiments the combined effects of acidification and food availability will be investigated. Therefore, sea urchin larvae will be raised under elevated $p\text{CO}_2$ levels and given different concentration of food. Oxygen consumption and ammonia excretion rates will be measured. Morphological and molecular characterizations including gene

expression analyses of midgut acid-base transporters and metabolic genes will be carried out to generate a link between organismic and the molecular responses. This comprehensive dataset will help to establish a physiological concept for explaining midgut plasticity of sea urchin larvae under ocean acidification and different food regimes.

P-28: Role of endothelium in mice thoracic aorta: Sex specific differences in the vasorelaxant effect of estrogen via different estrogen receptors

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Our study tries to reduce the gap of knowledge on the mechanisms governing sex differences in the estrogen vasorelaxant effect. Specifically, we investigated the role of endothelium in mediating the vasorelaxant effect of 17 β -estradiol through different estrogen receptors (ER α , ER β and GPER). Furthermore, the role of signalling pathways (PI3K, Wnt& Notch), which could modulate the vascular effect of estrogen, was addressed.

Rings of thoracic aorta prepared from 18 weeks male and female wild-type mice were mounted in a Mulvany myograph for functional assessment. The effects of 17 β -estradiol and specific estrogen receptor agonists were examined following vessel precontraction with phenylephrine. The role of endothelium was assessed by mechanical removal. The role of endothelial vasodilator substances (nitric oxide (NO), prostaglandins and endothelium-derived hyperpolarizing factor) as well as the signalling pathways (PI3K, Wnt& Notch) were addressed using established pharmacological inhibitors.

Generally, estrogen receptor stimulation caused a mild vasorelaxation (max. response 10-20%). Removal of endothelium diminished ER α agonist (PPT) vasorelaxation markedly in both sexes, but ER β agonist (DPN) and GPER agonist (G1) differently. Whereas the DPN effect decreased in female ($p < 0.01$), it was not significantly affected in male aorta. Conversely, G1 vasorelaxation was completely abolished in male ($p < 0.001$), but not in female aorta. L-NMMA, by inhibiting nitric oxide synthase, blunted the vasorelaxant effects of 17 β -estradiol in both sexes, whereas inhibitions of cyclooxygenase and Ca²⁺-dependent potassium channels

were ineffective. PI3k signalling pathway inhibition by LY 294002 considerably increased the concentration-dependent vasorelaxant effect of estrogen independent of the estrogen receptor subtype, sex and presence of endothelium.

Thus, the endothelium mediates estrogen vasorelaxant action mainly through ER α and ER β in female, while through ER α and GPER in male aorta. NO contributes to this vasorelaxation in male and female. Estrogen vasorelaxation is potentiated by inhibiting the PI3k signalling pathway unmasking an endothelium independent estrogen induced vasoconstriction.

Key words: 17 β -estradiol, estrogen receptors, NO, PI3k

P-29: Cigarette smoke causes a bioenergetic crisis in retinal pigment epithelium cells

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Age-related macular degeneration (AMD) is the most common blinding disease in the elderly population. In AMD, dysfunction and ultimately degeneration of retinal pigment epithelium (RPE) cells lead to photoreceptor degeneration in the macula. Cigarette smoke is a major exogenous risk factor for AMD, but the pathophysiological mechanisms at the molecular level are not understood. In the present study, we investigated the effects of cigarette smoke on a human RPE cell line (ARPE-19) with focus on cell metabolism. For this purpose, we treated ARPE-19 cells with cigarette smoke extract (CSE) and analyzed treatment effects by realtime cell metabolic analysis using a Seahorse Bioanalyzer, Western Blot, flow cytometry, and transmission electron microscopy. CSE treatment resulted in increased mitochondrial ROS and a reduction of mitochondrial oxidative phosphorylation as well as glycolysis compared to control cells. In healthy cells, reduced mitochondrial ATP production by oxidative phosphorylation leads to a metabolic switch to compensatory glycolysis, mediated by hypoxia-inducible

factors (HIFs), dimeric transcription factors with an oxygen-labile α -subunit and a constitutively expressed β -subunit. Consequently, we analyzed the α -subunits of the two main HIF isoforms HIF-1 and HIF-2 by Western Blot. In line with decreased basal and compensatory glycolysis, CSE treatment resulted in a downregulation of HIF-1 α protein levels. We restored basal and compensatory glycolytic function of CSE-treated ARPE-19 cells by induction of HIF-1 α accumulation using either hypoxic incubation or treatment with Roxadustat, a clinically approved HIF stabilizer. Our study provides novel molecular insights of metabolic regulations in the RPE driving AMD pathology.

