Nürnberg, March 2019

Dear colleagues,

Welcome to the 7th Symposium of the Young Physiologists and Pharmacologists, Nürnberg 2019. We very much appreciate your participation.

As with all the previous meetings of the Young Physiologists and this joint meeting, we hope that your experience in Nürnberg will be invigorating and interesting. This symposium allows us to learn and to talk freely about scientific work done by the participants, our achievements and possible failures, to share our knowledge and experiences with other colleagues in a friendly and fresh environment.

In this occasion we count with more than 70 early career scientists from every corner of Germany as well as participants from Switzerland, Austria, Egypt and Nepal. All of them pushing the limits of knowledge on a broad variety of fields converging on physiology: from elementary biophysics to complex organism homeostasis. This symposium, has the first time the official participation and collaboration of the *Forum Junge Wissenschaft* which merges Physiology with the closely related fields of Pharmacology and Toxicology, bringing additional scientific contributions to our event. Together, more than 26 talks and 39 posters have been submitted, which shows the major interest in this kind of symposiums.

As organizers, we expect you to get the maximal profit from this activity, to learn not only about your topic but about the other variety of subjects, too. Please motivate yourself to participate with discussions during talks or poster sessions. As well, you will have the chance to expand your network contacts with other participants during our city tour and the social dinner. We wish you all a nice stay in Nürnberg. Welcome on board!

Yours,

Annika Droste and Gustavo Chaves

Aknowledgments

To our sponsors:

- Nikon
- B. Braun
- Congress- und Tourismus-Zentrale Nürnberg
- Klinikum Nürnberg Medical School GmbH
- Deutsche Physiologische Gesellschaft (DPG)

Their economical support has been crucial for the development of this symposium.

To Prof. Ute Scholl for kindly accept to be our invited speaker, collaborating with a career workshop that we expect to be inspiring to all of us.

To the Deutsche Physiologische Gesellschaft (DPG) for providing travel grants to all active participants and to allow us to use its platform to promote the event. Special thanks to Pamela Finsterseifer for all her support and coordination.

To Andreas Ritzau-Jost for his help in maintaining the Young Physiologists' website.

To all members of the Institute of Physiology and Pathophysiology, PMU Nürnberg, Klinikum Nürnberg for all their help and support during the organization of this symposium.

And finally to all of you to participate.

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General Information

Nuremberg and the PMU

Nuremberg (German: Nürnberg; Nuremberg dialect: Närmberch) is the second-largest city in Bavaria. Nuremberg dates back to the middle ages. During World War II, the once magnificent city was almost completely razed to the ground by bombs. Many historical buildings, however, were rebuilt in the medieval style. Today, more than 500.000 people live here. The city is not only popular for the world's most famous Christkindelmarket, but also for "Lebkuchen"(gingerbread) and "Drei im Weggla", three sausages in a bread roll. Furthermore, the modern rail history officially began in Nuremberg with the opening of the steam-powered Bavarian Ludwig Railway between Nuremberg and Fürth on 7th December 1835!

There are many institutions of higher education in the city like the Friedrich-Alexander-Universität Erlangen-Nürnberg or the Technische Hochschule Georg Simon Ohm adding up to approximately 40.000 students. Another university is Paracelsus Medizinische Privatuniversität Nürnberg (PMU). This institution is organized as a private foundation with locations in Salzburg and Nuremberg. Teaching, research and patient care – these are the three pillars on which the university was founded in 2002 in Salzburg and in 2014 in Nuremberg. Solely 50 students per year (250 students in total) are admitted in Nuremberg which allows individual tutoring. The structure of the study content is oriented to the modern American medical schools. The successful completion of the first part of the American entrance examination (USMLE Step 1) is obligatory at Paracelsus University and is a mandatory condition for the completion of the course of study. The

study ends with the degree of "Dr. med. univ." and is internationally approved.

Venue

The address of the venue is:

Haus 57, Klinikum Nürnberg Nord,

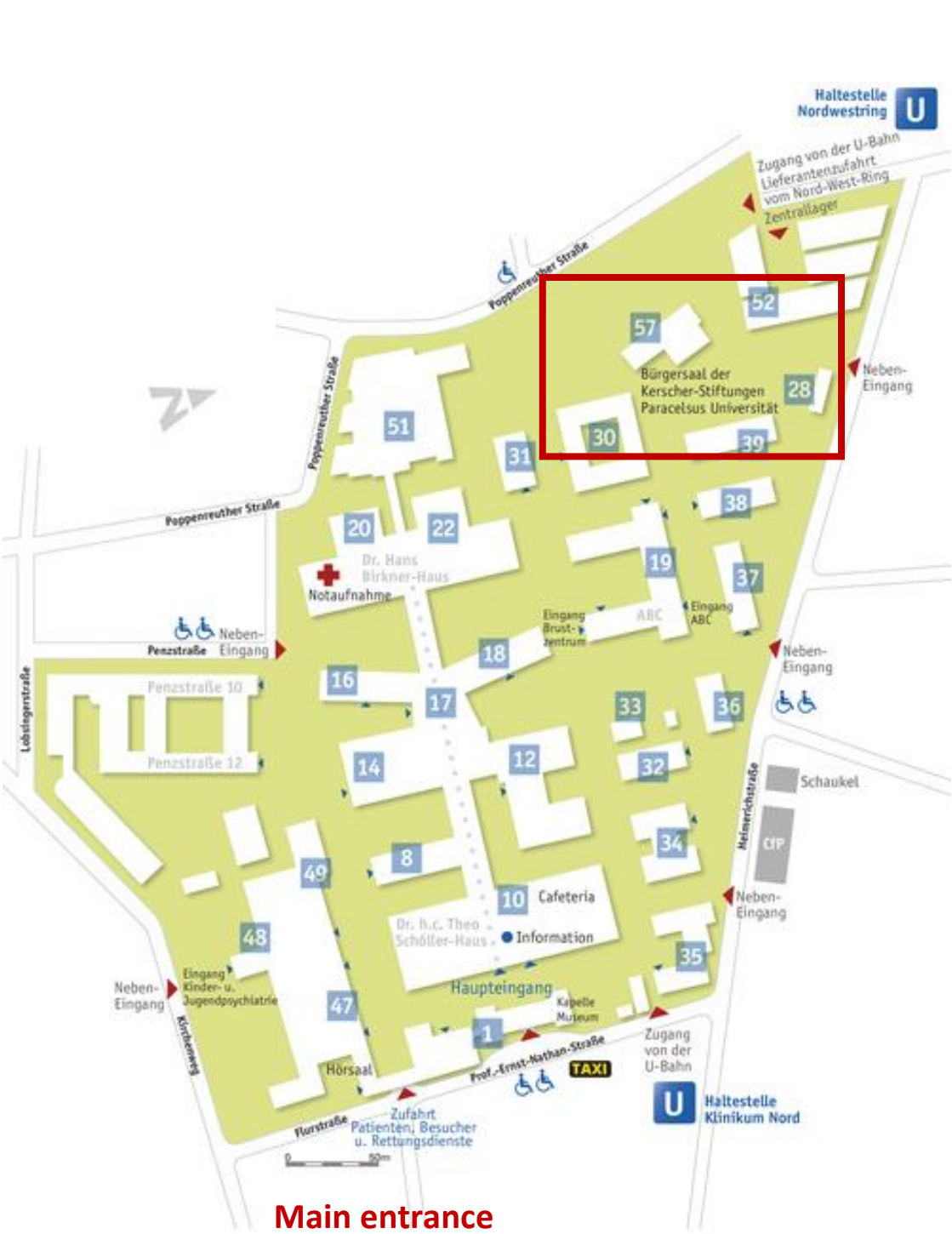
Prof.-Ernst-Nathan-Str. 1, 90419 Nürnberg

There are parking facilities in front of the hospital, but you can also come by bus or underground:

Use the U3 to Nordwestring, station: Klinikum Nord.

Detailed timetables can be found at https://www.vgn.de/.

Please check the map how to get to house 57.



Main entrance

Social Program

After the last talk on Thursday, we will spend some time in the city to show you the famous castle of Nuremberg, the Hauptmarkt (main market) where the Christkindelmarkt takes place and some other highlights. For this, we planned a guided city tour. Two out of three tours will be in German language and one in English. We will end the tour in front of the Heilig Geist Spital, the restaurant of our social dinner.

They offer a three-course menu consisting of either pork, beef, fish or vegetarian variations with dumplings and potatoes for around 29.00 € p.p. The buffet price plus drinks will be paid directly by you at the restaurant.

Poster Session

Please bring your poster in an A0 format and portrait orientation. The number of your displaying board is the same assigned to your contribution, please check the book of abstracts. Posters will be presented in two sessions, both of them at Haus 57, 1 OG. They will be presented by the participants on both days accordingly with the program and they can stay in the room overnight. Board pins will be available at the place.

Oral Presentations

The symposium is organized in seven sessions consisting of 3 - 6 talks each. The talks would have a duration of 12 min + 5 min of open discussion. VGA and HDMI cables will be provided, the same that a computer. Speakers could either bring presentations on a USB stick or on personal computers. Please approach to our staff in order to handle your presentation on the break before your session.

Prizes

Every participant will have the chance to vote for his favorite candidates in two categories: *Best Poster Award* and *Best Talk Award*. In both cases, the winners will be selected by direct voting using a ballot form that will be handled right after Oral Session 7. In case of a tie, the tiebreaker will be made by random selection among the candidates. The price for the Best Talk Award will be given to 3 candidates and consists in a slot to present the work at the German Physiological Society meeting in Ulm 2019. In case of the Best Poster Award, the two candidates with more

votes will receive a digital COOLPIX W100 waterproof camera sponsored by Nikon.

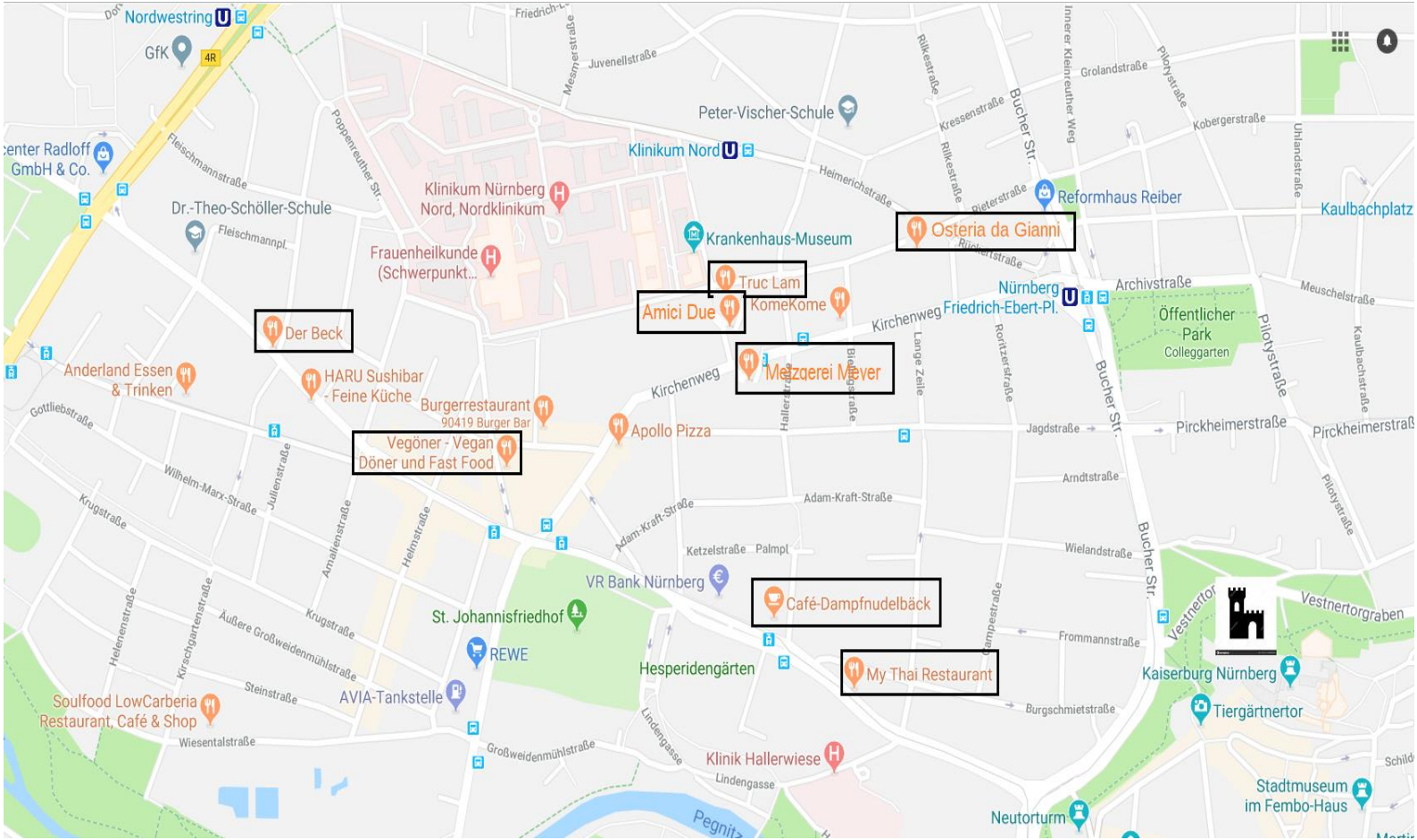
Certificate of Attendance

Certificates will be handled to every participant on the second day of the symposium around the lunch break. Please approach to the reception desk to claim yours. We encourage to all participants, passives and actives, to sign the attendance list on both days.

Breaks

We offer you some free drinks and snacks during coffee breaks and poster sessions. Please help yourself. Lunch runs by every participant though. A variety of restaurants around the clinic could be found just crossing the street. Please see map attach ed. You can choose between several restaurants/ fast food restaurants very close to the hospital:

- Truc Lam: An Asian restaurant with changing daily menus
- Palast Grillhaus: Kebab and other Turkish specialities
- Metzgerei Meyer: Butcher's shop with daily lunch menu and tasty burgers
- Vegöner: A special fast food restaurant that offers vegan Kebab
- Due Amici or Osteria da Gianni: Two great Italian restaurants in front of the hospital with one of the best pizzas in Nuremberg
- Crazy Nate's West Coast Mexican: Burritos and more (lunch only on Friday)


Nordwestring
GfK
4R
Juvenellstraße
Peter-Vischer-Schule
Klinikum Nord
Heimerichstraße
Reformhaus Reiber
Osteria da Gianni
Kaulbachplatz
Fleischmannstraße
Dr.-Theo-Schöller-Schule
Fleischmannpl.
Klinikum Nürnberg Nord, Nordklinikum
Krankenhaus-Museum
Frauenheilkunde (Schwerpunkt...
Truc Lam
Amici Due
KomeKome
Nürnberg Friedrich-Ebert-Pl.
Archivstraße
Öffentlicher Park
Colleggarten
Der Beck
Kirchenweg
Metzgerei Meyer
Anderland Essen & Trinken
HARU Sushibar - Feine Küche
Burgerrestaurant
90419 Burger Bar
Apollo Pizza
Jagdstraße
Pirckheimerstraße
Vegöner - Vegan Döner und Fast Food
Adam-Kraft-Straße
Arndtstraße
Wielandstraße
Ketzelstraße
Palmpl
VR Bank Nürnberg
Café-Dampfnudelbäck
St. Johannisfriedhof
REWE
Hesperidengärten
My Thai Restaurant
Frommannstraße
Kaiserburg Nürnberg
Tiergärtnertor
Vestnertorgraben
Soulfood LowCarberia Restaurant, Café & Shop
AVIA-Tankstelle
Steinstraße
Wiesentalstraße
Großweidenmühlstraße
Lindengasse
Klinik Hallerwiese
Burgschmietstraße
Stadtmuseum im Fembo-Haus
Neutorturm
Pegnitz
Bucher Str.
Pilotystraße
Krugstraße
Wilhelm-Marx-Straße
Gottliebstraße
Julienstraße
Amalienstraße
Hehnstraße
Helenenstraße
Kirschgartenstraße
Äußere Großweidenmühlstraße
Rollnerstraße
Lange Zeile
Hallerstraße
Bielingasse
Rilkestraße
Kressenstraße
Pieterstraße
Rückertstraße
Innerer Kleinreuther Weg
Grolandstraße
Kobergerstraße
Uhlandstraße
Meuschelstraße
Kaulbachstraße
Poppenreuther Str.
Messnerstraße
Friedrich-L

Detailed Program

Thursday, 14.03.2019

09:30 **Registration**

10:00 **Welcome**

10:45 **Oral Session 1: Tissue Physiology**

Chairs: Tobias Brügmann, Co-Chair: Annika Droste

Jannis Meents, Aachen

O-01 The role of increased phosphorylation in the hypersensitivity of iPSC-derived nociceptors from chronic pain patients

Thomas Seidel, Erlangen

O-02 Contribution of longitudinal components of the transverse-axial tubular system to Ca2+ release in ventricular cardiomyocytes

Anja Wenzl, Hannover

O-03 Micromechanical investigations of hiPSC-CMs and human ventricular heart tissue at myofibrillar level reveal functional differences

Anika Kraetzig, Leipzig

O-04 Effects of n-butyrate on porcine colon epithelium under hypoxia

Johannes Jägers, Duisburg-Essen

O-05 The use of perfluorocarbon based artificial oxygen carriers is a promising approach to sustain physiological properties of the kidney during isolated warm perfusion

Luisa Kaluza, Aachen

O-06 Loss-of-function mutation causes gain-of pain syndrome: neuropathy patient harbors a trafficking deficient sodium channel mutation

12:30 **Lunch**

13:30 **Oral Session 2: Receptors**

Chair: Marcus Schewe, Co-Chair: Karolina Najder

Christoph Klenk, Zürich

O-07 Crystal structure of the parathyroid hormone 1 receptor in complex with a peptide agonist

Alexandr Ilyaskin, Erlangen

O-08 Tauro-deoxycholic acid (t-DCA) inhibits human purinergic receptor P2X4 heterologously expressed in *Xenopus laevis* oocytes

Lisa Baaske, Leipzig

O-09 Regulation of free fatty acid transporters in ovine rumen

Saskia Cönen, Jülich

O-10 Molecular dynamics study oh hP2X_2 receptors desensitization

14:45 **Coffee Break**

15:00 **Oral Session 3: Biophysics**

Chair: Jan-Philipp Machtens, Co-Chair: Anja Wenzl

O-11 Marcus Schewe, Kiel

A Pharmacological Master-key Mechanism that Unlocks the Selectivity Filter Gate in K^+ Channels

O-12 Bettina Mertens, Jülich

Vesicular glutamate transporters (VGLUTs): channels, transporters, both?