P-30: Reduced anxiety-related behavior in rats lacking the capsaicin receptor TRPV1

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Background: The capsaicin receptor TRPV1 is particularly known for its role in the transduction of heat pain. Experiments in TRPV1-deficient mice (TRPV1^{-/-}) have established the contribution of TRPV1 to many areas of pain research. However, the TRPV1 receptor is also involved in processes beyond nociceptive signaling (e.g., anxiety) which in turn can influence pain behavior. In this work, we therefore investigated the anxiety-related behavior of the TRPV1^{-/-} rat in addition to pain behavior.

Methods: To demonstrate functional TRPV1 inactivation, calcium imaging experiments were performed with TRPV1 agonists from cultured dorsal root ganglia (DRG) neurons. To characterize pain behavior, heat pain thresholds were determined from 10 male and 10 female TRPV1^{-/-} rats (Envigo, The Netherlands) and 10 male Sprague Dawley wild-type rats (WT). Exploratory behavior was assessed with the Elevated Zero Maze (EZM) test and Open Field (OF) test.

Results: None of the 128 DRG neurons responded to TRPV1 agonists. The TRPV1^{-/-} animals showed increased heat pain thresholds compared to the WT rats. In the EZM, the TRPV1^{-/-} animals spent more time on the open arms (WT: 31%, TRPV1^{-/-}: 54.2%) and crossed the open arms more frequently (WT: 1.9, TRPV1^{-/-}: 8.2; $p=0.0012$, $d=1.4$). No significant difference was observed between the sexes. In the OF, there was no significant difference in the length of stay in the central zone of the OF between TRPV1^{-/-} and wild-type animals (WT: 13.3%, TRPV1^{-/-}: 15.3%, $p=0.33$, $d=0.35$).

Discussion: The data show that TRPV1 is essential, but not exclusive, to the detection of painful heat stimuli. The TRPV1^{-/-} rats explored the open arms of the EZM more intensely than the wild-type rats, suggesting that they have less anxiety. We confirmed in an open arena that the anxiety-inducing phenotype of lacking TRPV1 was not related to changes in locomotor activity. Together with the significantly reduced heat pain of TRPV1^{-/-} animals, their reduced anxiety behavior must also be considered when interpreting evoked pain behavior in TRPV1^{-/-} animals

P-31: Unraveling the role of the Ca²⁺-activated K⁺ channel K_{Ca}3.1 in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is linked to the physiology and microenvironment of the exocrine pancreas. The cellular "Ca²⁺ toolkit" includes ion channels that indirectly affect Ca²⁺ signaling such as the Ca²⁺-activated K⁺ channel K_{Ca}3.1. In PDAC, this channel is massively overexpressed and indicates a poor prognosis. Despite some *in vitro* information regarding K_{Ca}3.1 role in PDAC progression, *in vivo* data are missing. The current project is aimed at reducing this gap of knowledge.

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 inhibitor TRAM-34 or a combination of gemcitabine and TRAM-34. Retrieved pancreata were sliced, stained, and analyzed to assess tumor size and the extent of fibrosis. This was complemented through the assessment of migration in a spheroid model of PDAC composed of pancreatic tumor cells and pancreatic stellate cells.

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. This could mean that the combined effect makes the tumor tissue more accessible to treatment. In PDAC spheroids, K_{Ca}3.1 inhibition in combination with gemcitabine decreases the invasive potential. This is accompanied by a change from an elongated to a round morphology of the migrating cells. Nevertheless, this inhibition does not reach the level of inhibition of gemcitabine alone. More interestingly, the inhibition of plasma membrane K_{Ca}3.1 by maurotoxin decreases

the invasive potential of the PANC-1/PS1 spheroids more efficiently than the combination of TRAM-34 and gemcitabine.

Collectively these results point to a function of K_{Ca}3.1 channels in complex tumor models that goes beyond the well described roles of the channels in individual cell types from the tumor microenvironment.

P-32: Transcriptome analysis of endothelial cells with linked T-786C and A-922G single nucleotide polymorphisms in the promoter of the *NOS3* gene

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A single nucleotide polymorphism (SNP) at position -786 in the promoter of the *NOS3* gene (T-786C) in people homozygous for the C allele leads to a decreased ability of the *NOS3* gene to respond to unidirectional shear stress. In addition, a second SNP at position -922 (A-922G) in the *NOS3* gene that is in strong linkage disequilibrium with the T-786C SNP has been identified. Even though the T-786C SNP has been shown to be functionally relevant –constituting an independent genetic risk factor for chronic inflammatory diseases in which endothelial dysfunction plays a crucial role – individuals harboring the CC genotype do not become diseased earlier or more severe than people with the TT genotype. This led to the assumption that there must be powerful mechanisms to compensate for the relative lack of nitric oxide (NO).