O-13 Dominik Lenz, Marburg

Insights into the gating pathway of prestin (SLC26A5)

16:00 **Poster Session + Coffee**

17:00 **Oral Session 4: Hypoxia and Inflammation**

Chair: Christoph Klenk, Co-Chair: Heidemarie Dobias

O-14 Franziska Dengler, Leipzig

Expression levels of HIF prolyl 4-hydroxylase enzymes in murine jejunum epithelium

O-15 Yoshiyuki Henning, Essen

Thyroid hormones in ocular angiogenesis and inflammation – implications for retinal degeneration

O-16 Micol Rugi, Münster

The role of sodium transporters for neutrophil chemotaxis

18:00 – 19:00 **Break**

19:00 **Guided City Tour**

20: 00 **Social Dinner at Heilig Geist Spital**

(Spitalgasse 16, 90403 Nürnberg)

Friday, 15.03.2019

09:00 **Oral Session 5: Protons**

Chair: Franziska Dengler, Co-Chair: Jannis Meents

O-17 Stefan Bernhard, Ulm

Interleukin 8 alters the intracellular pH and glucose uptake of neutrophil granulocytes

O- 18 Zoltan Pethö, Münster

Environmental pH and mechanical stress orchestrate pancreatic stellate cell activation

O-19 Gustavo Chaves, Nürnberg

Mechanism of zinc inhibition on the voltage-gated proton channel

10:00 **Poster Session + Coffee**

11:00 **Oral Session 6: Ion Channels and Biophysics**

Chair: Gustavo Chaves, Co-Chair: Luisa Kaluza

O-20 Indra Schröder, Darmstadt

Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral K^+ channel

O-21 Sarah Thull, Aachen

Resurgent-like sodium currents induced by insecticide in the absence of pore-blocker

O-22 Navin K. Ojha, Jena

Membrane potential manipulation with visible flashlight illumination of targeted superparamagnetic beads

O-23 Karolina Najder, Münster

TRPM2 channel sustains low intracellular sodium concentration in activated neutrophils

12:15 **Career Workshop: Prof. Ute Scholl**

13:15 **Photo + Lunch Break**

14:15 **Oral Session 7: Neurophysiology**

Chair: Dominik Lenz, Co-Chair: Bettina Mertens

O-24 Andreas Ritzau-Jost, Leipzig

Potassium channel-independent action potential amplitude in small nerve terminals revealed by high-resolution current clamp recordings

O-25 Matthias Deutsch, Jülich

Knockdown of HCN channels in mouse hippocampal neurons by virus delivered gene-interfering tools

O-26 Kim Le Cann, Aachen

Efficient RNA transfer by fusogenic liposomes into induced-pluripotent stem cell-derived neurons for the study of a Huntington's Disease in vitro model

15:15 **Coffee + Voting**

15:45 **Award Ceremony**

O-01 The role of increased phosphorylation in the hypersensitivity of iPSC-derived nociceptors from chronic pain patients

Jannis Meents[1], Clara Kerth[1], Petra Hautvast[1], Angelika Lampert[1]

[1]Institute of Physiology, RWTH Aachen University Hospital, 52074 Aachen Germany

The chronic pain syndrome inherited erythromelalgia (IEM) is attributed to mutations in the voltage-gated sodium channel (Nav) 1.7. In the past, we have investigated induced pluripotent stem cell (iPSC)-derived nociceptors from IEM patients with the Nav1.7/I848T mutation. These nociceptors display enhanced action potential characteristics that may well explain the pain phenotype described by the patients. Furthermore, we could show in these human nociceptors that the pain-causing Nav1.7/I848T mutation leads to a hyperpolarized Nav channel activation compared to unmutated controls. This enhanced activation leads to a lowering of the nociceptor's action potential firing threshold and potentially leads to increased pain sensation. As the I848T mutation creates a possible novel phosphorylation site for threonine kinases, we tested whether phosphorylation might play a role in the observed shift in Nav1.7 voltage dependence of activation. Indeed, we find in heterologous expression systems that kinase inhibitors reduce the shift in activation. Using phosphomimetics, we confirm a role of protein kinases in the enhanced activation of Nav1.7. In future experiments we will investigate, which exact protein kinase is responsible for the observed shift of Nav1.7 activation and whether inhibitors of that particular kinase can be used in iPSC-derived nociceptors of IEM patients to return action potential firing behavior to normal levels. We believe that our findings may open new avenues for pharmacological intervention in pain syndromes.

O-02 Contribution of longitudinal components of the transverse-axial tubular system to Ca2+ release in ventricular cardiomyocytes

Thomas Seidel, Dominik Fiegle, Tilmann Volk

FAU Erlangen, Institute of Cellular and Molecular Physiology, 91054 Erlangen, Germany

Background: Chronic heart failure leads to remodeling of the transverse-axial tubular system (TATS) of ventricular cardiomyocytes in human heart failure and animal models of cardiac disease, leading to changes in t-tubule shape and orientation. However, the contribution of longitudinal or sheet-like TATS components to EC coupling in ventricular myocytes is unclear.

Methods: Ventricular cardiomyocytes were isolated from adult rats or from samples of human failing hearts. Rat myocytes were kept in culture to induce remodeling of the TATS. We acquired 2D and 3D confocal images, correlating them with confocal line scans of fieldstimulated rat and human myocytes. Time and velocity of local Ca2+ release were correlated with the distance to the closest TATS component. Ca2+ release parameters were also correlated with established and novel 2D and 3D measures of the TATS, comprising spectral density, mean distance, standard deviation of distance, volume density, and morphology of TATS components. To measure Ca2+release synchrony we calculated the standard deviation of Ca2+release times.

Results: Local Ca2+ release time and maximum Ca2+ release velocity (dF/F0dtmax) correlated significantly with local distance to the closest TATS component in both rat and human cardiomyocytes. In rat myocytes, Ca2+ release times and velocity near longitudinal components were comparable to Ca2+ release near transverse components, suggesting the presence of functional EC coupling in longitudinally oriented tubules. When correlating tsystem measures with Ca2+ release in linear and quadratic models, Ca2+ release synchrony was predicted best by mean and standard deviation of 3D intracellular distance to the closest TATS component, whereas conventional 2D measures, especially spectral TATS density, correlated poorly. This also applied to human failing cardiomyocytes. Interestingly, Ca2+ release time near sheet-like components was lower and velocity higher than near tubular components.

Conclusions: Longitudinal TATS components in rat and t-sheets in humans contribute to EC coupling in ventricular cardiomyocytes. We suggest that 3D measures of intracellular TATS distance are suitable predictors in both rat and human myocytes.

O-03 Micromechanical investigations of hiPSC-CMs and human ventricular heart tissue at myofibrillar level reveal functional differences

Anja Wenzl[1], Kristin Schwanke[2], Tim Holler[1], Birgit Piep[1], Ulrich Martin[2], Robert Zweigert[2], Theresia Kraft[1], Bogdan Iorga[1]

[1]Deparment of Molecular and Cell Physiology, Hannover Medical School, Hannover, Germany

[2]Department of Cardiac, Thoracic, Transplantation and Vascular Surgery, Leibniz Research Laboratories for Biotechnology and Artificial Organs, Hannover Medical School, Hannover, Germany

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) exhibit some immature functional and morphological features compared to human adult ventricular cardiomyocytes (CMs). Subcellular myofibrils directly determine the contractile force achieved by CMs. To understand whether the immature contractile function of hiPSC-CMs can be attributed to myofibrillar function, we measured steady-state and kinetic force parameters of myofibrils within hiPSC-CMs and compared these parameters to those of human ventricular myofibrils (hvMFs) isolated from adult donor heart.

At saturating calcium concentration [Ca^{2+}] hiPSC-CMs reach a maximum isometric force of 43±33 kPa, which was slightly smaller than the maximum force generated by hvMFs (54±30 kPa). At submaximal [Ca^{2+}], to simulate physiological conditions, myofibrillar force response to calcium was significantly higher for myofibrils of hiPSC-CMs (pCa_{50}=5.85±0.08) than for hvMFs (pCa_{50}=5.45±0.11). By applying a rapid slack-restretch maneuver of Ca^{2+}-activated myofibrils, we measured the rate constants (k_{TR}) of the cycling cross-bridges determining force re-development. k_{TR} for hiPSC-CMs (1.13±0.34 s^{-1}) was significantly faster than k_{TR} for hvMFs (0.77±0.38 s^{-1}). Upon rapid Ca^{2+} removal, kinetics of the first phase of relaxation (k_{LIN}) was not significantly different between hiPSC-CMs and hvMFs, while during the second phase myofibrils of hiPSC-CMs (k_{REL}=5.32±2.20 s^{-1}) relax much faster than hvMFs (k_{REL}=3.38±1.44 s^{-1}).

A significant faster k_{TR} and similar k_{LIN} suggest that cross-bridges enter force generating states faster in hiPSC-CMs than in hvMFs. Because both contractile systems express the ventricular β-myosin isoform, the data suggest that a distinct isoform pattern of other sarcomeric proteins induce the observed functional divergences between myofibrils within hiPSC-CMs and hvMFs. Further analyses of sarcomeric protein isoforms in both types of myofibrils are required to clarify this point. Based on previous work we assume that hiPSC-CMs are rather at a pre-natal stage regarding their sarcomeric protein isoforms and therefore could be used to understand mechanisms of early cardiac diseases affecting contractile function.

O-04 Effects of n-butyrate on porcine colon epithelium under hypoxia

A. Kraetzig, F. Dengler, G. Gäbel

Institute of Veterinary Physiology, University of Leipzig, 04103 Leipzig, Germany

The key energy source for colonocytes, butyrate, is produced in the colonic lumen by microbial fermentation. Previous studies on rumen epithelium raise suspicion that butyrate may have protective effects on epithelial integrity under hypoxia, a common complication of several diseases. Hence, we hypothesized a similar effect of butyrate on the porcine colon epithelium.

Porcine colonic epithelia were mounted in Ussing chambers and gassed with 100% oxygen for 45min to equilibrate. Epithelia were incubated with buffer solutions containing either 50mM Na-butyrate ('butyrate') or 50mM NaCl ('control') instead. After equilibration, chambers of each incubation group were gassed differently: 100% oxygen ('normoxia'); 1% oxygen + 99% nitrogen ('hypoxia'). Electrophysiological parameters (short circuit current (I_{sc}) and tissue conductance (G_t)) were measured. Subsequently, mRNA expression in the differently incubated epithelia was measured using RT-qPCR for the following genes: monocarboxylate transporter (MCT)1, MCT2, sodium-coupled MCT (SMCT)1, down-regulated in adenoma (DRA), zonula occludens (ZO)1 and claudin1.

Initial I_{sc} of the butyrate treated epithelia tended to be lower than of the control group. In both buffer, butyrate containing and free solution, a significant decrease of I_{sc} under hypoxia was detectable, but the relative decrease was diminished by butyrate. Hypoxia caused an increase of G_t compared to normoxia. However, the hypoxia induced increase was significantly lower under butyrate incubation compared to the control group. Gene expression of the targets examined showed no significant difference neither between different gassing nor incubation conditions.

An increased G_t illustrates a reduced epithelial integrity under oxygen depletion. In turn, a decreased I_{sc} represents a reduced electrogenic transport across the epithelium under hypoxia. Butyrate diminished the effects of hypoxia on G_t and I_{sc}. Consequently, butyrate seems to have protective short-term effects on porcine colon epithelium under hypoxia and may prevent tissue from damage. Missing effect on gene expression levels may be due to short incubation time.

O-05 The use of perfluorocarbon based artificial oxygen carriers is a promising approach to sustain physiological properties of the kidney during isolated warm perfusion

Johannes Jägers[1], Katja Bettina Ferenz[1]

[1]Institute of Physiology, University of Duisburg-Essen, University Hospital Essen, Germany

Objecting the extension of the transplantable organ-pool, we want to regenerate organs, especially kidneys, from donors after circulatory death. Using normothermic perfusion, we follow the approach of a cell free perfusion. Therefore, a formulation of perfluorocarbon-based artificial oxygen carriers (AOC) has been developed. Advantages of synthetic oxygen carriers over red blood cell concentrates or full blood are the independence of donors, long-term stability and the certainty of sterility, which is important in the case of immunosuppressed recipients of organs.

For our studies, we use the isolated perfused rat kidney (IPRK) in a recirculating mode. This model allows a realistic prospect on the later application of the perfusate we develop. Main physiological and biochemical parameters are easily observable. During the experiment data about the flow, pressure and pH are easily obtained. The pO2 of the perfusate is measured online via chemical-optical sensors before and after the kidney. The oxidative state of the respiratory chain cytochromes is observed via a non-invasive photometric method. Furthermore, cell damage is determined by measuring LDH in the perfusate. The urine analysis contains check for albuminuria- and GFR-measurement via Bromocresolgreen staining of the urine and via the Inulin-Anthron method.

First results show we are able to maintain a physiological GFR over several hours as well as to prevent albuminuria in that time in kidneys that are perfused with AOCs over a period of 6h. The oxidative state of the cytochromes of the respiratory chain remains physiological over that period. In addition, the cell survival according to LDH measurements of the perfusate is improved in kidneys perfused with AOCs in comparison to those kidneys that were treated with a perfusate free of any oxygen carriers.

To conclude, our synthetic AOCs show promising results to substitute red blood cell concentrates in perfusate used for normothermic machine perfusion of explanted organs.

O-06 Loss-of-function mutation causes gain-of pain syndrome: neuropathy patient harbors a trafficking deficient sodium channel mutation.

Luisa Kaluza[1], Jannis E. Meents[1], Martin Hampl[1, 2], Corinna Rösseler[1], Petra A. I. Hautvast[1], Silvia DetroDassen[3], Ralf Hausmann[3], Günther Schmalzing[3], Angelika Lampert[1]

[1]Institute of Physiology, RWTH Aachen University, 52074 Aachen, Germany.

[2]Institute of Physiology and Pathophysiology Friedrich-Alexander University Erlangen-Nürnberg, 91054 Erlangen, Germany.

[3]Department of Molecular Pharmacology, RWTH Aachen University, 52074 Aachen, Germany.

Mutations in the voltage-gated sodium channels (Nav) 1.7, 1.8, and 1.9 are linked to human pain syndromes. Most of the mutations studied to date present with an intuitive pathomechanism, a direct link between the function of the channel and the phenotype of the patient.

In this study, we investigate the D1639N mutation in the SCN10A gene encoding Nav1.8, that has previously been described in a patient suffering from the chronic pain syndrome small fiber neuropathy (SFN). We show that the mutation leads to a significant reduction in current density, but does not alter any other tested biophysical gating properties of the channel. Thus, the D1639N mutation causes loss-of-function of the channel even though it has been identified in a gain-of-pain patient. Neither co-expression of ß-subunits, nor overnight incubation at lower temperature (27°C) rescued the current density. However, incubation with 1 mM lidocaine overnight restored the current density of Nav1.8/D1639N transfected cells. Ion channel blockers have been reported to support channel trafficking to the membrane by stabilizing trafficking deficient proteins, and thus the rescuing effect of lidocaine suggests a trafficking deficit of the D1639N mutation. Using a GFP linked construct of Nav1.8, we show that the mutation likely accumulates in cell organelles in the cytoplasm, such as the endoplasmatic reticulum (ER).

The accumulation of misfolded or trafficking deficient proteins in cell organelles in the cytoplasm, e.g. the ER, is known to possibly result in ER stress. Thus, the accumulation of Nav1.8/D1639N may result in ER stress causing the systemic disease SFN. Lidocaine could potentially be a suitable therapeutic option for this SFN patient carrying the D1639N mutation, because it likely dissolves the accumulation in cell organelles. However, further clinical testing is needed.

O-07 Crystal structure of the parathyroid hormone 1 receptor in complex with a peptide agonist

Janosch Ehrenmann[1]*, Jendrik Schöppe[1]*, Christoph Klenk[1]*, Mathieu Rappas[2], Lutz Kummer[1], Andrew S. Doré[2], and Andreas Plückthun[1].

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Parathyroid hormone 1 receptor (PTH1R) is a class B multi-domain G protein-coupled receptor and the major regulator of mineral ion homeostasis and bone metabolism. It is activated by parathyroid hormone and the closely related parathyroid hormone-related protein. Teriparatide and abaloparatide are analogues of these hormones and approved for use in the clinic to increase bone formation and mineralisation, representing one of the most effective, yet costly, treatments available for osteoporosis. Despite its high pharmacological relevance and tremendous efforts put into uncovering the molecular function, to date only little is known about the structural properties of this receptor.

Wild-type human PTH1R suffered from poor biophysical properties precluding purification and crystallization of native receptor. To improve receptor expression and thermo-stability we performed a directed evolution approach comprising of cell surface display in yeast and selection of improved receptor variants by fluorescence-activated cell sorting with an engineered fluorescent ligand. The best receptor variant thereof was optimized further by rational protein engineering and was used for crystallization in lipidic cubic phase in complex with a peptide analogue of parathyroid hormone. The crystal structure was solved at 2.5 Å, and the high resolution allowed us to precisely delineate the binding mode of parathyroid hormone and revealed molecular details within conserved structural motifs critical for class B receptor function. Moreover, it provides a structural rationale for receptor-activating mutations underlying the severe phenotype of Jansen's metaphyseal chondrodysplasia. Together, our findings extend the understanding of the mechanisms of class B receptor ligand binding and activation, and serve as a basis for structure-based drug design for the treatment of osteoporosis and disorders of calcium homeostasis.

O-08 Tauro-deoxycholic acid (t-DCA) inhibits human purinergic receptor P2X4 heterologously expressed in *Xenopus laevis* oocytes

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The P2X receptor family comprises non-selective cation channels activated by ATP. One member of this family, P2X4, was found to be abundantly expressed in the apical membrane of bile duct epithelial cells. Recently, we have demonstrated that bile acids, especially tauro-deoxycholic acid (t-DCA), stimulate several members of the epithelial sodium channel (ENaC)/degenerin (DEG) family of ion channels including the acid-sensing ion channel 1 (ASIC1). Interestingly, comparison of P2X4 and ASIC1 crystal structures revealed a high degree of structural similarity of the channels. Therefore, we hypothesized that bile acids may also affect P2X4. In the current study, we investigated whether t-DCA may modulate human P2X4 heterologously expressed in *Xenopus laevis* oocytes. Whole-cell and single-channel currents were measured using the two-electrode voltage clamp and outside-out patch-clamp technique, respectively. Application of t-DCA (250 μM) had no effect on P2X4 activity *per se*, but significantly and reversibly reduced ATP-activated P2X4-mediated currents by ~70%. The inhibitory effect of t-DCA on P2X4 was concentration dependent with an apparent IC_{50} of about 160 μM. The affinity of P2X4 to ATP (EC_{50}=2.4±1.3 μM) in the presence of t-DCA
was similar to that under control conditions (EC_{50}=4.8±1.2 μM). In outside-out single-channel patch-clamp recordings we demonstrated that the estimated channel open probability was significantly reduced in the presence of t-DCA due to stabilization of the channel closed state by t-DCA. Finally, site-directed mutagenesis and a molecular docking approach provided evidence that a putative t-DCA binding site may be localized within the transmembrane domain of P2X4. We conclude that t-DCA inhibits ATP-activated P2X4 currents by interacting with a specific site of P2X4 and stabilizing its closed state.

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O-09 Regulation of free fatty acid transporters in ovine rumen

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Recent studies in colon of monogastric animals point to a regulation of transport proteins for short chain fatty acids by G-protein-coupled receptors (GPRs), which can modify levels of cyclic adenosine monophosphate (cAMP). Influence of cAMP on monocarboxylate transporter-1 (MCT-1) and Na^+/H^+-exchanger (NHE) were shown in a Caco2-subclone cell line and ruminal tissue, respectively. We wanted to investigate, whether MCT-1 and NHE could be influenced by activation of GPRs in ruminal epithelium.

Ovine ruminal epithelia were mounted in Ussing chambers and incubated with forskolin (activator of adenylyl cyclases which raises cAMP level), butyrate or niacin (a GPR109A agonist). After incubation, cAMP concentration was analysed in epithelium. Butyrate incubation following forskolin application lowered cAMP levels significantly in comparison to only forskolin stimulated tissues. This effect was even more pronounced, when tissues were incubated at a mucosal pH of 6.5 and butyrate was administered only mucosally. Niacin failed to provoke a significant reduction of cAMP levels.

Subsequently, ^{14}C-acetate fluxes were analysed under the influence of forskolin and MCT-1-inhibitors. The inhibitor p-hydroxymercuribenzoic acid led to a significant reduction of ^{14}C-acetate fluxes. An influence of forskolin and consequently cAMP could not be observed.

Finally, pH_i was determined in primary cultured ruminal epithelial cells of sheep. They were undergoing an acidification challenge and NHE activity was assessed by incubation with forskolin and/ or 5-(N-ethyl-N-isopropyl)-amiloride (EIPA), an NHE-inhibitor. NHE function was confirmed as EIPA induced a significant inhibition of counter-regulation after acidification. Nonetheless, only a slight, not significant diminution of counter-regulation was noticed after forskolin administration.

Our results suggest, that the diminishing effect of butyrate on cAMP levels is rather not generated by activation of GPR109A. Potentially, a different GPR, e.g. FFAR2, might be the key factor, mediating changes of intracellular second messengers. However, changes of cAMP did not show any effect on transport proteins in our models, therefore indicating different ways of their regulation.

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O-10 Molecular dynamics study oh hP2X$_2$ receptors desensitization

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P2X receptors belong to the class of ligand-gated ion channels and are activated by extracellular ATP. The subtypes named P2X$_1$ to P2X$_7$ assemble in homo- or heterotrimeric complexes and are permeable to mono- or divalent cations. The seven mammalian family members of P2X are widespread through various cell types and are involved in important physiological processes like nociception or muscle contraction. Therefore, P2X receptors represent promising targets for drug development due to their physiological functions. The recently determined X-ray crystal structures of the human P2X$_3$ receptor in its three conformational states: open, desensitized, and apo-resting. The intracellular domains form a complex structure termed the cytoplasmic cap, which is assumed to dynamically build during receptor activation. We used all-atom molecular dynamics simulations of hP2X$_3$ to investingate conformational changes and pore closure during desensitization. We demonstrated that the cytoplasmic cap markedly stabilizes the transmembrane domains in the open state, thereby preventing pore closure. Starting from the open state, the unfolding of the C-terminal residues already occured on the microsecond timescale of our simulation, whereas the N-terminal domains remained stable, preventing the conformational transition of the transmembrane domains towards the desensitized state. Thus we propose that hP2X$_3$ receptor desensitization is a three-step process: It is characterized by an initial fast detachment of the C-terminal domains and a subsequent slow unfolding of the N-terminal residues. Afterwards, conformational changes of the transmembrane domains lead to pore closure.

O-11 A Pharmacological Master-key Mechanism that Unlocks the Selectivity Filter Gate in K^+ Channels

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K^+ channels openers are promising pharmacological tools for the treatment of many disorders including epilepsy, pain, inflammation, arrhythmias and hypertension. Consequently, a large number of compounds have been developed. However, in most cases their binding sites and precise mechanisms of action are unknown hampering a rational drug design. Compound selectivity is thought to arise by the targeting of a specific activation mechanism as a great many of distinct mechanisms exists in K^+ channels. These various mechanisms, however, converge finally on one of two gates in K^+ channels, a lower gate at the cytoplasmic pore entrance (helix-bundle crossing) and an upper gate at the selectivity filter (SF). Here we report on a new class of negatively charged activators (NCAs) that universally target K^+ channels operated by the SF gate including many K_{2P} channels, voltage-activated hERG channels and Ca^{2+}-activated BK channels. Functional analysis combined with crystallographic studies and molecular dynamics (MD) simulations revealed a conserved NCA effector mechanism that involves electrostatic coordination of K^+ ions beneath the SF and stabilization of the open SF configuration. These results disclose an unrecognized poly-pharmacology for many known K^+ channel openers and a unique drug interaction principle, highlight a SF gating machinery conserved across K^+ channel families and advance our general understanding of drug action in ion channels with specific implications for drug design.

O-12 Vesicular glutamate transporters (VGLUTs): channels, transporters, both?

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Vesicular glutamate transporters (VGLUTs) accumulate the excitatory neurotransmitter glutamate in synaptic vesicles and are thus major determinants of the strength of excitatory synapses. They are not only of high physiological importance, but also functionally unique: VGLUTs are secondary active glutamate transporters as well as anion channels and Na^+-coupled phosphate transporters [1].

In order to characterize VGLUT1 by electrophysiological techniques, we mutated targeting signals in amino- and carboxy-terminal regions [2]. $VGLUT1_{SM}$ was heterologously expressed as GFP fusion protein in HEK293T cells and studied via a combination of whole-cell patch clamping, noise analysis and fluorescence intensity measurements. We found that VGLUT1 forms a pH dependent chloride channel with a lyotropic anion selectivity and a unitary current conductance around 1 pS. To compare permeabilities of various anions, we measured the whole-cell fluorescence amplitude for each studied cell and normalized whole-cell currents to this value that is proportional to the number of transporters in the surface membrane. Such experiments revealed that VGLUT anion channels are permeable to multiple large anions. Comparing normalized currents from cells dialyzed with Cl^-- based or with $glutamate^-$- based internal solutions, revealed 30 times lower transport rates for $glutamate^-$ than for Cl^-. A point mutation that was shown to abolish glutamate transport [3], H120A, exhibits altered single channel amplitudes and changed anion selectivity of the $VGLUT1_{SM}$ anion channel.

Our experiments provide novel insights into the multiple transport functions of vesicular glutamate transporters.

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O-13 Insights into the gating pathway of prestin (SLC26A5)

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Prestin (SLC26A5) is a member of the SLC26/SulP anion transporter family. Its unique quasi-piezoelectric mechanical activity generates fast cellular motility of cochlear outer hair cells (OHC), a key process underlying active amplification in the mammalian ear. By combining homology modelling, MD simulations and cysteine accessibility scanning, a structure was derived, proposing a 7+7 TM inverted repeat architecture with the domains TM3 and TM10 as central gating cavity [Gorbunov et al., *Nature Comm.* 2014]. Under physiological conditions, the electromotility of mammalian prestin orthologues depends on binding of intracellular anions, suggesting that the underlying dynamics may be derived from an ancestral anion transport mechanism, which is still present in non-mammalian orthologues [Schaechinger and Oliver, *PNAS* 2007].

To address the molecular mode of action of anions in electromechanical function, we conducted a glutamate scan of the putative anion binding site in TM3 and TM10. While introduction of glutamate at most positions probed was disruptive for protein function, glutamate at the central position Ser396 (TM10) was tolerated. Moreover, S396E rendered prestin anion-independent and insensitive to competitive anionic blockers. Using the CRISPR/Cas9 methodology, the *r*Pres-S396E-mutation was also introduced into transgenic mice, validating the effects detected upon expression in CHO cells.

We conclude that S396E reveals location and coordination of substrate anions. Anion binding may enable the voltage-dependent conformational transition by perturbing a local hydrogen or electrostatic network.

O-14 Expression levels of HIF prolyl 4-hydroxylase enzymes in murine jejunum epithelium

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The HIF prolyl 4-hydroxylases 1-3 (HIF-P4Hs) are the main players of an oxygen sensing mechanism mediating the adaptation of cells to hypoxia[1,2]. An additional transmembrane P4H (P4H-TM) also contributes to it[2]. Hydroxylation of the hypoxia inducible factor (HIF) α subunit by the HIF-P4Hs leads to its rapid proteasomal degradation under normoxic conditions. In hypoxia, the hydroxylation is inhibited, HIFα escapes degradation and modulates the expression of more than 300 genes involved in cellular adaptation to hypoxia[1]. The activation of HIF via inhibition of HIF-P4Hs may have vast therapeutic value in the treatment of pathological conditions characterized by insufficient O_2 levels but also in (chronic) inflammatory conditions like inflammatory bowel disease.

Due to their increasing therapeutic importance, a differentiation of the roles that each of the three HIF-P4H isoforms and P4H-TM plays in the target tissues is necessary. Thus, we assessed the expression of these enzymes in the murine jejunum epithelium using droplet digital PCR (ddPCR). Then we used HIF-P4H-1 knock out and HIF-P4H-2 hypomorph mice (and their respective wild types) to identify the effects on HIF activation by analyzing the jejunum epithelium for the expression of classical HIF-target genes.

In general, we found similarly high levels of HIF-P4H-1, -2 and -3, while P4H-TM was not expressed in jejunum epithelium. This finding may hint at redundant roles of the isoforms securing a stable regulation of the adaptation to hypoxia in spite of the profoundly changing oxygenation of the gastrointestinal tract within the physiological range. In the genetically modified mice, however, only HIF-P4H-2-hypomorphism but not HIF-P4H-1 knock out showed effects on gene expression level and thus HIF activation, indicating a predominant role of HIF-P4H-2 in jejunum epithelium.

[1] Semenza 2014

[2] Myllyharju und Koivunen 2013

O-15 Thyroid hormones in ocular angiogenesis and inflammation – implications for retinal degeneration

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Many people suffer from retinal degenerative diseases with different levels of vision loss up to complete blindness. Currently, thyroid hormones (TH), in particular triiodothyronine (T3) and its precursor thyroxine (T4), gain broader attention as crucial factors for retinal development and photoreceptor function. For instance, alterations of the TH status influence color vision, and excess TH signaling was previously linked to degeneration of cone photoreceptors. However, the molecular mechanisms causative for these degenerative processes are not clearly identified thus far. Retinal diseases are often associated with neovascularization and inflammatory processes, such as in age-related macular degeneration, the most common cause of blindness in elderly people, where central vision is lost. Since TH have proangiogenic and proinflammatory effects, we are currently investigating a potential link between TH signaling, neovascularization and inflammation in the retina. In the presented project, we are focusing on the role of the retinal pigment epithelium (RPE), a monolayer of pigmented cells located adjacent to the retina, in photoreceptor degeneration, because the RPE is crucial for maintaining visual function by regulating photoreceptor homeostasis. To obtain the first insights of TH signaling in the RPE, we treated an immortalized RPE cell line (ARPE-19) with T4 and T3. Here we present data on TH treatment effects with focus on angiogenesis and inflammation, which were observed in a dose-dependent manner. These effects might be driven by hypoxia-inducible factor 1 (HIF-1), a key regulator of proangiogenic processes under hypoxic conditions. In line with this hypothesis, we found that HIF-1α, the α-subunit of HIF-1, was altered between different treatment conditions. For further systemic analyses, we will take advantage of a state-of-the-art imaging technique, termed *EyeCi*, which we have previously developed. EyeCi allows us to make the whole eye transparent in order to analyze ocular vasculature within intact eyes in its native, three-dimensional organization.

O-16 The role of sodium transporters for neutrophil chemotaxis

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Neutrophils are the first line of defense against pathogens. They are able to reach the site of inflammation and fight the intruder because of their ability to chemotax, i.e. to move directionally along a gradient of chemoattractant molecules. Our group has previously shown that chemotactic stimulation of neutrophils leads to an increased Na^+ load in neutrophils. This is accentuated in an acidic environment. Chemotaxis in turn is compromised in an acidic environment.

My work is focused on the role of the ionic balance in chemotaxis and in particular on the proteins which are required for coping with and responding to the Na^+ load during chemotactic stimulation, respectively. Na^+/K^+ ATPase and Na^+/Ca^{2+} exchanger (NCX) are two of those proteins that are intimately linked to the intracellular Na^+ balance.
I analyzed chemotaxis of murine neutrophils embedded in a 3D collagen I matrix in a gradient of C5a. Chemotaxis was evaluated with live-cell imaging for one hour. Images were acquired in 5 s intervals and the stacks were analyzed with Amira and ImageJ software. I probed two different pH values, physiological (pH7.4) and acidic (pH6.5). NCX was inhibited with KB-R7943, and the Na/K ATPase with ouabain.
Chemotaxis is strongly impaired at pH 6.5, while undirected motility is hardly affected by the extracellular pH.
NCX inhibition affects mostly chemotaxis at pH 7.4, and this inhibition is less effective at pH 6.5. Interestingly, KB-R7943 does not affect the undirected motility. On the contrary ouabain affects both motility and chemotaxis.
Our results point out the importance of Na^+ balance in neutrophil chemotaxis and the role of Na^+/Ca^{2+} exchanger and Na^+/K^+ ATPase in sustaining sodium homeostasis.