To identify such compensatory mechanisms, differences in the transcriptome were examined utilizing RNA sequencing. To this end primary cultures of ⁻⁹²²GG/⁻⁷⁸⁶CC genotype human umbilical vein endothelial cells (HUVECs) (n=3) and ⁻⁹²²AA/⁻⁷⁸⁶TT genotype HUVECs (n=6) derived from different individuals were exposed to unidirectional shear stress for 24 hours before their RNA was isolated and sequenced.

RNA sequencing analysis revealed twelve differentially expressed genes (DEG) between ⁻⁹²²GG/⁻⁷⁸⁶CC genotype and ⁻⁹²²AA/⁻⁷⁸⁶TT genotype HUVECs after 24 hours of exposure to unidirectional shear stress. When comparing the static controls, 29 DEG were found. Enrichment analysis emphasized differences in the extracellular matrix organization and cell-cell adhesion under the influence of unidirectional shear stress. The analyses indicate that organization of the extracellular matrix (EM) through cell-cell interaction and EM remodeling are altered in ⁻⁹²²GG/⁻⁷⁸⁶CC endothelial cells and may contribute to counteracting the impaired NO

formation/release. Thus, these may be important components in the prevention of atherosclerosis and associated diseases.

P-33: Modulation of Acid-Sensing Ion Channel 1a by Thyroid Hormones

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Acid-sensing ion channels (ASICs) are receptors for extracellular protons and are expressed mainly in the central and peripheral nervous system. ASICs are involved in different physiological processes, such as synaptic plasticity, learning, pain detection, and fear conditioning. So far, seven ASICs-isoforms (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, ASIC4 and ASIC5) have been identified, with ASIC1a being the most widely expressed in the CNS. ASIC1a is inhibited by different substances, such as amiloride, diminazene and ibuprofen and modulated by several peptide toxins.

In this study, we found that the thyroid hormones T3 and T4 modulate ASIC1a channels. ASIC1a currents were potentiated 3-fold by T3 and 1.5-fold by T4, with EC₅₀ values of 3.3 μ M and 2.4 μ M, respectively. We found that both thyroid hormones (T3, T4) did not alter the affinity of ASIC1a for protons, nor did they indirectly affect the channel by altering cell membrane properties. Currently, we are studying the effects of thyroid hormones on ASIC1a ion selectivity and are trying to locate their binding site on ASIC1a.

In subsequent studies, the physiological significance of the effect of T3 on ASIC1a channels will be addressed. Since ASICs are expressed in the pituitary gland and thyroid hormones are important in early brain development, we speculate that modulation of ASIC1a by thyroid hormones may be involved in negative feedback regulation of thyroid hormones and may affect brain development.

P-34: Molecular pharmacology of the K_{2P} channel TRESK

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K_{2P} channels play an important role in regulating the membrane potential and thus in cellular excitability. The K_{2P} channel TRESK is mainly expressed in the central and peripheral nervous system but has been also found in non-neuronal tissue such as lung, heart, testicles, liver, kidney, thymus and spleen. Due to the high expression in neurons of the dorsal (DRG) and trigeminal ganglion (TG), the channel has become the focus of pain research in recent years. Currently, selective and highly potent modulators for the K_{2P} channel TRESK are sought due to its physiological role in nociception. Here, we describe using two-electrode voltage clamp (TEVC) a dihydropyridine derivative which is the first known TRESK blocker which combines high selectivity and potency (TRESK EC₅₀: 53.5 ± 4.2 nM). Noteworthy, the compound has a slow wash-out rate, an effect which we recently described for highly potent TASK-1 channel blockers that are trapped in the central cavity. Channel inhibition is independent from external potassium concentrations. This lack of an “knock-off” effect suggests that the drug could bind in the central cavity. To further investigate a possible binding site of the blocker in the central cavity, a systematic alanine mutagenesis scan of mTRESK was performed. Consistently, this functional alanine scan primarily identified amino acids that putatively face into the central cavity. Aromatic residues facing the central cavity of TRESK are unique within the K_{2P} channel family. Strikingly, these residues appear to be a prerequisite for the high affinity of the compound. Moreover, we studied the putative role of TRESK in nociception by electrophysiological experiments in DRG neurons, using this novel pharmacological tool. Strikingly, only small DRG neurons were drug-sensitive, indicating a predominant functional expression restricted to these neurons. Besides the treatment of TRESK-related channelopathies and pain, specific TRESK modulators could be especially useful in regards to the basic research, identifying the physiological role of TRESK in different native tissues.