O-17 Interleukin 8 alters the intracellular pH and glucose uptake of neutrophil granulocytes

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Introduction: We recently reported that alterations in membrane potential (MP)[1], intracellular pH (pHi)[2], cell shape as indicated by forward scatter (FSC)[3], and glucose uptake (GlcU)[2] are mediated by the chemotaxin C5a. These responses are substantially impaired in hemorrhagic shock or sepsis, respectively, and thus might contribute to immune dysfunction in systemic inflammation. In the present study, we hypothesized that similar changes can be evoked by the chemotactic agent Interleukin 8 (Il8).

Methods: MP, pHi and GlcU were monitored by flow cytometry using the dyes DiBAC(4)3, SNARF, and 2-NBDG, respectively. 10 000 cells were measured per donor in RPMI titrated to an extracellular pH (pHe) of 7,4 at 37°C if not indicated otherwise.

Results: Exposure of PMN from five donors with Il8 (50 ng/ml) resulted in a significant increase in GlcU (+119% ±17%, $p < 0,05$), FSC (+102% ±16%, $p < 0,05$) and pHi (+0,40 ±0,03 ± $p < 0,05$) but no significant change in MP after 5 minutes as measured by flow cytometry. Extracellular acidification to pHe 6,6 or 7,0 to mimic the inflammatory microenvironment changed baseline pHi of PMN but did neither impair relative alkalization (+0,31 respectively +0,37) nor GlcU (+90% respectively +89%).

At physiological pHe, kinetics of internal pH changes were biphasic with minimal acidification during the first 60 s upon Il8 exposure followed by an alkalization. FSC and GlcU displayed an initially fast increase (49%/min and 36%/min, respectively) during the first minute followed by a continuous slow increase (6%/min and 5%/min, respectively).

Conclusion: To our knowledge, this is the first report of simultaneous kinetic measurements of metabolic and electrophysiological quantities in human PMN upon pro-inflammatory Il8 exposure. Further studies need to elucidate the possible contribution of Il8 to systemic inflammation associated hyperlactemia.

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O-18 Environmental pH and mechanical stress orchestrate pancreatic stellate cell activation

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Pancreatic stellate cells (PSCs) are stromal cells of the pancreas that can become activated in various diseases such as pancreatic cancer. Pancreatic cancer is coupled to changes in the extracellular environment of the stroma, including mechanical stiffness and interstitial pH. The tumorous transformation of the pancreatic ductal cells leads to impaired ductal HCO_3^- secretion, which in turn leads to a relative alkalinization of the interstitial pH of the pancreas. In this study we aim to elucidate how changes in extracellular pH (pH_e) and mechanical stress affect the intracellular pH (pH_i) homeostasis and activation of pancreatic stellate cells.

We isolated PSCs from healthy adult wild-type C57/BL6 mice. Following isolation, we cultured PSCs for up to 120 hours in media buffered to different pH_e on a stiff substrate or on polyacrylamide gels of physiological stiffness. Cellular viability, activation and proliferation were assessed using immunocytochemistry and flow cytometry. To investigate the functional expression of potential pH sensors, regulators and pH-regulated transporters of PSCs, we performed RT-qPCR, Western blot and pH_i measurements.

Alkalization of the extracellular milieu substantially PSC activation as evidenced by an increase in cell size and α-SMA positivity, especially on a rigid substrate. Similarly, cell proliferation, cell cycle progression and nuclear Yap localization are also facilitated in an alkaline pH_e environment. Multiple pH sensors and regulators are highly expressed in activated PSCs cultured in pH_e 7.4 compared to freshly isolated cells and cells cultured in pH_e 6.6. Also, NHE1 activity is enhanced in activated PSCs at pH_e 7.4 as compared to inactive PSCs at pH_e 6.6. When PSCs are cultured in an acidic pH_e environment, the pH_i acidifies to $pH_i \leq 7.0$. In summary, alkaline pH_e is a potent activator of PSCs, that may enable cell cycle progression through Yap/Taz and HIF1a mediated pathways.

O-19 Mechanism of zinc inhibition of voltage-gated proton channel

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Zinc is a physiological inhibitor of voltage-gated proton channels (H_V1). A substantial number of reports have shown that inhibition of H_V1 by zinc changes the physiology of several cell types; e.g. diminishing the oxidative burst in phagocytes, limiting histamine secretion in basophils, reducing invasiveness of cancer cells and preventing the final maturation of human sperm.

We investigated the zinc inhibition of a new member of the voltage-gated proton channel family, NpH_V1, from the insect *Nicoletia phytophila* [1]. We found that NpH_V1 is inhibited less by external zinc than the human channel hH_V1 at pH_o 7. Both slowing of activation (τ_{act}) and the shift in voltage threshold are diminished. Aligning the insect channel with the human channel reveals that NpH_V1 possesses only one of the two externally accessible histidines. Instead of a histidine in the second extracellular loop NpH_V1 expresses an aspartate (Asp^{145}). Asp has a very low pK_a value which leaves it deprotonated in solution at pH_o 5. The effects of zinc were abolished at pH_o 5. This contradicts the hypothesis that Asp permits channel inhibition even at low pH. Substitution of Asp^{145} with His^{145} restores zinc sensitivity of NpH_V1 comparable to hH_V1. Substitutions of the His^{92} to Ala^{92} and His^{92}/Asp^{145} to Ala^{92}/Ala^{145} render the channel almost zinc insensitive.

The data support the idea that strong zinc inhibition of the proton channel is dependent on a histidine in the S3-S4 linker. The detailed investigation of the coordination sites of zinc ions might advance the development of a medical proton channel inhibitor.

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O-20 Site-specific ion occupation in the selectivity filter causes voltage-dependent gating in a viral K^+ channel

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Many K^+ channels exhibit selectivity filter gating, often voltage-dependent and independent from dedicated voltage-sensor domains. A plethora of experimental and theoretical literature has shown that this is likely modulated by the occupation of K^+ binding sites within the selectivity filter. We approached this topic by analyzing single-channel gating kinetics and by revealing details on ion occupation from the same set of lipid bilayer experiments.

Kcv channels are viral pore-only K^+ channels; their selectivity filter gating, as observed from in vitro expressed proteins in planar lipid bilayers, is modulated by both voltage and K^+. This sub-ms gating is beyond the canonical resolution of the experiments and was thus analyzed by fitting extended beta distributions to amplitude histograms (1). A global fit of the rate constants and the single-channel IV curves based on available atomistic models of ion transport was performed for different voltages and K^+ concentrations. The rate constant of channel closing correlates with the probability of three ions being in the filter instead of two (2).

The experimental results further point to a crucial role of flexibility in modulating selectivity filter closure rather than a direct conformational change. The role of the inherent flexibility for gating in Kcv channels is currently further explored by anisotropic network modelling.

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O-21 Resurgent-like sodium currents induced by insecticide in the absence of pore-blocker

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Voltage-gated sodium channels are responsible not only for the fast upstroke of the action potential, but they also modify cellular excitability via persistent and resurgent currents. We are beginning to understand the molecular basis of the latter: it is commonly accepted that an open channel blocker is necessary to interfere with the channel's inactivation process to produce resurgent currents. Here, we applied the classical type-II pyrethroid deltamethrin on human cardiac Nav1.5 expressed in HEK cells during whole-cell patch-clamp experiments. Surprisingly, we observed resurgent-like currents at very negative potentials in the absence of any open-channel blocker. Deltamethrin is predicted to bind with and stabilize domain II of the channel in the activated position to inhibit sodium channel deactivation. In contrast, activation of domain IV is associated with binding of the inactivation particle that terminates ion flow. Our experiments suggest that in deltamethrin-modified channels, resurgent-like currents are generated because the movement of domain IV and the resulting unbinding of the inactivation particle occur quicker than deactivation via movement of domain II. Notably, when activation of domain IV was inhibited by co-application of the sea anemone toxin ATx-II, these resurgent-like currents were eliminated although persistent currents were still observed. This indicates that the channel is permeable even if domain IV does not activate and generation of resurgent-like currents requires domain IV movement. By illuminating additional conducting sodium channel gating states and giving evidence for an alternative way of resurgent current induction in sodium channels, our findings shed new light on the complex sodium channel gating.

O-22 Membrane potential manipulation with visible flashlight illumination of targeted superparamagnetic beads

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The electrical membrane potential (V_m) is a crucial parameter determining the physiological properties of cells – in particular of excitable cells. Undoubtedly, optogenetics is a non-invasive and powerful method of V_m modulation, however, the method requires the genetic manipulation of cells. Therefore, a recently published approach to manipulate V_m by direct optical stimulation of gold nanoparticles (AuNPs, dia. 20 nm) targeted to unmodified cells received substantial attention [1]. However, NPs are not visible under a conventional microscope, and hence, their precise location and binding cannot be assessed. We therefore introduce a complementary method to manipulate V_m in a spatio-temporal fashion by visible flashlight stimulation (Xenon discharge lamp, 385-485 nm,~ 500 µs) of superparamagnetic beads (polystyrenecoated iron oxide-core beads, dia. 4.5, 2.8, 1 µm) targeted to unmodified cells. Flashlight stimulation of a single bead targeted to a cell resulted in the transient inward current sufficient to trigger action potential under whole-cell patch-clamp control. The waveform of the current measured upon flashlight stimulation of membrane-targeted bead reflected the time course of the local temperature excursion (first derivative) induced by the absorbed light and subsequent heat dissipation. Moreover, this apprach of local heating of a single cell using flashlight illumination of superparamagnetic bead(s) may serve as a precise and promising method of investigating preconceived ideas of the cellular thermodynamic phenomena in details.

[1]. Carvalho-de-Souza et al. "Photosensitivity of neurons enabled by cell-targeted gold nanoparticles." Neuron 86(1) (2015): 207-217.

O-23 TRPM2 channel sustains low intracellular sodium concentration in activated neutrophils

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Neutrophils are the most abundant immune cells in the body and stand for pivotal defense mechanisms. Their functions are intimately linked to ion fluxes and thus, depend on intra- and extracellular ion concentrations as well as on the respective transport proteins.
TRPM2 is a non-selective cation channel, permeable for both Ca^{2+} and Na^{+} and highly expressed in neutrophils. TRPM2 is gated by products of oxidative stress, which in neutrophils are robustly produced by activated NADPH oxidase. Despite this direct link to neutrophil function, the impact of TRPM2 activation on innate immune response still needs to be elucidated. In contrast to calcium, not much is known about sodium regulation in neutrophils. Yet, disruption of the intracellular sodium homeostasis affects not only Ca^{2+} and H^{+} export, but also membrane potential and volume regulation. Thereby, the intracellular Na^{+} homeostasis is crucial for neutrophils.
Using murine WT and TRPM2$^{-/-}$ bone marrow-derived neutrophils we analyzed changes in intracellular sodium concentration ($[Na^{+}]_i$) upon neutrophil activation and consequences of the TRPM2 knock-out on neutrophil chemotaxis and ROS production.
In TRPM2$^{-/-}$ neutrophils ionic homeostasis is disrupted. Lack of the channel presumably attenuates membrane depolarization upon activation and allows for uncontrolled Na^{+} influx. High intracellular sodium concentration in activated TRPM2$^{-/-}$ neutrophils affects NCX1.3 transport, triggering NCX1.3 reverse mode.
Despite altered $[Na^{+}]_i$, chemotaxis of TRPM2$^{-/-}$ neutrophils in 3D matrix is not significantly different when compared to WT neutrophils and probed at pH 7.4. Basal ROS production and its increase upon PMA stimulation is similar in both genotypes. However, stimulated siTRPM2-HL-60 cells have diminished ROS production. This indicates that TRPM2 can differentially regulate ROS production, depending on cell type.
High $[Na^{+}]_i$ in TRPM2$^{-/-}$ neutrophils can be detrimental in inflamed, acidic environment. Therefore, our ongoing studies focus on the involvement of TRPM2 channel and other Na^{+} transport proteins in sensing chemoattractant in low pH.

O-24 Potassium channel-independent action potential amplitude in small nerve terminals revealed by high-resolution current clamp recordings

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The amplitude and duration of presynaptic action potentials are central parameters determining the strength of synaptic transmission. In general, the amplitude of presynaptic action potentials is determined by the sodium channel availability and the duration by the potassium channel availability. Recently, potassium channels were shown to substantially decrease and dynamically control action potential amplitudes in small nerve terminals[4]. This conflicts particularly with results from large nerveterminals suggesting that small nerve terminals operate differently. Therefore, we established high-resolution current-clamp recordings with quartz glass pipettes from small nerve terminals of cultured cortical neurons. Using electrical circuits we systematically investigated errors related to pipette capacitance and amplifier performance. We found large action potentials with ~120 mV amplitude. Pharmacologically blocking potassium channels prolonged action potentials but did not affect their amplitude. Thus, our data indicate canonical potassium channel-independent action potentials at small nerve terminals.

O-25 Knockdown of HCN channels in mouse hippocampal neurons by virus delivered gene-interfering tools

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Pacemaker ion channels, also known as hyperpolarization- and cyclic nucleotide-gated (HCN) ion channels, are frequently expressed in various neuronal tissues. On the cellular level they contribute to the regulation of the resting membrane potential, integration of synaptic input at the dendrites, regulation of presynaptic neurotransmitter release, as well as generation of rhythmic activity. Thus HCN-channel dysfunction or altered gene expression levels are considered to be involved in several pathological conditions including epilepsy, neuropathic pain, or an age-related decline in the working memory.

To investigate consequences of HCN-channel dysfunctions in single neurons and neuronal networks, we interfered with HCN-channel expression levels. Therefore, we specifically targeted the different channel isoforms using two independent gene-interfering techniques. First, we took advantage of a cell-autonomous RNA-interference process. It mediates the breakdown of target mRNA by the application of short-hairpin RNAs. As a second approach we used an enzymatically inactive Cas9 variant. This protein binds specifically to transcriptional start regions of *hcn* genes, thereby directly interfering with the gene transcription. Both techniques were delivered to hippocampal neurons in a primary cell culture system and in an organotypic slice culture system by recombinant adeno-associated viruses (AAVs). We monitored the specificity and efficacy of *hcn* gene knockdown by immunological, and quantitative PCR assays. By electrophysiological recordings of virus transduced neurons, we validated changes of HCN-current responses and neuronal activity. The knowledge achieved by these experiments provides further insight in HCN-channel functions, in particular their contribution to the activity of neuronal networks.

O-26 Efficient RNA transfer by fusogenic liposomes into induced-pluripotent stem cell-derived neurons for the study of a Huntington's Disease in vitro model

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Huntington's disease (HD) is a devastating neurodegenerative disorder caused by a mutation of the huntingtin gene, which leads to a degeneration of medium spiny neurons (MSNs) in the striatum.

We used induced-pluripotent stem cells (iPS cells) from healthy controls and HD patients, which we differentiated into MSNs. Electrophysiological recordings show only minor effects of the HD mutation in our iPS cell-derived MSNs, suggesting that the cells either display an early non-pathological developmental stage, or that they miss some HD pathological features.

In this study, we aim to optimize the iPS cell-derived MSNs to better recapitulate HD-induced molecular changes. Previous studies have shown a striatal down-regulation of the gene encoding the voltage-gated sodium channel b4 subunit (SCN4B). b subunits are auxiliary components modulating the electrophysiological function of voltage-gated sodium channels (Navs), which are responsible for the generation of action potentials.

To investigate the potential pathological consequences of a b4 subunit down-regulation, we aim to efficiently knock-down b4 in our iPS cell-derived MSNs using short-hairpin RNA (siRNA). The transfection techniques developed so far are unfortunately not very effective in neurons. Nucleic acid transfer efficiency is low and the long incubation times of lipofection systems dramatically impair cell function and morphology. Therefore, we aim to use fusogenic liposomes that transfer directly and free nucleic acid into the cell cytoplasm. The direct RNA incorporation circumvents endosomal uptake-based challenges since repetitive fusion events are neither affecting cell viability nor morphology or specific functions of cells and are greatly effective.

We show that the iPS cell-derived MSNs successfully and quickly integrate eGFP mRNA. In addition, the electrophysiological functions of neurons treated with fusogenic liposomes are not impaired.

We will present during the symposium current-clamp data of different iPSC-derived MSNs as well as the first encouraging results of the new fusion's technique to knock-down b4 and more closely mimic HD conditions.

P-01 In-vitro assessment of force-frequency relationship and catecholamine response in failing human ventricular myocardium

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Background: The loss of transverse tubules (t-tubules) as part of the phenotypic change in failing human cardiomyocytes (CMs) is currently gaining increasing recognition. It goes hand in hand with a decreased contractile force and defective excitation-contraction coupling. The discovery of mechanisms that drive the recovery of failing hearts holds enormous therapeutic potential. However, the cultivation and functional testing of human heart tissue has so far posed an insurmountable challenge in heart research, due to the short-lived nature and instability of human CMs in vitro. Here, we demonstrate an approach to functionally assess human failing myocardium and correlate function to the degree of t-tubule remodeling.

Methods and Results: Human heart tissue obtained from heart surgeries and transplants was cut on a vibratome and cultured in a biomimetic setup, allowing for stable long-term cultivation under continuous stimulation and simultaneous recording of contractile force. Automated signal processing was applied to handle large data sets of contractile force and to analyze functional responses to treatments without bias. Force-frequency relationship (FFR) was determined by series of different stimulation frequencies, using a programmed protocol. Furthermore, slices were treated with isoproterenol and corticosteroids to investigate catecholamine-response and potential recovery pathways. While most samples showed a negative FFR, both isoproterenol and dexamethasone had positive effects on recovery of contractile force. After 1-3 weeks in culture, slices were fixed and imaged by confocal microscopy. Computational image processing was then applied to examine and quantify changes in tissue structure and t-tubules and to correlate them to the functional data.

Conclusion: We demonstrate a workflow for functional assessment of human myocardium in vitro. We suggest that our approach will help to shine new light on structure-function relationship in human failing hearts and to discover mechanism of pathological remodeling and recovery.

P-02 Aldosterone infusion induces renal damage and activation of Nrf2 a key regulator of the antioxidant response in kidney cells of mice

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Epidemiological studies described an increased risk of kidney cancer for hypertensive patients. Many of these patients have increased levels of the blood pressure regulating mineralocorticoid aldosterone (Ald). We recently showed that Ald induces oxidative stress and DNA-damage in kidney cells in vivo and in vitro. As a protection against the induced damage, kidney cells are capable to upregulate key regulators of the antioxidant defence, such as nuclear factor-erythoid-2-related factor 2 (Nrf2). The aim of the present study is to investigate the aldosterone-induced DNA-damage and Nrf2 activation in the kidney cells of mice.

Male C57BL/6-mice received three different concentrations of Ald (75, 125 and 250 µg/kg x d) combined with 1% NaCl in the drinking water. After 4 weeks the kidneys were isolated for further analysis.

Ald-exposed mice did show a changed blood pressure compared to the control. Kidney and heart weights were significantly higher in Ald-treated mice. The kidney function of the Ald-treated mice was impaired, as detected via an increase in the albumin/creatinine ratio, as well as fibrosis and atrophy in cortical kidney sections of all treatment groups compared to the control animals. A decrease in the creatinine clearance could not be shown. Additionally, kidneys of Ald-infused mice exhibited more DNA damage, visualized by γ-H2AX staining on histological sections. Moreover, an increased Nrf2 activation could be found in the kidney cells of all Ald-treated mice via an antibody specific for the phosphorylated and thus activated Nrf2 protein on histological sections.

Mice, like rats show increased kidney injury and DNA-damage after Ald treatment. The visualized Nrf2 activation in the kidney cells proves the upregulation of Nrf2 due to kidney injury initiated by the Ald infusion.

P-03 Molecular physiology of muscle type ionotropic glutamate receptors in Drosophila melanogaster

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The glutamatergic neuromuscular junction (NMJ) of Drosophila Melanogaster is a well characterized model synapse to investigate synaptic transmission, development and plasticity. In contrast to vertebrates, the Drosophila NMJ uses glutamate as excitatory neurotransmitter. The principal structure of ionotropic glutamate-receptors is conserved from prokaryotes (GluR0, Chen et al., 1999) to Homo Sapiens (NMDA, AMPA, Kainate receptors). It comprises a big extracellular amino-terminal domain (ATD), three membrane spanning helices (TM1, TM3, TM4), a p-loop (TM2) and an intracellular carboxy-terminal domain (CTD). In Drosophila, four subunits assemble to one muscle receptor where GluRIIC, GluRIID, GlurIIE and either GluRIIA or GluRIIB are essential (Qin et al., 2005; Peterson et al., 1997). Presence of either GluRIIA or GluRIIB gives rise to functionally distinct receptors, GluRIIB showing faster desensitization kinetics (DiAntonio et al., 1999). Furthermore, removal of GluRIIA leads to the induction of presynaptic homeostatic potentiation (PHP, Frank et al., 2006; Davis and Müller, 2015). Using CRISPR/Cas9 technology we started creating genomic editing platforms for each muscle receptor subunit, enabling us to modify these genes by inserting tags, point mutations and chimeric receptor constructs. This approach allows us to nail the region responsible for the induction of PHP down to a few amino acids. For characterization we will apply localization microscopy. In addition, we will study candidate regions of the Drosophila receptors by heterologous expression in HEK cells and using fast application electrophysiology (Heckmann et al., 1996). To investigate chimeric homomers the mammal kainate receptor GluK2 will be used as an expression backbone and certain domains will be replaced by Drosophila sequences. Finally, bioinformatics like structure prediction with Phyre2 will be helpful.

P-04 Investigating the molecular interaction between ASIC1a and Big Dynorphin

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In a number of pathological diseases acid-sensing ion channel 1a (ASIC1a) activity promotes neurotoxicity during prolonged acidosis. The opioid neuropeptide Big Dynorphin (BD) shows an over-lapping expression pattern with ASIC1a in the nervous system and has also been implicated in neuronal damage.

Recent work demonstrated that BD shifts ASIC1a steady-state desensitization to more acidic pH values, thus enhancing ASIC1a activity and acidosis-induced neuronal death. We set out to investigate the molecular mechanism of the ASIC1a-Big Dynorphin interaction by identifying the binding site of the ligand on the receptor.

Here we performed extensive mutagenesis of 11 amino acids, that are located in the acidic pocket of ASIC1a and made electrophysiological recordings with the two electrode voltage clamp method (TEVC) on Xenopus laevis oocytes. It was suggested that the peptide binds directly to the acidic pocket of ASIC1a because the spider toxin PcTx1 prevents the effect of BD and binds to the acidic pocket.

We confirmed that BD reduces steady-state desensitization at pH 7.15 with an EC50 of 3.5µM. It was striking that all glutamate residues, that were mutated showed a slightly decrease of the BD effect, but only one of them, mutant E235R, residing just in the center of the acidic pocket, revealed a significant loss-of-function of the peptide. Interestingly, five mutants enhanced the effect of BD. It gives us a hint that glutamate residues, located in the acidic pocket are crucial for BD modulation of the channel.

Further experiments will provide insight whether the apparent loss-of-function mutation lowered the affinity of ASIC1a for BD and hence may be part of the binding site, and how the gain-of-function of mutations can be explained.

P-05 The potential of inhibition of acid-sensing ion channels (ASICs) to impact tumor growth in glioblastoma

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Glioblastoma multiforme (GBM) is the most malignant form of brain cancer with a median survival of 15 months, despite of multi-modal therapy including surgery, radiotherapy and chemotherapy. Because of its fast proliferation and high metabolic rate, GBM is often observed thriving in an acidic microenvironment. A better understanding of the physiology of GBM stem cell lines and their signaling cascades under acidic conditions could reveal possible drug targets and is therefore essential.

Our group recently characterized functional acid sensing ion channels ASIC1 and ASIC3 in two glioblastoma stem cell lines and published microarray data that revealed improved survival of patients with higher ASIC1/3 mRNA expression. Acid sensing ion channels (ASICs) are neuronal proton-gated, voltage-insensitive sodium channels of the Deg/ENaC superfamily, found in both the central and the peripheral nervous system. They were shown to be neuroprotective when inhibited in mouse models of focal ischemia. In 2015 it was published that ASICs may induce acidotoxicity independent of their ion conduction but via conformational changes that lead to recruiting of serine/threonine receptor kinase interacting protein 1 (RIP1) and to consecutive necroptosis, a form of programmed cell death. Thus, ASICs could provide GBM cells with the ability to detect the pH of their surrounding environment and may potentially impact tumor growth.

In our current project, we characterize the role of ASICs in the two glioblastoma stem cell lines R54 and R8 (CD133+ and CD133-, respectively). We hypothesize that activated ASICs are at least partially responsible for the decreased viability under acidic pH and that their activation induces necroptosis. First results in a clonogenic assay show that acidic pH reduces sphere growth, which can be partially restored by the necroptosis inhibitor Nec-1. We are currently examining whether the same effect can be achieved by the ASIC1-specific toxin inhibitor Psalmotoxin or siRNA targeting ASICs.

P-06 Interactome of the Cavβ2-subunit of L-type voltage-gated calcium channels in cardiomyocytes

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L-type voltage-gated calcium channels (LTCCs) are heteromultimeric membrane proteins which allow Ca^{2+} entry upon plasma membrane depolarization. The b-subunit of voltage-dependent calcium channels (Cavβ) interacts with the alpha interaction domain in the pore-forming α1 subunit and regulates the trafficking and biophysical properties of these channels. Of the four Cavβ isoforms, Cavb2 is predominantly expressed in cardiomyocytes. This subunit is known to associate with diverse proteins besides LTCCs, but the molecular composition of Cavb2 nano-environments in the heart is yet unresolved. Here, we used two different proteomic strategies to identify Cavb2 interacting proteins. The first strategy was pulldown assays coupled with high-resolution quantitative mass spectrometry (MS). Whole mouse heart lysates and recombinant Cavb2 were incubated together with streptavidin beads and the eluates analyzed by MS. In parallel, we used a protein-labeling technique in living cells based on an engineered ascorbate peroxidase (APEX). In this strategy, Cavb2 was fused to APEX and expressed in adult rat cardiomyocytes using an adenovirus system. APEX oxidizes biotin-phenol to phenoxyl radicals, which covalently react with electron-rich amino acids (Tyr, Trp, His, Cys) in the proteins. Thus, proteins in a radius under 20 nm are covalently labelled with biotin-phenol, purified using streptavidin beads and identified by MS. Unlike the pulldown assay, APEX-labeling has the main advantage that labeling is performed in living cells, preserving their architectural integrity and allowing for pulldown-associated false positives to be discarded. Analysis of pulldowns by MS revealed 126 proteins associated with Cavb2. Using the in situ APEX-based biotin labeling we confirmed that 13 proteins are located in the nano-enviroments of Cavb2, with a high specificity and consistency in all the replicates. Candidates include translational factors, Z-disc components and proteins involved in cardiac contraction in cardiomyocytes. Currently, we are elucidating the functional role of some of these molecular interactions.

P-07 Knockdown of HCN channels in mouse hippocampal neurons by virus delivered gene-interfering tools

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Pacemaker ion channels, also known as hyperpolarization- and cyclic nucleotide-gated (HCN) ion channels, are frequently expressed in various neuronal tissues. On the cellular level they contribute to the regulation of the resting membrane potential, integration of synaptic input at the dendrites, regulation of presynaptic neurotransmitter release, as well as generation of rhythmic activity. Thus HCN-channel dysfunction or altered gene expression levels are considered to be involved in several pathological conditions including epilepsy, neuropathic pain, or an age-related decline in the working memory.

To investigate consequences of HCN-channel dysfunctions in single neurons and neuronal networks, we interfered with HCN-channel expression levels. Therefore, we specifically targeted the different channel isoforms using two independent gene-interfering techniques. First, we took advantage of a cell-autonomous RNA-interference process. It mediates the breakdown of target mRNA by the application of short-hairpin RNAs. As a second approach we used an enzymatically inactive Cas9 variant. This protein binds specifically to transcriptional start regions of hcn genes, thereby directly interfering with the gene transcription. Both techniques were delivered to hippocampal neurons in a primary cell culture system and in an organotypic slice culture system by recombinant adeno-associated viruses (AAVs). We monitored the specificity and efficacy of hcn gene knockdown by immunological, and quantitative PCR assays. By electrophysiological recordings of virus transduced neurons, we validated changes of HCN-current responses and neuronal activity. The knowledge achieved by these experiments provides further insight in HCN-channel functions, in particular their contribution to the activity of neuronal networks.

P-08 Establishing the cardiotonic steroid ouabain as a potent anti-cancer drug against biliary tract cancer – Step one

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Background: Biliary tract cancer (BTC) is a malignancy of epithelial bile duct cells with dismal prognosis. Hence, identification of therapeutically relevant substances and description of their cellular actions is of utmost important.

The Na^+/K^+-ATPase (NKA) is a primary active ion transporter of all eukaryotic cells establishing the intra- and extracellular ionic distribution. Hence, NKA is involved in virtually all physiological processes.

Cardiotonic steroids (CTS) are NKA inhibitors and some of them are used for treatment of heart insufficiency. CTS bind to the α-subunit and inhibit NKA at saturating (millimolar; mM) concentrations, resulting in increased intracellular calcium concentrations (ionic pathway).

However, recent publications reported anti-tumor effects of ouabain in subsaturating (nanomolar; nM) concentration, which are not explainable by oubain-caused inhibition of the "ionic pathway". These effects uncover a novel receptor function and identify CTS, as endogenous ligands which cause a conformational change of the NKA resulting in its interaction with other signaling receptors and activation of intracellular pathways regulating cell proliferation and apoptosis ("signaling pathway").

Aim and Methods: Therefore we aim to

- evaluate a dose-dependent and time-dependent cytotoxic effect of ouabain by performing classical resazurin cytotoxicity assays (on different BTC cell lines n=10)
- clarify cell-death-related events via FACS cell cycle analysis and caspase acitivity assay.
- measure the expression of all 14 NKA subunits in different BTC cell lines to unravel possible cell-line specific composition and try to correlate them with cytotoxicity data
- characterize subsaturating concentrations of ouabain electrophysiologically by measuring cell membrane potential and rheogenic outward currents

Results and foresight: We found that Ouabain has a cell line-dependent cytotoxic effect at nanomolar concentrations in all tested cell lines (n=10). Moreover, RT-PCR-based NKA subunit expression analysis of all 10 cell lines revealed cell type specific expression of different NKA subunits, with some subunits (e.g. atp1a1, atp1a3) being expressed in all tested cell lines, and others only expressed in some cell lines. Whether cell type specific expression is correlated with other physiological processes will be further investigated. However, correlations between NKA subunit composition and cytotoxicity could not be found.

P-09 Detailed Comparison of H_2O_2 Production of Human PMN and HL-60 derived Cell Lines

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The interplay between the voltage-gated proton channel and the activity of the NADPH oxidase is still unsolved. Several attempts have been made utilizing numerous inhibitors, blockers, ionophores, and activators to elucidate the interconnection. We plan to decipher this question by genetic manipulation of voltage-gated proton channels in PMN. The oxidative burst of human PMN and human cell lines are compared to identify the most suitable cell line representing PMN for further genetic manipulation. Here, we are using a fluorescence-based assay to measure H_2O_2 production from human PMN and four cell lines representing human PMN. Activation kinetic and maximal production are compared as well as inhibition due to zinc. NADPH oxidase is not affected by zinc concentrations used in the assay. However, voltage-gated proton channels are inhibited by [Zn^{2+}] applied. Our results strongly suggest that voltage gated-proton channels modulate reactive oxygen species (ROS) production. Furthermore, effects of zinc on human PMN and cell lines are identical, supporting a proposed general mechanism of oxidative burst in human phagocytes.

P-10 Effect of glycine on induced stress in BV-2 microglia cells

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Microglia are antigen-presenting immune cells in the central nervous system and act as first line of defense in the brain. Activated microglia release pro-inflammatory cytokines and can trigger an oxidative burst against pathogens. In many neurodegenerative diseases inflammation processes of microglia seem to play an important role. The aim of this study was to investigate if the amino acid glycine is able to modulate the inflammatory response of BV-2 microglia cells. Inflammatory cell stress was induced by lipopolysaccharide (LPS) and interferon-γ (IFN-γ) incubation for 24 hours in the absence or presence of 1 or 5 mM glycine. Cells were analyzed by flow cytometry technique (FACS) for cell volume and side scatter, Annexin-V and 7-AAD staining for detection of apoptosis and necrosis and surface markers were analyzed to characterize the cell activation status. Treatment with LPS/IFN-γ induced apoptosis. In the presence of 5 mM Gly the fraction of living cells was significantly increased and early-apoptotic cells were decreased in comparison to LPS/IFN-γ control. Surface markers of CD54 and CD11b showed a significant glycine dose dependent increase under treatment with LPS/IFN-γ. Glycine is able to counteract the effects of LPS/IFN-γ and leads to increased cell viability and a decrease of apoptosis in BV-2 microglial cells.

P-11 Evaluation of the efficiency of Sargassum Virgatum in infertility treatment induced by gamma rays in male rats

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Gamma radiation is a widely used and highly effective radio therapeutic agent. One of the limiting side effects of Gamma radiation is infertility where testicular failure is highly manifested. In the present work, the regenerative potential of Comparison treatment comprising water extract of Sargassum virgatum algae in two concentrations (100 and 400 mg/kg (bw) or ethanol extract of Sargassum virgatum algae in two concentrations (100 and 400 mg/kg (bw) in testicular failure induced in rats by a single dose of 3.5 gray of gamma radiation was investigated. Infertility associated biomarkers, serum hormones, Caspase-3 in testes, Antioxidant enzymes, Semen Characteristic Analysis, in addition, to histopathological examination, were performed for control group and after gamma radiation dose to confirm testicular failure induction, and also after receiving the proposed treatments of water & Ethanol extracts of Sargassum virgatum.