Key words: TRESK, pharmacology, electrophysiology, nociception, DRG neurons

P-35: Uremic toxins from CKD patients impact on endothelial nanomechanics and function

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Patients with chronic kidney disease (CKD) have a markedly increased incidence of cardiovascular events and mortality. Among others, a high concentration of circulating uremic toxins in CKD patients leads to vascular inflammatory responses,

thereby inducing endothelial dysfunction. During the last years, a correlation between endothelial (dys)function and the mechanical properties of the endothelium (endothelial stiffness) could be demonstrated. Here, we hypothesized that uremic serum, derived from CKD patients, changes the nanomechanical properties of endothelial cells (ECs), leading to endothelial dysfunction and vascular inflammation. In addition, the influence of CKD on mineralocorticoid receptor (MR) expression and nitric oxygen (NO) bioavailability was studied, which is linked to EC function.

Primary human ECs were cultured with uremic sera derived from children with stage 3–5 (predialysis) CKD or adult hemodialysis patients or healthy controls. The height of the endothelial glycocalyx (eGC) and mechanical stiffness of the actin cortex was monitored using Atomic Force Microscopy-based nanoindentation and verified by fluorescence stainings. MR and eNOS (endothelial NO synthase) expression were analyzed via qPCR and NO bioavailability was quantified.

Uremic sera lead to a significant increase of cortical stiffness and an incremental reduction of the eGC height in a CKD stage-dependent manner. AFM data could be associated with individual pulse wave velocity and serum concentrations of gut-derived uremic toxins. In addition, uremic sera lead to 4-fold MR expression compared to control serum. Stimulation with uremic serum also significantly decreased eNOS expression and NO bioavailability. Current approaches identified single uremic toxins like p-cresyl-sulfate and indoxyl-sulfate as potential mediators.

Our data identify the endothelial glycocalyx and actin cortex as important targets of uremic toxins. Progressive structural damage of the endothelial surface with diminishing kidney function is indicated and we identify the MR as a mediator of CKD-induced endothelial dysfunction.

P-36: The role of Piezo1 in endothelial nanomechanics and vascular inflammation

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Endothelial cells (ECs) lining blood and lymphatic vessels are involved in the regulation of peripheral blood pressure and are constantly exposed to mechanical and biochemical forces. Their mechanical properties, especially the ones of the top surface layer (the endothelial glycocalyx (eGC) and underlying cell cortex) are highly flexible and can be correlated with proper endothelial function. In addition,

structures like the eGC and mechanosensitive ion channels are important endothelial mechanosensors. One candidate is the mechanosensitive nonspecific cation channel Piezo1. In this project, we aim to understand the role of Piezo1 in endothelial nanomechanics and vascular inflammation.

ECs were stimulated either with the Piezo1 agonist Yoda1 or TNF α to induce *invitro* vascular inflammation. To quantify the effect of Piezo1 on the nanomechanics of eGC the atomic force microscopy was used. We additionally quantified adhesion forces between human monocyte and ECs. Furthermore, we investigated the effects of Piezo1 absence on nanomechanical characteristics using CRISPR-Cas9 induced Piezo1 knockout in ECs.

Our results show that TNF α -induced inflammation and activation of ECs leads to eGC damage and consequently to a decrease in eGC height. Simultaneous activation of Piezo1 with Yoda1 abrogates the negative effect of TNF α . By staining with wheat germ agglutinin (WGA), we were able to confirm the eGC nanomechanics measurements. Cell-cell adhesion analysis show that TNF α leads to an increase in adhesion forces between monocytes and ECs, whereas simultaneous activation of Piezo1 by Yoda1 prevents the TNF α effect. This was confirmed by demonstrating TNF α increases the number of monocytes adherent to ECs. Accordingly, CRISPR-Cas9 mediated knockout of Piezo1 leads to a decrease of eGC height.

Our results suggest that Piezo1 is an important regulator of endothelial nanomechanics. Its activity has beneficial effects on eGC and improves the vasoprotective function of the EC surface. Therefore, Piezo1 could serve as a pharmaceutical target in cardiovascular disease.

P-37: Frequency dependency of the QT interval and its correction in student-generated measurements

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Background: The QT interval is a well-established marker for ventricular repolarization. As the QT interval is inversely regulated with the heart rate (HR), several formula are established to calculate frequency corrected QTc intervals. In

practical physiology courses, students determine QT and calculate QTc intervals at rest and following exercise. Hereby, they learn the autonomous regulation of the QT interval and the effect of frequency correction formulas. However, it remains unclear, whether these learning objectives can be reliably demonstrated on the data generated by untrained students.

Methods: A full cohort of 3rd year medical students (n=380) participated in a practical ECG course. In each course (~20 students), ECGs of volunteers were recorded in small groups (2-4 students) under resting conditions and immediately following physical exercise. On both ECGs, students measured HR and QT intervals and calculated QTc intervals using the Bazett formula. For a subgroup of individuals, all parameters were reevaluated by an expert clinical electrophysiologist. The student-generated data were analysed on the level of individual experiments and as pooled data regarding their representation of the learning objectives and compared to the expert's measurements.

Results: In 110 experiments, students reported a mean HR of ~70 bpm, a QT interval of ~380 ms and a QTc interval of ~410 ms. Following exercise, students found an average increase of HR by ~75%, a decrease of the QT interval by ~25% and an increase of the QTc interval by ~5%. In these pooled data, the inverse correlation between HR and QT interval and the frequency correction by the Bazett formula could be visualized. In individual data sets, however, these aspects could not always be demonstrated. For the reevaluated subgroup (n=47), the students' and the expert's HR data were highly concordant. The average QT and QTc intervals were comparable, however, in the student-generated data, the variance was substantially higher.

Conclusions: The QT and QTc intervals determined by untrained students from ECGs at rest and following exercise demonstrate the autonomous regulation of this important (bio)marker and the efficacy of the Bazett formula on the level of pooled data. Due to a high variance of the individual QT and QTc intervals, however, the interpretation of individual data is critical. Therefore, we recommend a discussion of the experimental results based on the aggregated data of each course.

P-38: HIF-1 and p53 – friends or foes in the colorectal carcinoma?

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The colorectal carcinoma (CRC) belongs to the most common cancers in developed countries. CRCs have been associated with mutations in the tumour suppressor p53 and often develop hypoxic areas. P53 plays a key role for cell cycle arrest and apoptosis. Besides, invasive and metastasizing tumour growth strongly depends on hypoxia-inducible factors (HIF) which accumulate in hypoxic regions of the tumour. Several studies have provided evidence for crosstalk between HIF-1 α and p53 in CRCs, although the exact interaction between HIF-1 α and p53 remains controversial. We investigate the interaction between HIF-1 and p53 in human CRC cells (HCT116). Therefore, we knockout one transcription factor to find out how this knockout influences the activity and stability of the other transcription factor (Western Blot, qPCR) and what these changes mean for cell viability (Annexin assay) and metabolism under normoxia and hypoxia. In wt cells, p53 seems stabilized and gains transcriptional activity after 24 hours of severe hypoxia (0.1% O₂). Knockout of p53 does not change HIF-1 α expression after 4 and 24 hours of moderate and severe hypoxia in comparison to wt cells. However, in p53 ko cells HIF-1 target genes are significantly downregulated after 4 hours of hypoxia compared to wt cells. Under normoxia, p53 ko cells perform higher rates of anaerobic glycolysis than wt cells. Under hypoxia, no difference concerning glucose consumption and lactate production can be observed. Interestingly, the main cell death mechanism of p53 ko cells under hypoxia is apoptosis. On the other hand, a HIF1A ko reduces p53 mRNA expression under normoxia. After 24 hours of hypoxia, HIF1A ko cells stabilize higher P53 levels and die significantly more often than HIF1A wt cells. In conclusion, we are able to show that HIF-1 α and p53 influence each other concerning basal expression and stabilization under hypoxia. To prove the hypothesis that a p53 ko might reduce the transcriptional activity of HIF-1 we will perform a transcription factor assay. Besides, we disagree with the hypothesis that p53 loses activity under hypoxia and that a p53 ko leads to survival advantages of CRC cells under hypoxia. Our results indicate that apoptosis under hypoxia can also be p53-independent. Finally, we found out that a HIF1A ko leads to an increase in apoptotic death of CRC cells under hypoxia. In future studies, we would like to examine whether this increased cell death is dependent on p53 and whether HIF1 inhibitors might be a treatment option for CRCs with LOF mutations of p53 by inducing a HIF1A ko in p53 ko cells.

P-39: Heterotrimeric concatamers of ionotropic P2X4 and P2X7 receptors

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P2X4 and P2X7 receptors are cation channels expressed in immune cells. They are activated by binding ATP released from damaged and inflamed tissue and are involved in inflammation and pain sensation. Whether P2X4 and P2X7 receptors assemble exclusively as homotrimers or also as P2X4-P2X7 heterotrimers is controversial. To clarify this, we cloned concatamers encoding heterotrimers of P2X4 and/or P2X7 subunits in permuted arrangement. We expressed these concatamers in *X. laevis* oocytes and investigated their function by recording ATP-induced whole cell currents using two-electrode voltage clamp.