P-12 Structural and functional assessment of isolated cardiomyocytes from vibratome-cut and cultured human ventricular myocardium

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Background: Translational cardiac research requires verification of findings from cell or animal models in human cells and tissues, but this is commonly hampered by the low availability of human heart tissue and unsatisfactory in-vitro methods for culture. However, recent advances, using vibratome-cut slices of living myocardium, enable for extended culture of functional human myocardial tissue and continuous assessment of contractile force. To investigate electrophysiology and excitation-contraction coupling on the single cell level, subsequent isolation of cardiac myocytes is necessary from minimal amounts of tissue, i.e. <50 mg. Conventional low-yield, chunk isolation procedures from human myocardial samples commonly require large amounts of heart tissue, i.e. >1 g. Thus, for comprehensive structural and functional characterization of cultured and fresh human cardiomyocytes improved isolation protocols are required.

Methods: Human and animal cardiac myocytes were isolated from vibratome-cut tissue slices. We optimized the protocol with adult rat heart tissue and quantified yields of Ca^{2+}-tolerant myocytes by flow cytometry, comparing them to yields from Langendorff perfusion and conventional chunk isolation methods. Myocardial slices of 300µm thickness were generated with a high-precision vibratome and then subjected to two-step enzymatic digestion. This was followed by gentle tissue dissociation and reintroduction of Ca^{2+} (2mM). Subsequently, cells were fixed to analyze the rod-shaped, striated fraction of cardiomyocytes via flow cytometry, taking into account size and granularity. Viability of unfixed cells was verified by field stimulation and Ca^{2+} imaging.

Results: Isolation from rat vibratome slices resulted in 39.8±6% Ca^{2+}-tolerant cardiomyocytes, compared to 7.3±2.8% for the chunk isolation approach and 71±6.2% for Langendorff perfusion (n=3). Vibratome slices of 20-40mg wet weight yielded 1827 to 2817 rod shaped, Ca^{2+}-tolerant cardiomyocytes. Translating the protocol to samples of human myocardium from end-stage failing hearts, we obtained Ca^{2+}-tolerant cardiomyocytes from less than 50 mg of tissue. Assessment of structure and function of isolated human cardiomyocytes by confocal microscopy, Ca^{2+} imaging and Patch-Clamp experiments indicated functional excitation-contraction coupling.

Conclusion: Cardiomyocyte isolation from vibratome-cut tissue slices is superior to conventional chunk isolation, probably due to atraumatic slicing and higher surface/volume ratio.

P-13 Controlled Clinical Pilot Study Neurodermatitis and Effectiveness of Radon Speleotherapy

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Curative Radon (Rn) treatments for patients who suffer from inflammatory diseases of the musculoskeletal system or chronic disorders of the skin and respiratory system have a long tradition in Europe. Several clinical studies could demonstrate that Rn-therapy can cause a significant reduction of pain and enhancement of functionality as well as a positive shift in molecular blood parameters. Neurodermatitis is an inflammatory skin disease that is manly characterized by pruritus and eczema. A number of studies showed that Rn-therapy has a positive effect on various inflammatory diseases, the purpose of this study is, to evaluate if patients who suffer from neurodermatitis can evidently benefit from Rn-therapy.

The study recruitment was started in 2017. Until now 19 study participants passed a cure stay of two weeks in Bad Gastein. The intervention group receives eight Low-Dose Radon- and Hyperthermia Treatments (LDRnHT) in the Gastein Healing Gallery. The control group receives sauna therapies, with the same controlled humidity and temperature conditions. This comparison of Hyperthermia Treatment versus LDRnHT ensures that the effect of the nobel gas Radon can be evaluated. The aim of the study is to assess the modification of skin condition and molecular blood parameters, as well as quality of life after Rn-therapy compared to the situation before. Long term effects are documented in follow-up visits after three, six and nine months. At all timepoints blood samples and questionnaires will be collected as well as a physical examination by a dermatologist is scheduled. Before cure and three months after cure additional skin biopsy for histological analyses will be collected.

The recruitment of at total number of 32 patients should be completed by the end of 2019. It seems that neurodermatitis patients can benefit from LDRnHT, but of course outstanding parameters have to be considered to come to a final conclusion.

P-14 Differential presynaptic short-term and homeostatic plasticity of excitatory and inhibitory transmission

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Activity in neuronal circuits arises from the interplay of excitatory and inhibitory synaptic signaling, which is delicately balanced to maintain stable network activity[1]. There is evidence that excitatory and inhibitory synaptic transmission show diverging short-term plasticity[2]. However, which of the multiple factors governing synaptic transmission cause differential short-term plasticity is not fully understood. Additionally, long-term perturbation of network activity is known to homeostatically affect excitatorytransmission[3,4], while the impact on inhibitory transmission remains largely elusive. Here, we explore short-term plasticity by extracellular stimulation of excitatory and inhibitory inputs onto whole-cell patch-clamped neurons in cortical cell culture. We isolate presynaptic short-term plasticity by pharmacologically alleviating postsynaptic contributions. Excitatory and inhibitory synapses demonstrate fundamentally different short-term plasticity, with stronger synaptic depression due to higher vesicular release probability in inhibitory compared with excitatory synapses. Furthermore, 48 hours of activity deprivation by Tetrodotoxin inversely affects synaptic transmission, leading to strengthening of excitatory and weakening of inhibitory transmission mediated by several mechanisms including differential changes in release probability. In contrast to activity deprivation, Forskolin potentiates both excitatory and inhibitory synapses. Thus, our data reveal differential mechanisms of plasticity and biophysical adaptations stabilizing activity in neuronal networks.

P-15 Sensory parameters for the prediction of a migraine attack: a psychophysical study

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Aims: Migraine attacks are still unpredictable, which makes everyday life difficult for many patients. In a previous pilot study reduced habituation to a painful electrical stimulus was found to be altered in the preictal phase of migraine.

Methods: In this study we assessed the perception of nociceptive and non-nociceptive sensory stimuli in 20 healthy subjects on five consecutive days and in 20 migraine patients in different phases of the migraine cycle (Interictal: >24 hours before, preictal: <24 hours before, ictal, postictal). Electrical stimulation at the forehead and temple was controlled by the subjects via a mobile phone app. Depending on the individual threshold, a supra-threshold 10s long electrical stimulus was rated in the beginning, middle and end on a numerical pain rating scale. The size of the axon-reflex-erythema was determined using a Laser-Doppler-Imager. The intensity and hedonic estimates (pleasant-unpleasant) of various odours in "Sniffin Sticks" were recorded.

Results: The pain thresholds tend to decrease in patients until its minimum ictally, while in healthy subjects they tend to increase. The axon-reflex erythema was larger in migraineurs than in healthy subjects. Patients show preictally a reduced habituation to supra-threshold painful stimuli in comparison to ictal/postictal phases. Ictally the migraineurs rate the intensity of odours higher than in other phases and compared to healthy subjects more unpleasant.

Conclusion: Reduced habituation to a painful electrical stimulus is present preictally also with a system, that is easy to handle by patients. Odour perception was altered only ictally. The CGRP release or vessel reactivity seems to be increased in migraineurs generally in comparison to healthy. This makes habituation to an electrical stimulus so far the only parameter with predictive value of an attack. Mechanistically there may be specific changes of the habituation in the nociceptive system specifically in the preictal phase, but no general sensory hypersensitivity.

P-16 Role of volume-regulated chloride channels in biliary tract cancer cisplatin resistance

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Background: The term Biliary tract cancer (BTC) describes a rare group of malignancies of epithelial bile duct cells with dismal prognosis due to a lack of effective therapies. A major reason for poor treatment outcome is the development of resistance towards common therapies like the chemotherapeutic drug cisplatin which is part of the reference chemotherapeutic regimen in BTC treatment. The cytotoxicity of cisplatin is based on accumulation and activation of the substance in the cytoplasm of the target cell, followed by DNA-interaction and subsequent induction of apoptosis. Tumour cells counteract such cytotoxic effects by various mechanisms such as diminished cisplatin uptake and activation, enhanced cisplatin efflux or resistance to apoptosis. Recently, a specific subunit composition (LRRC8) of the volume-regulated anion channel (VRAC; a chloride (Cl^-)-exporting channel) has been shown to serve as a cisplatin uptake pathway. Moreover, it is known that Cl^- loss upon VRAC activation under normotonic conditions drives apoptotic volume decrease (AVD), which is prerequisite for successive steps of apoptosis. Intracellular activation of cisplatin is more likely in an environment with a low Cl^- concentration, meaning that VRAC activity may facilitate intracellular cisplatin activation. In a feed forward mode VRACs might thus foster cisplatin cytotoxicity and on the contrary loss of VRAC function might favour chemoresistance in tumour cells.

Aim and Methods: Using a comprehensive panel of BTC cell lines, we aim to identify a connection between cisplatin responsiveness, VRAC subunit composition, VRAC activity, as well as intracellular Cl^- concentrations in BTC. Moreover, we will determine the effect of VRAC-associated changes in the intracellular Cl^- concentration on the cytotoxicity of cisplatin in BTC cells.

Results and foresight: We were able to establish the cytotoxic effect of cisplatin on a panel of ten BTC cell lines in a cell line and dose- and time-dependent manner. Other than that, we tested the cytotoxic effect of the Cl^- channel inhibitor DCPIB on BTC cells. RT-PCR analysis of LRRC8 subunits (LRRC8A-E) revealed cell type specific differences regarding the expression levels. Additionally, we started to characterize VRAC currents of specific BTC cell lines (three cell lines with different levels of resistance) electrophysiologically by patch clamp recordings under stimulation with hypotonic solutions and subsequent current inhibition with DCPIB. A next step will be to assess a possible combinatory effect of cisplatin and DCPIB by performing resazurin assays with the three representative BTC cell lines.

P-17 Meningeal arterial vasoconstriction by potassium and capsaicin

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Introduction: High concentrations of potassium depolarize excitable membranes and lead to contraction of vascular smooth muscle fibers through the activation of voltage-gated calcium channels (1). In contrast, capsaicin elicits nociceptive afferents by activating calcium-conducting TRPV1 receptor channels (2). Less is known about TRPV1 receptors expressed by vascular smooth muscle cells, whose activation can trigger vasoconstriction (3). Using a new combination of calcium imaging and vasomotor monitoring, partly combined with immunohistochemistry, we investigated the effect of potassium and capsaicin on meningeal arteries of the murine dura mater encephali.

Methods: The dura mater encephali of C57BL/6 wild type and TRPV1 -/- knockout mice was dissected from the bone and fixed on the bottom of custom-made transparent dishes using mild negative pressure. After incubation with the calcium indicator Fluo8-AM, arterial vessels of the dura mater were observed with a fluorescence microscope. Solutions of 60 mM KCl and 0.3 µM capsaicin were applied for 30 s and the fluorescence signals were documented. Selected preparations were immunohistochemically stained for TRPV1 protein and evaluated with a confocal laser scanning microscope.

Results: In wild type mice, within a few seconds KCl and capsaicin superfusion elicited calcium signals in arterial vessels associated with vascular constriction, whereas in the TRPV1 -/- mice only KCl provoked this response. KCl often led to a migrating calcium signal and constriction wave, especially at arteriole origins. In contrast, capsaicin typically triggered point-like responses. The localization of calcium signals revealed only a slight overlap of the vascular sections activated by KCl or capsaicin. Immunofluorescence stains showed colocalization of the calcium signal with TRPV1 expression in wild-type mice indicating the presence of TRPV1 receptors on smooth muscle cells.

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P-18 Elucidating Zinc Binding to the Voltage-Gated Proton Channel hHv1 Using Computer Simulations

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H_V1 voltage-gated proton channels are proton-specific ion channels with unique properties. For example, they are massively expressed in human sperm where they are necessary for maturation and motility, hence essential for conception.

Voltage-gated proton channels are strongly inhibited by Zn^{2+}. Experimental studies revealed that histidine residues are essential for Zn^{2+} binding. However, the two accessible histidine residues H140 and H193 are too far apart to coordinate simultaneously one Zn^{2+} in a structural model of the monomeric channel. It was thus hypothesized that two Zn^{2+} binding sites can be formed between pairs of equivalent histidine residues (H140-H140, and H193-H193) at the interface of a H_V1 homodimer. The consecutive experimental measurements were also in agreement with this hypothesis.

We tested this hypothesis and investigated the determinants of Zn^{2+} binding at the molecular level using computational approaches: molecular modeling, molecular docking, and molecular dynamics simulations.

Our results support the hypothesis enunciated above: The modeling and docking simulations show that the hH_V1 channels can form homodimers that present an appropriate interface for two Zn^{2+} binding sites, each involving a pair of equivalent histidine residues from each monomer. The molecular dynamics simulations reveal that two Zn^{2+} can stably be accommodated in the proposed binding sites. The zinc ions are coordinated by the histidine and two acidic residues. Essentially, the glutamate residues E192 play an essential role in Zn^{2+} binding. Comparison with another possible dimer conformation and with the monomeric form of the channel also reveals why the dimer conformation hypothesized above is more able to coordinate zinc ions.

P-19 Evaluation of cobalamin-receptor expression in dogs with idiopathic inflammatory bowel disease

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Causes of serum cobalamin deficiency in dogs can vary greatly. Selective cobalamin malabsorption is a rare genetic disorder, whereas chronic gastrointestinal or pancreatic disease are the main reasons for dogs to develop cobalamin deficiency. Hypocobalaminemia is also known as a risk factor for a negative clinical outcome in dogs with chronic inflammatory enteropathy. With cobalamin acting as an essential cofactor for intracellular enzymes, cobalamin deficiency can lead to disorders in cobalamin metabolism and patients may not respond to the treatment initiated to address the underlying disease process unless being administered supplemental cobalamin. However, the pathophysiology of cobalamin deficiency has not been completely investigated in dogs with chronic enteropathies to date.

We hypothesize that intestinal mucosal inflammation is associated with changes in the expression of the ileal cobalamin-intrinsic factor (IF)-receptor, known as CUBAM-receptor, resulting in a decreased gastrointestinal uptake of cobalamin. Thus, our aim is to quantify the expression of the two cobalamin receptor subunits, CUBN and AMN, in ileal biopsies from healthy dogs and dogs with idiopathic inflammatory bowel disease (IBD).

We have collected intestinal mucosal biopsies from dogs, each of which was assigned to one of the following groups: groups 1-3 comprised of dogs (n=8 each) diagnosed with IBD which were further divided in (1) dogs with severe hypocobalaminemia (serum cobalamin concentration below the lower limit of the reference interval), (2) dogs with suboptimal cobalamin status (i.e., serum cobalamin concentration within the lower reference interval), and (3) dogs with normocobalaminemia (i.e., serum cobalamin concentration within the upper range of the reference interval). The 4th group included healthy control dogs. Formalin-fixed and paraffin embedded sections of these intestinal biopsies will be stained for the CUBN and AMN protein using commercially available polyclonal antibodies and immunofluorescent dyes, and the receptor expression will be quantified using laser scanning microscopy. Cobalamin expression will be compared among the different groups of dogs and among the different segments of the gastrointestinal tract (i.e., ileal mucosal expression compared to that in the stomach, duodenum, and colon), and will be evaluated for a potential correlation with the patients' serum cobalamin status.

P-20 Increased phosphorylation causes a gain-of-function in the chronic pain mutation $Na_v1.7$/I848T

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Mutations in voltage-gated sodium channels (Na_vs) are known to cause a variety of chronic pain syndromes, such as inherited erythromelalgia (IEM). In our study we focus on the gain-of-function mutation $Na_v1.7$ p.I848T that causes IEM. Patients carrying this mutation suffer from sudden pain attacks and synchronous erythema of the distal extremities, often triggered by exercise or exposure to higher temperatures.

Earlier studies have shown that the I848T mutation causes a hyperpolarizing shift of activation of $Na_v1.7$. Since the mutation to threonine potentially creates a novel phosphorylation site, we investigated if the observed shift of activation can be explained by increased phosphorylation. We performed whole-cell patch-clamp electrophysiology on HEK293T cells, transiently transfected with $Na_v1.7$ wildtype or its mutant form. To test the role of phosphorylation in the gain-of-function of the I848T mutation, we applied the nonspecific kinase inhibitor, staurosporine. The results showed a significant reduction of the hyperpolarizing shift of activation in I848T-transfected cells. This suggests that increased phosphorylation is at least partially responsible for the observed gain-of function of the channel. Additionally, we performed phosphomimetics and replaced threonine by glutamate (I848E), which mimics a phosphorylated amino acid. This mutation should therefore have a similar effect as the I848T mutant. The results of the I848E mutation underline our previous finding by showing a hyperpolarizing shift in activation compared to the Nav1.7 wildtype.

So far, we have shown that phosphorylation changes the activation of the $Na_v1.7$/I848T channel, potentially explaining the increased pain sensation in the patients. In further experiments we will determine the involved protein kinase by using specific inhibitors (H-89, calphostin C) and activators (forskolin, PMA) of Protein Kinases A and C. These results might open new pharmacological options in the treatment of patients with erythromelalgia.

P-21 Effect of music on pain perception: a study in healthy individuals by experimentally induced pain

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Music has been used for pain treatment from ancient time. It is found that listening to music resulted in significant activation of a network of subcortical structures including the nucleus accumben, ventral tegmental area and hypothalamus. There are many evidences to show that music can relieve pain. They suggest that pain has number of effect at physiological level. Though pain is subjective response, for experimental basis, both subjective and physiological measurements become necessary for precise results. We measured pulse rate, respiratory rate, galvanic skin response and pain intensity providing experimentally induced pain by immersing hand in cold water. This study was conducted to see if music can be used as an adjunct to analgesia. A total of 30 under graduate students under went cold pressor test. Pulse rate, respiratory rate, galvanic skin response and pain intensity on visual analogue scale (VAS) were compared without music and with music. There was significant decrease in Pulse rate (Threshold p=0.000), (Tolerance p=0.000), Respiratory rate (Threshold p=0.001), (Tolerance p=0.003), Galvanic skin response (Threshold p=0.029), (Tolerance p=0.005) and Pain intensity (p=0.000) with music in comparison to without music under cold pressor pain. Threshold time and tolerance time were significantly increased while listening to music.($p<0.05$).

It can be concluded that music can reduce pain and it can be used as an adjunct to analgesia.

P-22 Simultaneous optical recording of rapid cellular signaling events

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Human sperm gather chemical cues to navigate across the female reproductive tract. Chemosensory signalling in human sperm involves changes in the membrane potential (V_m) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) that are controlled by the sperm-specific ion channels Slo3 and CatSper, respectively. The channels are gated in a non-genomic fashion by the female steroid progesterone. We established a novel fluorimetric multiplexing approach to record rapid V_m and $[Ca^{2+}]_i$ responses simultaneously. This technique revealed that the rapid, non-genomic progesterone-signalling pathway in human sperm involves an interplay of $[Ca^{2+}]_i$ and V_m, i.e. the Ca^{2+} influx via CatSper triggers a Ca^{2+}-induced hyperpolarization that decreases the open probability of CatSper, thereby curtailing further Ca^{2+} influx. The Ca^{2+}-induced hyperpolarization might be mediated by the activation of Slo3 by $[Ca^{2+}]_i$.

Furthermore, using chemosenory signalling in sea urchin sperm as a model, we demonstrate that our unique multiplexing technique is amenable to various combinations of fluorescent indicators and can monitor up to three signalling modalities simultaneously. Finally, we show that the technique is also applicable to live single-cell microscopy. Taken together, we introduce and demonstrate the application of a powerful optical multiplexing appraoch to delineate signalling events and interrogate signalling interplay.

P-23 Crosstalk between the acid-sensitive outwardly rectifying (ASOR) and the volume-sensitive outwardly rectifying (VSOR) anion channel in microglial cells

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Objective: The process operating in living organisms to preserve a viable acid-base balance is a vital homeostatic function shared in nearly all tissues. Mechanisms regulating pH are especially important in the brain because predominant high electrical activity elicits rapid H^+ movements to acidify or alkalize its compartments. In case of ischemic brain damage or neuronal damages, pH regulating mechanism fail to maintain a constant pH and consequently, acidosis occurs. Acidification has been shown to influence the electrical behaviour of numerous cell types, including microglial cells in the brain.

Methods: Cl^- currents and cell membrane potentials (V_{mem}) were measured in BV-2 cells using whole-cell patch clamp. The mean cell volume (MCV) was assessed using a Z2 Coulter counter.

Results: Lowering the extracellular pH to 4.5, quickly induces an outwardly rectifying current in BV-2 microglial cells, displaying high amplitudes and time-dependent activation at positive potentials and smaller values and an initial peak at negative potentials. This current can be inhibited by Cl^- channel blockers like DCPIB, NPPB and DIDS. Moreover, the application of an external acidic solution depolarizes V_{mem} and increases the MCV. Regarding the relation between ASOR and VSOR current, we could show several similarities, including pharmacological profiles, the ion permeability sequence and outward current rectification. Current activation of the ASOR is, however, clearly different from VSOR. Concurrent exposure to hypotonicity and acidic conditions shows a short time frame in which both currents apparently coexist, but in a long run, the current switches to a pure ASOR phenotype, indicating a predominance of ASOR over VSOR.

Conclusions: We show that in microglial cells extracellular acidification elicits an outwardly rectifying Cl^- current (ASOR) with several similarities and also differences to the volume-sensitive Cl^- current (VSOR), but if both currents are mediated by the same or different channel entities, needs to be further investigated.

P-24 Functional analysis of Swiprosin-1, a putative Ca^{2+}-dependent actin filament bundler in immune cell migration

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The actin cytoskeleton is crucial for cell morphology and cell migration. We established Drosophila macrophages as an excellent model system for studying conserved gene functions regulating cell shape, directional cell migration, phagocytosis and wound response in a physiological environment.

Here, we use Drosophila macrophages to dissect the function of Swiprosin-1 (Swip-1), a highly conserved actin binding protein in immune cell migration and immune cell response. Drosophila Swip-1 shares high homology (63% identity) to Swiprosin-1/EF-hand domain containing 2 (EFhd2), one of two similar putative Ca^{2+}-dependent actin filament bundler in humans. EFhd2 was first identified in human B-lymphocytes, but it has also been linked to Alzheimer's disease and other neurological disorders.

Like their human counterparts, Swip-1 is highly expressed in Drosophila macrophages. High-resolution structured illumination microscopy (SIM) reveals that endogenous Swip-1 localizes to the lamellipodium of polarized isolated Drosophila macrophages. Live cell spinning disk imaging of an EGFP-tagged protein further confirmed lamellipodial localization of Swip-1 suggesting a possible role in regulating Ca^{2+}-dependent cellular protrusion dynamics. Importantly, macrophage-specific RNAi-mediated knock-down efficiently reduces its expression. Thus, we want to employ cell type-specific RNAi knock-down, CRISPR/Cas9 deletion mutants and tissue specific overexpression of Swip-1 using the Gal4/UAS-system to dissect the conserved role of Swip-1. Additional biochemical experiments (e.g. gel filtration and co-sedimentation analysis) will further help us to better understand the regulation of this putative Ca^{2+}-dependent actin filament bundler in cellular dynamics.

P-25 Spatial contrast theory of itch: a psychophysical study

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Chronic itch is a severe sensory experience prevalent in patients with skin diseases, systemic and neuropathic conditions affecting quality of life. The mechanism of non-histaminergic itching is still not fully understood. Therefore there's no mechanistically based treatment available. Different theories of non-histaminergic itch exist: 1. The „labelled-line-theory" describes a specific way using itching-specific receptors and neurons (i.e.Histamine) 2. An unspecific pathway is described by the „spatial-contrast-theory-of-itch ". This theory postulates that itching is based upon the contrast of signal input to the CNS of individual nociceptors, which fire fiercely, and their neighbours innervating the same skin area that are silent. Pain arises if a similar signal magnitude is transmitted from all nerve fibers innervating the same skin area.

In this project either the skin of healthy human volunteers was soaked with a pruritic/algogenic substance via intracutaneous microinjection or applied focally via little spicules. The magnitude of itch and pain were rated on a numerical rating scale by 15 healthy volunteers. The used substances are well characterized on cellular level: β-Alanin (MrgprD), BAM8-22 (MrgprX1), Cowhage/Cowhage-extract (PAR2?), Methylglyoxal (TRPA1, Nav 1.8) and SIF (syntheticinterstitial-fluid) as control.

Our results show, that there is more pain than itch if the substances have been applied using intradermal injection. Focally applied substances provoke a higher rating of itch than pain. Solely β-Alanin caused predominantly itch irrespective of the application mode, because β-Alanin activates the MrgprD-receptor which is expressed only in a small subset of primary afferents.

In conclusion the results of this project support the „spatial-contrast-theory-of-itch", because the focal application causes more itch than pain in contrast to the injection causing more pain. In some cases in which a receptor is expressed only in a small subset of primary afferents, the spatial contrast is reached not by focal application, but by sparse innervation density of these specialised neurons.

P-26 A dysregulation of the prolactin/vasoinhibin axis appears to contribute to preeclampsia

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Preeclampsia is a hypertensive disorder of pregnancy affecting 3-5 % of all pregnancies. There is no curative treatment and the pathomechanisms of preeclampsia are poorly understood. Studies demonstrated altered levels of antiangiogenic factors in patients with preeclampsia. One such factor is the antiangiogenic and antivasodilatatory peptide hormone vasoinhibin, which is higher in the circulation, urine and amniotic fluid of women with preeclampsia. Normal pregnancy is characterized by elevated circulating prolactin and placental lactogen levels, both of which can serve as vasoinhibin precursors when they are enzymatically cleaved. A dysregulation in vasoinhibin generation during preeclampsia is indicated by higher vasoinhibin levels detected in patients, the elevation of prolactin and placental lactogen above the levels of normal pregnancy, and by high levels and activity of vasoinhibin-generating enzymes. Various molecular effects of vasoinhibin correlate with mechanisms of possible relevance in preeclampsia: the alteration of PAI-1 levels, the regulation of eNOS, the regulation of the growth factors VEGF and bFGF needed for proper implantation and an adequate fetal growth rate, as well as the induction of endothelial cell apoptosis via caspases and the Bcl-2 family proteins. Clinical consequences of an alteration of these mechanisms may be high blood pressure, a reduced fetal growth rate, and a limited success of implantation, all of which occur in preeclampsia. The present theory integrates molecular effects of vasoinhibin, reported measurements of vasoinhibin in biological samples from patients, and clinical characteristics of preeclampsia showing that a dysregulation of vasoinhibin generation may be causally linked to the development of preeclampsia. Consolidation of this theory will demonstrate whether the assessment of vasoinhibin levels, its precursors and regulation can contribute to estimate the risk of preeclampsia, or to improve its treatment.

P-27 Sugars heavily impact on Reactive Oxygen Species production during the Respiratory Burst of phagocytes

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Reactive oxygen species (ROS) are produced by the action of the NADPH oxidase complex during the respiratory burst of phagocytes. This has many physiological implications in human health. However, ROS may not be always beneficial for health. For example, their release can increase tissue damage in patients after suffering an ischemic stroke. Moreover, the regulatory mechanisms for ROS production by phagocytes are not fully understood. ROS are produced by the reduction of molecular oxygen (O_2) located extracellularly which leads to H_2O_2 formation. Our investigation was focused on measurement of H_2O_2 production of a genetically modified leukemia cell line PLB-985 X-CGD SgpslBW (shortly BW) as well as human polymorphonuclear cells (PMN) and human peripheral blood mononuclear cells (PBMC).

ROS generation is an energy dependent process and usually the central role in the generation of ATP molecules belongs to glucose. Here, we have investigated the dependence of glucose and other 6C sugar substrates on ROS production. Sugars as: glucose, fructose, galactose, as well as the clinically relevant 2-deoxyglucose were tested. To visualize H_2O_2 generation, a fluorometric amplex red method was used. Our results suggest that H_2O_2 production by the BW cell line and PBMC is solely dependent of external glucose. Whereas, PMN cells in contrast, are mildly dependent of external glucose, indicating possibly a lack of glycogen stores in BW and PBMC. Fructose and galactose exhibited no production of any detectable ROS for BW, PBMC or PMN. Two possible scenarios seem reasonable, either the absence of specific sugar transporters for other 6C sugars in the cell membrane or incompatibility of these substrates in NADPH production.

Interestingly, 2-deoxyglucose demonstrated a concentration dependent inhibition of ROS production in PMN and BW. Indicating functional transport of 2-deoxyglucose across the membrane. In PMN ROS production was inhibited by 2-deoxyglucose without presence of external glucose. In comparison to BW which exclusively showed detectable ROS production under presence of external glucose. Our results clearly show that the sugar metabolism plays a substantial role in ROS production and might be one key to microvascular complications in diabetes.

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P-28 Miniaturization of the Clonogenic Assay Using Confluence Measurement

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Objective: The abstract is based on [1]. The clonogenic assay is a widely used method to study the ability of cells to 'infinitely' reproduce. However, the standard protocol using 6-well only allows measurement of a limited number of samples on one plate. Moreover, interpretation of the clonogenic assay is based on endpoint-analysis. We here describe a new protocol that miniaturizes the assay in the 96-well microplate format and utilizes the confluence detection method for non-endpoint and continuous measurement of clonogenic growth.

Methods: Bile duct cancer cells were seeded in the 96-well microplate at low seeding numbers. Clonogenic growth was evaluated after 5, 6 and 7 days using the confluence detection function of a Spark multimode reader. PTC-209 was used as a model substance to demonstrate the applicability of our protocol. For evaluation, confluence pictures were analyzed with ImageJ, using appropriate settings for size and circularity.

Results and Conclusions: We were able to demonstrate that the confluence detection function of the multimode reader reliably detected colonies and that non-endpoint measurement using the confluence detection function in the same well allows for resolution of (expected) time-dependent effects of clonogenic growth such as an increase of the colony mean size over time. Importantly, using our new protocol, we were able to resolve the concentration-dependent cytotoxic effect of PTC-209 on clonogenic growth. The effect was similar to the effect of PTC-209 on clonogenic growth in the standard 6-well format, excluding format-specific effects.

In summary, we show that miniaturization in the 96-well microplate format and utilization of the confluence measurement function of a multimode reader serve as a cost- and time-efficient option for measurement of clonogenic growth. Our protocol allows for meaningful time-resolved and non-endpoint measurement and the inclusion of appropriate controls and technical duplicates in the same plate.

1. Mayr C, Beyreis M, Dobias H, et al. Miniaturization of the Clonogenic Assay Using Confluence Measurement. Int J Mol Sci. 2018 Mar 3;19(3). doi: 10.3390/ijms19030724. PubMed PMID: 29510509; PubMed Central PMCID: PMCPMC5877585.

P-29 The histone methyltransferase G9a: a new therapeutic target in biliary tract cancer

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Objective: The abstract is based on [1]. G9a is a histone methyltransferase that is associated with cancer development. Several studies demonstrated an association between high G9a expression in cancer tissues and unfavorable clinicopathological parameters.

Biliary tract cancer (BTC) is a deadly disease with dismal outcome and limited therapeutic targets. In the current study, we aimed for initial investigation of G9a as a contributor to BTC.

Methods: Sixty-eight (68) cases of formalin-fixed, paraffin-embedded BTC samples archived at the Institute of Pathology (Paracelsus Medical University, Salzburg, Austria) were immunostained for G9a, E-Cadherin and Vimentin. In vitro expression analysis using established BTC cell lines (n = 9) was performed using Western blot and real-time PCR. The cytotoxic effect of commercially available G9a inhibitors BIX01294, BRD4470 and UNC0642 was investigated using the resazurin assay.

Results and Conclusions: G9a was expressed in about 90% of the BTC samples. We observed a significant increase of G9a expression in G3 versus G2 tumors. Epithelial-to-mesenchymal transition (EMT) is a key process in tumors to gain invasion characteristics. Double staining revealed that high G9a expression significantly correlated with high Vimentin (positive EMT effector) and low E-Cadherin expression (negative EMT effector), respectively. High G9a expression was significantly associated with poor survival (median survival 66.87 months in the G9a-low group versus 13.71 months in the G9a-high group). In vitro experiments demonstrated G9a (mRNA and protein) as well as H3K9me2 expression in BTC cells. Treatment of cells with three G9a inhibitors had a significant cytotoxic effect and resulted in diminished G9a and H3K9me2 protein levels.

We demonstrated that G9a is expressed in BTC and associated with higher tumor grade and lower overall survival. Moreover, in vitro experiments showed a cytotoxic effect of G9a expression in BTC cells. We conclude that G9a might be a candidate for pharmacological intervention in BTC.

1. Mayr C, Helm K, Jakab M, et al. The histone methyltransferase G9a: a new therapeutic target in biliary tract cancer. Hum Pathol. 2017 Nov 10. doi: 10.1016/j.humpath.2017.11.003. PubMed PMID: 29133140.

P-30 The cancer stem cell inhibitor napabucasin (BBI608) shows general cytotoxicity in biliary tract cancer cells and reduces cancer stem cell characteristics

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Objective: Biliary tract cancer (BTC) is a deadly disease with poor survival. The involvement of cancer stem cells (CSC) in BTC is likely. Napabucasin is a newly developed CSC inhibitor that is currently used in numerous clinical trials. However, data regarding the effect of Napabucasin in BTC are missing.

Methods: Cytotoxic effect of Napabucasin was evaluated using resazurin assay and Annexin V/7-AAD staining. We used soft agar assay, clonogenic growth assay, aldehyde-dehydrogenase-1 assay and CD326 surface expression measurement to investigate the effect of Napabucasin on functional CSC traits. The effect of Napabucasin on CSC expression was evaluated by Western Blot and a RT-PCR-based human CSC array.