We found that heterotrimeric P2X4-P2X7 concatamers have ATP binding site affinities intermediate between those of non-concatenated P2X4 and P2X7 receptors, which have high and low ATP affinity, respectively. The pK_d values for ATP depended on the order of the P2X4 and P2X7 subunits in the heterotrimer. The whole-cell currents of non-concatenated P2X4 receptors showed an inactivating phenotype, whereas non-concatenated P2X7 receptors showed slowly increasing currents upon longer ATP applications of several seconds. Heterotrimeric concatamers showed current kinetics in between the P2X4 and P2X7 phenotype, also depending on the P2X4-P2X7 subunit order. The whole cell kinetics remained unchanged during repeated ATP application indicating the existence of stable concatamers.

We conclude that P2X4 and P2X7 heterotrimers are functional, with their ATP concentration dependence and current kinetics depending on the specific order of the individual P2X4-P2X7 subunits.

P-40: Nocifensive behavior responses to laser-delivered heat stimuli reveal thermal hypoalgesia in both glucose-driven and methylglyoxal-driven larval zebrafish models of diabetes

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Diabetic peripheral neuropathy (DPN) affects approximately 50% of diabetic patients leading to increased heat pain thresholds in quantitative sensory testing (Themistocleous et al., 2016). We developed a novel method to investigate nocifensive responses to heat stimuli in larval zebrafish, which we applied to both glucose-driven and methylglyoxal-driven diabetes models to determine the heat pain threshold as a marker of DPN.

Wildtype zebrafish embryos were injected with either *pdx1*, *glo1* or control morpholino oligonucleotides to disrupt pancreatic development (*pdx1*) or methylglyoxal scavenging by glyoxalase 1 (*glo1*). Alternatively, larvae were incubated in 60 mM glucose, 60 mM mannitol or 200, 500 or 1000 μ M methylglyoxal starting 24 hours post fertilization (hpf). *Pdx1* morphants and glucose incubated larvae were additionally treated with the antihyperglycemic compound PK11195 (10 μ M). At 96 hpf radiant heat stimuli (150 ms, 18-88 mW) were delivered to freely moving larvae with a near-infrared diode laser (Schäfter+Kirchhoff, Germany) to determine a stimulus response function. Statistical analysis was conducted with Prism 9 (GraphPad, USA).

Both *pdx1* morphants (threshold 47.4 mW) as well as glucose incubated larvae (threshold 43.6 mW) show a statistically significantly ($p < 0.0001$) increased heat pain threshold when compared to control morphants (threshold 33.4 mW), wildtype (threshold 29.7 mW) or mannitol incubated animals (threshold 23.2 mW), which was completely prevented by PK11195 treatment. Slightly smaller but significant effects ($p < 0.0001$) were observed in *glo1* knockdowns (threshold 44.4 mW) and methylglyoxal incubated larvae (threshold for 200 μ M 39.1 mW, 500 μ M 36.7 mW, 1000 μ M 37.8 mW).

We show that nocifensive responses to short laser heat stimuli can be quantitatively analyzed in larval zebrafish and we were able to translate the thermal hypoalgesia observed in patients with DPN to all tested zebrafish models of diabetes. Further, we show that the reactive dicarbonyl methylglyoxal is an important mediator downstream from glucose leading to DPN.

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P-41: Sex-related pain behavioural differences due to adolescent stress can predispose low back pain in adulthood

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Background: Non-specific low back pain (LBP) is a global burden that is associated with adverse childhood events with pathophysiological mechanisms still unknown. Here, we investigated if restraint stress in adolescent rats causes mechanical hyperalgesia in adulthood and whether these effects differ between the sexes.

Methods: Adolescent Wistar rats experienced restraint stress from post-natal day PD21 to PD32 and controls were handled. In adulthood, two injections of NGF/Saline were administered in the lumbar multifidus muscle on PD85 and PD90 to establish the experimental model of low back pain. Pressure pain threshold (PPT) and Paw Withdrawal Threshold (PWT) were measured for deep muscular hyperalgesia and cutaneous mechanical hyperalgesia, respectively.

Results: Stress during adolescence moderately lowered PPT in females (Cohens $d=0.6$) whereas males experienced a significant drop in PPT ($p<0.0001$) two days after stress. PWT was lowered in both males ($p=0.008$) and females ($p=0.038$) on PD36, but it was only persistently significant in males in adulthood ($p=0.039$). However, in the stressed group followed by saline and NGF injection, moderate mechanical hyperalgesia ($d=0.5$) of the low back was reported in females, but a higher effect was seen in males ($d=0.8$).

Conclusions: Stress-induced long-lasting muscle hyperalgesia and short-lasting cutaneous hyperalgesia in both males and females. However, males have persistent mechanical hyperalgesia throughout adulthood, but females show a faster recovery to stress in adulthood. Hence, dorsal horn neurons were primed in both males and females. Due to the test-retest paradigm, the Wistar rats developed a stronger habituation to the interventions.

P-42: T- and L-type calcium channels maintain calcium oscillations in the murine zona glomerulosa

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The hormone aldosterone is produced in the zona glomerulosa of the adrenal gland. Aldosterone plays a crucial role in maintaining serum electrolytes and blood pressure. The two major stimuli of aldosterone synthesis are angiotensin II and hyperkalemia. Stimulation leads to membrane potential depolarizations which activate voltage-gated calcium channels. This influx of Ca^{2+} provides the signal for aldosterone production. It was previously suggested that the T-type calcium channel $\text{Cav}3.2$ is required for voltage oscillations and calcium influx in response to these stimuli.

However, observations from $\text{Cav}3.2$ knock-out (KO) mice could not corroborate these findings, as aldosterone levels were unchanged between KO and wildtype (WT).

In our study, we investigated alternative calcium entry pathways in murine ZG cells by using calcium imaging, RNA-seq and patch clamp.

We found that intracellular Ca^{2+} oscillations in ZG KO cells resembled those of WT despite the loss of $\text{Cav}3.2$. ZG KO cells showed similar calcium levels in response to stimulation as WT.

RNA-Seq showed that no calcium channels or transporters were upregulated to compensate for the loss of $\text{Cav}3.2$.

Application of T- and L-type Ca^{2+} channel antagonists revealed that the Ca^{2+} spikes observed in the KO cells are not maintained by any T-type but were instead dependent on L-type Ca^{2+} channels.

We found these channels also in WT ZG cells where they are vital components for physiological calcium signaling in addition to $\text{Cav}3.2$. Blockade of both, T- and L-type calcium channels completely abolished oscillatory calcium signals and increase of intracellular calcium evoked by angiotensin II.

Our results indicate that both, T- and L-type calcium channels play a crucial role in the response of the ZG to external stimuli. Our data suggests that blockade of T- or L-type channels alone will not lower aldosterone levels but a combined inhibition may provide a promising strategy to investigate.

P-43: An allosteric inhibition mechanism that governs the selectivity filter gate in TRESK K_{2P} channels

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TRESK two-pore domain potassium (K_{2P}) channels are strongly expressed in sensory neurons of the dorsal root ganglia (DRG) and trigeminal ganglia (TG) but also in other cell types such as T-lymphocytes and control their electrical activity. Thereby, TRESK channel function is linked to different (patho-)physiological conditions like pain perception and transmission, (neuro-)inflammation and immunological processes rendering them as important drug targets. For some pharmacological modulators molecular determinants have been identified but detail mechanistic understanding of pharmacological gating is so far elusive.

Here, we report on a class of small compound modulators activating many other K_{2P} channels (i.e. negatively charged activators (NCAs) that, however, inhibit TRESK K_{2P} channel activity. Our experiments that included systematic cysteine-scanning mutagenesis, electrophysiological measurements (i.e. two-electrode voltage-clamp and inside-out patch-clamp recordings), blocker competition experiments and permeant ion effects suggest an allosteric mechanism of inhibition. Key residues (e.g. G140, F145, E349, F352, F255 and K356) with a reduced apparent affinity for the NCA BL-1249 in TRESK suggest a binding site similar to that reported in TREK-1 K_{2P} channels located in side-pockets branching off from the inner cavity under the selectivity filter (SF). These residues partially overlap with that for binding of classically open pore blockers like quaternary ammonium ions (e.g. tetrabutylammonium, TBA). Indeed, the presence of TBA reduced the apparent affinity for several NCAs (DC-PIB, ML67-33, BL-1249). However, the inhibitory effect of the NCAs was strongly antagonized when TRESK channel conducted Rb⁺ instead of K⁺ suggesting that they not directly block the pore but exerted an inhibitory allosteric effect onto the SF as Rb⁺ is known to stabilize the conductive conformation of the filter gate. In contrast, inhibition by the open pore blocker loratadine and chlorpromazine was similar for TRESK channels conducting Rb⁺ or K⁺ ions.

In conclusion, we report that several NCAs produce inhibition in TRESK channels via an allosteric mechanism involving the SF in contrast to other TRESK inhibitors that appear to block the permeation pathway directly. Further, our results suggest binding for the NCAs in side-pockets branching off from the permeation pathway which might provide a basis for drug design of specific TRESK inhibitors.

P-44: Mechanosensitive ion channels are vital sensors and transducers of durotaxis in pancreatic stellate cells

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by an increased tissue stiffness that creates a rigidity gradient between healthy and tumorous tissue. Cells recognize this gradient and undergo durotaxis, a process where cells migrate towards the areas of higher stiffness. Pancreatic stellate cells (PSCs) play a major role in producing the rigid environment in PDAC promoting cancer invasiveness. The molecular sensors behind durotaxis are largely unknown. This study aimed to examine the importance of mechanosensitive ion channels in the durotaxis of PSCs.

We created a two-dimensional model system using ultraviolet polymerized polyacrylamide gels with a linear rigidity gradient. The influence of ion channels Piezo1, TRPC1 and TRPV4 in durotaxis was studied using pharmacological and genetic methods on primary murine PSCs.

Our results indicate that PSCs migrate towards a more rigid substrate. Inhibiting or activating Piezo1 suppressed durotaxis, as did knocking out the TRPC1 channel with simultaneous TRPV4 inhibition. These findings indicate that mechanosensitive ion channels, particularly Piezo1, are involved in sensing and transmitting the mechanical microenvironment to guide the migration of PSCs. The study suggests that Piezo1 relies on TRPC1 or TRPV4 for mediating the stiffness-directed migration of the cells.

NOTES

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LIST OF PARTICIPANTS

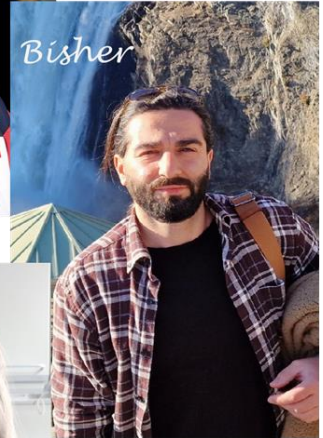
1. Ambekar, Mayuresh
2. An Dinh, Hoang (O-08)
3. Badali, Constance (O-02)
4. Bellón, Noelia
5. Berthold, Malte (P-39)
6. Blechschmidt, Vivian (P-30)
7. Blurton, Christine (O-12)
8. Borutta, Johanna-Theres (P-36)
9. Bossen, Judith (O-18)
10. Dreger, Kimberley (P-24)
11. Elena Friesen-Klengel (P-03)
12. Elsaid, Basant (P-28)
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29. Knop, Mirjam (O-11)
30. Kühle, Jan-Philip (O-25)
31. Kuspiel, Sven (O-20)
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33. Lenz, Dominik
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37. Matt, Ann-Sophie
38. Milewski, Moritz (P-11)
39. Müller, Xenia (P-23)
40. Musinszki, Marianne (O-22)
41. Nagel, Anika (P-06)
42. Narayanan, Udhaya (P-16)
43. Neelsen, Lea Christine
44. Petersen, Inga (O-13)
45. Pethö, Zoltan (P-44)
46. Proske, Alina (P-17)
47. Prüser, Merten (O-07)
48. Przibylla-Diop, Catrin
49. Qin, Lu (P-33)
50. Questino, Annalisa (P-19)
51. Quintanova, Catarina (O-14)
52. Quinting, Theresa (P-05)
53. Rapedius, Markus (O-09)
54. Roggendorf, Clara (P-22)
55. Röhrdanz, Niels (O-04)
56. Ruf, Isabel (P-14)
57. Saoub, Stefanie (P-38)
58. Schewe, Marcus
59. Schild, Yves Leon (O-26)
60. Schneider, Pascal (P-01)
61. Schönbauer, Stefanie (P-43)
62. Schönberger, Tina (P-04)
63. Schubert, Annika (P-15)
64. Schütte, Sven (P-34)
65. Seiler, Johannes (O-01)
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67. Soret, Benjamin (P-31)
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75. Windhaber, Christina (O-15)
76. Wrobeln, Anna (P-10)
77. Yao, Xujin (P-09)
78. Zuo, Meilan (O-05)



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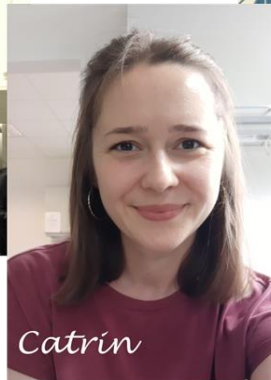
Marcus



Bisher



Inga



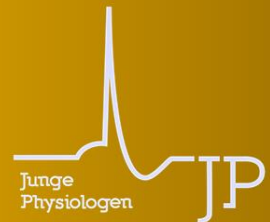
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