Results and Conclusions: Napabucasin reduced viable BTC cells in a cell line- and concentration-dependent manner and increased apoptotic and necrotic cells. We observed a significant reduction of tumor spheres and clonogenic growth following Napabucasin treatment. Moreover, Napabucasin reduced CD326 surface expression and the number of aldehyde-dehydrogenase-1-positve cells. Protein levels of established CSC markers were significantly reduced after Napabucasin treatment. In addition, a comprehensive array-based gene expression analysis showed that Napabucasin downregulated mRNA levels of CSC-related genes.

In summary, our study provides first data about Napabucasin as a potential substance for treatment of BTC. We show that Napabucasin has a profound cytotoxic effect in BTC cells and reduced functional CSC characteristics as well as CSC-related protein and mRNA expression. Our study might be the base for future more detailed studies regarding Napabucasin and BTC as well as for studies investigating Napabucasin as a potential adjuvant substance alongside conventional chemotherapeutics.

P-31 Kinase signaling mediates mesenchymal stem cell conditioned medium-induced Na^+ channel activity in fetal lung cells

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Background: Pulmonary complications including respiratory distress syndrome are the leading cause of preterm morbidity and mortality. Impaired alveolar fluid clearance (AFC) and structural lung immaturity can lead to respiratory failure in preterm neonates. AFC is driven by vectorial Na^+ transport accomplished by the epithelial Na^+ channel (ENaC) and the Na,K-ATPase. Mesenchymal stem cells (MSCs) are suggested to harbor therapeutic potential for respiratory diseases, although effects on lung maturation have not been addressed. Beneficial effects of MSCs are attributed to paracrine signaling, i.e. growth factors involved in fetal lung development.

Aim: We addressed whether MSC conditioned medium (MSC-CM) is able to stimulate lung branching morphogenesis and Na^+ transport in primary rat fetal distal lung epithelial (FDLE) cells and if inhibition of growth factor signaling attenuates the effect of MSC-CM.

Methods: MSCs were isolated from full- and preterm umbilical cord. Effects of MSC-CM on Na^+ channel activity and expression were determined by Ussing chamber analysis and real time qPCR in primary rat FDLE cells. Lung maturation and branching morphogenesis were analyzed in fetal rat lung explants.

Results: Fetal lung explants cultivated in MSC-CM displayed an enhanced structural maturation. Furthermore, MSC-CM significantly ameliorated ENaC and Na.K-ATPase activity and gene expression. Inhibition of hepatocyte growth factor (HGF) signaling downregulated the effect of MSC-CM on ENaC activity, although HGF itself did not enhance ENaC activity. Thus, we analyzed phosphoinositide 3-kinase (PI3-K) signaling which is activated by HGF receptor and known to stimulate ENaC activity. Inhibitory studies of PI3-K downstream targets showed a significant reduction of MSC-CM mediated ENaC activity.

Conclusion: The results demonstrate that MSC-CM increases Na+ transport in FDLE cells, possibly attributable to PI3-K signaling, and improves branching morphogenesis. Therefore, MSC-CM can stimulate lung structural and functional maturation in vitro and might represent a future therapeutic option for preterm infants.

P-32 Outward rectification in a viral K^+ channel is based on ion depletion in the selectivity filter

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Voltage-dependence is an important property of potassium channels in a variety of physiological processes such as neuronal excitability and muscle contraction. Canonically, voltage-sensitivity is attributed to conformational changes in a specialized transmembrane voltage-sensing domain (VSD), which is covalently linked to the pore module. However, there is increasing evidence that voltage-sensitivity is also an intrinsic property of potassium channels lacking any canonical VSD. Here, we investigate the kinetics and mechanism of a pronounced voltage-dependent gating process in a member of the family of viral pore-only Kcv channels. $Kcv_{NH\ S77G}$ exhibits in multi-channel bilayer experiments in response to membrane hyperpolarization a time-dependent, ultra-slow inactivation, resulting in an outwardly-rectifying current-voltage relationship. Single-channel measurements demonstrate that this inactivation is caused by the voltage-dependent transition from an active state, in which the channel exhibits an open probability of about 90%, to an ultra-long-lasting, voltage-insensitive inactive state. The transition into the inactive state is sensitive to both the external K^+ concentration and the electrochemical driving force, supporting the idea that inactivation is directly linked to the permeation of potassium ions through the channel pore. These results provide a plausible mechanistic explanation on how ion channels without a VSD can sense a change in membrane voltage.

P-33 Function of mitochondrial $K_{Ca}3.1$ channels in lung cancer

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Among all malignant neoplasms, lung cancer has the highest incidence and mortality worldwide. A pivotal step for its lethality is the metastatic spread. Elevated expression of a calcium-activated potassium channel, $K_{Ca}3.1$, in lung cancer tissue predicts poor patient survival. Inhibition or knock-down of $K_{Ca}3.1$ leads to increased ICAM-1-dependent adhesion between non-small cell lung cancer cells and endothelium but diminishes the overall transmigration. Mechanisms underlying $K_{Ca}3.1$-dependent ICAM-1 expression are so far unknown.

This study analysed the expression of mitochondrial $K_{Ca}3.1$ in lung cancer and endothelial cell lines. Moreover, the effects of channel blockage on the mitochondrial membrane potential ($\Delta\Psi m$) were measured, because hyperpolarisation of $\Delta\Psi m$ upon mitochondrial $K_{Ca}3.1$ channel inhibition would cause a higher ROS production and thus, ICAM-1 presentation on the cell surface.

Western Blots were performed with antibodies against $K_{Ca}3.1$ and against the mitochondrial marker Bak in mitochondrial isolates and whole cell lysates. For a quantification of channel expression via immunofluorescence, the same channel antibody was applied in combination with MitoTracker™ CMTMRos. $\Delta\Psi m$ measurements were conducted employing the fluorescent dye rhodamine 123.

The expression of $K_{Ca}3.1$ in isolated mitochondria of carcinomatous A549-3R and H1975 cells could be demonstrated in Western Blot experiments. In superposition with the mitochondrial fluorescence signal, channel density was elevated by circa 40 - 50 % in carcinomatous A549-3R, H1975 and endothelial HMEC-1 cell lines. Application of TRAM-34, a $K_{Ca}3.1$ blocker, to A549-3R cells hyperpolarised $\Delta\Psi m$ significantly in comparison to DMSO treatment.

Taken together our results reveal the presence of functional mitochondrial $K_{Ca}3.1$ channels in lung cancer cells. They are consistent with the idea that ICAM-1 upregulation upon $K_{Ca}3.1$ inhibition is due to $\Delta\Psi m$ hyperpolarisation and increased ROS production.

P-34 A restricted QA+ blocker accessibility of TALK-2 K2P channels uncovers a structural constriction at the inner pore entrance

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Two-pore domain potassium (K_{2P}) channels play an important role in cellular electrical excitability and are regulated by divers physiological stimuli including voltage, temperature, protons, and certain permeant ions (i.g. Rb^+ and Cs^+) as well as pharmacological compounds such as 2-APB. All stimuli are thought to finally converge at the selectivity filter representing the principal gate in K_{2P} channels. Accordingly, K_{2P} channels are thought to lack gating at the intracellular pore entrance (helix-bundle- crossing gate) employed by many other types of K^+ channels. The existence of the latter can be probed by testing for the state dependence of inhibition by pore blockers (e.g. quarternary ammonium (QA) ions or modification of cysteine in the pore using MTS- reagents. Accordingly, in e.g. TREK-1 channels QA inhibition or pore cysteine modification is state independent. We report here that the non-activated TALK-2 channels are insensitive to inhibition by the large QA ion Tetrapentylammonium (TPA) unlike all other K_{2P} channels. However, TPA sensitivity increased dramatically when TALK-2 channels were activated by 2-APB but not when activated by the permeant ion Rb^+. However, inhibition by smaller QA ions was similar with and without activation. Furthermore, the modification rate of a pore cysteine with the voluminous modifying reagent MTS-TBAO was much faster when the channels were activated by 2-APB compared to non-activated or Rb^+ activated channels.

From that, we conclude a dilatation of the intracellular pore entrance removing a constriction for TPA or MTS-TBAO upon 2-APB-activation.

These findings identify a unique structural gating behaviour within the K_{2P} channel family, rising interesting questions towards further gating mechanisms and structural diversity in K_{2P} channels.

P-35 The viral potassium channel $Kmpv_{SP1}$ - an inherent inward rectifier

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Channel proteins and in particular potassium channels occur in almost every known organism, even in viruses. The phycodnaviruses, which belong to the dsDNA viruses, carry for example sequences for extremely small potassium channels in their genome. Among these so-called Kmpv channels (potassium channel Micromonas pusilla virus) are the smallest known potassium channels with less than 80 aa per subunit. Their small size and their function make them suitable model systems for a better understanding of basic structure and function relationships in K^+ channels, which can even be extrapolated to more complex potassium channels.

For electrophysiological characterization of the single channel currents of $Kmpv_{SP1}$, $Kmpv_1$ $Kmpv_{SP1}$F72I and Chimera $Kmpv_{SP1}$/TM 1-1 $Kmpv_1$ the planar lipid bilayer technique and the patch clamp technique in whole cell configuration was used.

The aim was to investigate and characterize the potassium channels $Kmpv_1$, $Kmpv_{SP1}$ and $Kmpv_{PL1}$, which are similar in their primary amino acid sequence at the level of their single-channel fluctuations. The particular focus of this study was on the analysis of structure/function correlates, which are responsible for the linear i/V relation in $Kmpv_1$ and the inward rectification in $Kmpv_{SP1}$ and $Kmpv_{PL1}$. The experiments show that the inward rectification of $Kmpv_{SP1}$ is an intrinsic property of the channel protein, which does not depend, unlike in the case of Kir channels, on any intracellular block. $Kmpv_{SP1}$F72I acquires a linear symmetrical i/V relationship. The rectification of the time averaged current seems to be generated by a voltage dependency of the open probability.

Chimera $Kmpv_{SP1}$/TM 1-1 $Kmpv_1$ weakens the inward rectification and shows also gating events with positive voltages.

P-36 ClC-3 Cl^-/H^+ exchangers in pain perception

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Within the central nervous system ClC-3 Cl-/H+ exchangers play a crucial role by regulating synaptic neurotransmission. Its function, however on sensory neurons has not yet been fully investigated. Here we study the role of ClC-3 in pain perception, by using tail flick and hot plate tests on $Clcn3^{-/-}$ and WT mice. We observed a significant increase in thermal sensitivity in $Clcn3^{-/-}$, suggesting that ClC-3 transporters are key regulators of neuronal pain signaling. To understand the molecular basis of this phenotype, we performed electrophysiological experiments on cultured dorsal root ganglion (DRG) neurons. Using patch-clamp, we examined the current threshold necessary to elicit action potentials (APs) in small DRG neurons. Current injection sufficient to trigger the first AP was significantly smaller in $Clcn3^{-/-}$ than in WT neurons.

Firing rate, defined as the number of APs per injection of five-fold threshold current was significantly increased in mutant neurons. Moreover, $Clcn3^{-/-}$ neurons were unable to maintain repetitive firing upon injection of depolarizing stimuli further than 250 pA.

Since sodium channels are mainly responsible for AP generation and DRG neurons almost exclusively express tetrodotoxin (TTX)-resistant $Na_v1.8$ and $Na_v1.9$ and TTX-sensitive $Na_v1.7$ channels, we measured sodium currents in the presence and in the absence of TTX. In agreement with the inability to maintain the continuous repetitive firing, $Clcn3^{-/-}$ DRG neurons exhibited a significant reduction of the TTX-resistant Na_v current. In contrast, TTX-sensitive Na_v channels show an increased current density, consistent with the lower current threshold, required to elicit APs in the $Clcn3^{-/-}$ condition. Taken together, our results demonstrate that ClC-3 transporters can modulate nociception by regulating the density of TTX-sensitive Na_v channels at the plasma membrane.

P-37 Calcium Signals in the Murine Zona Glomerulosa

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The adrenal gland is a functionally bipartite organ with a neuroendocrine medulla and a multilayered, steroid hormone-producing cortex. Within the cortex, androgens are synthesized in the innermost zona reticularis, cortisol in the zona fasciculata and aldosterone in the outer zona glomerulosa. Aldosterone is important for the regulation of blood pressure and salt/water homeostasis by regulating the reabsorption of NaCl and water as well as the secretion of K^+ in the kidney. Its synthesis is subject to two major stimuli, the serum concentrations of angiotensin-II (AT-II) and K^+. On the cellular level, following an electrical depolarization, stimulation of zona glomerulosa cells results in an increase of the intracellular calcium concentration, which is the signal for aldosterone synthesis.

Loading acute slice preparations of murine adrenal glands with an intensiometric fluorescent calcium indicator allowed us to record cytosolic [Ca^{2+}] signals for up to 60 minutes. We could confirm that calcium signals in the murine zona glomerulosa are mostly silent in the absence of extracellular AT-II at either 3 or 5 mM [K^+]. Upon increasing [AT-II], low frequency cytosolic calcium spikes could be observed in isolation or in bursts. Furthermore, the number of cells with visible activity increased with higher [AT-II]. Our results show that extracellular stimuli for aldosterone production are translated into calcium oscillations in zona glomerulosa cells. Information about the magnitude of these stimuli appear to be encoded in the properties of calcium oscillations, especially within bursts. Further work is needed to elucidate the source of the calcium increase as well as changes to absolute cytosolic concentrations.

P-38 Hypoxia inducible factor-1α and p53 in transition from ulcerative colitis to colorectal cancer

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Worldwide the number of newly reported patients affected by ulcerative colitis is steadily increasing (1). Most of the patients showed an increased risk to develop colorectal cancer. It appears essential to have an eye on the transition from chronically inflammatory disease to cancerogenic processes. Detailed knowledge of this transition might enable one to find new targets for therapeutic interference during early stages of disease development. Because chronically inflamed and cancerogenic tissues are hypoxic, our study focuses on the role of hypoxia inducible factor-1α (HIF-1α) on the one hand and its interaction with p53 (frequently mutated in colorectal diseases (2)) on the other hand. Because epithelial colorectal cancer development is a close interaction between the gut epithelium and immune cells we will investigate the interplay between epithelial cells and monocytes/macrophages and dendritic cells. For that purpose mice with a conditional knockout in epithelial cells of HIF-1α (VilCre/HIF-1α$^{+f/+f}$), p53 (VilCre/p53$^{+f/+f}$) or both (VilCre/HIF-1α+p53$^{+f/+f}$) and their HIF-1α (HIF1α$^{+f/+f}$), p53 (p53$^{+f/+f}$) and HIF-1α and p53 (HIF-1α+p53$^{+f/+f}$) expressing siblings will be examined in vivo in an Azomethane (AOM) - Dextran Sodium Sulfate (DSS) induced model of colitis and tumor development (3). Colon tissue will be used for RNA and protein examinations as well as immunohistochemical investigations. Furthermore the effect of the different knock-outs on the microbiome will be examined. There is evidence that HIF-1α knock-out in epithelial cells during experimental colitis has a protective role; therefore we expect tumor growth to be reduced in HIF-1α knock-out. On the other side a p53 knock-out should increase the tumor development (4). Which effect in process of tumor development is predominant is currently unknown and will be the focus of this study.

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P-39 Genetic and phenotypic analysis of daptomycin resistant isolates of Staphylococcus aureus

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Daptomycin (Dap) is a highly potent drug against the Gram positive human pathogen *Staphylococcus aureus*. Mutations in a number of genes cause Dap resistance (Dap^R). The aim of this work was to assess a possible association between Dap^R and mutations in genes *floA*, *pitA* and *mprF*. 40 *S.aureus* isolates with verified Dap^R were provided by the Robert Koch Institute (RKI). Dap sensitivity (Dap^S) was checked and quantified using E-test strips. *S.aureus* DNA was extracted and the three genes of interest were amplified fully (*floA* and *pitA*) or partially (*mprF*) by PCR and subjected to sequencing.

A mutation in the putative phosphate transporter PitA had been associated with Dap insensitivity before. However, we did not detect any mutations in this gene throughout the 40 isolates. FloA is involved in formation of lipid rafts and antibiotic susceptibility of meticillin resistant *S.aureus*. One exchange at aminoacid position 14 was observed in 12 of the analyzed strains but we found that other gene bank reference strains also harbor this exchange. In case of MprF, a number of mutations are associated with Dap^R.

Interestingly, we found six unpublished mutations of MprF that might be causative of the Dap^R phenotype. Whereas *pitA* and *floA* analyses did not yield new insights into *S.aureus* Dap^R, the new mutations in *mprF* have so far not been associated with that phenotype. It would be worthwhile to further characterize these mutations, preferably by the construction of deletion and complementation strains and investigation of their sensitivity to Dap.

List of Active Participants

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26. Christophe Jardin (P-18)
27. Kaluza, Luisa (O-06)
28. Kather, Stefanie (P-19)
29. Kerth, Clara (P-20)
30. Khadka, Pratiksha (P-21)
31. Kierzek, Michelina (P-22)
32. Kittl, Michael (P-23)
33. Klenk, Christoph (O-07)
34. Kraetzig, Anika (O-04)
35. Le Cann, Kim (O-26)
36. Lehne, Franziska (P-24)
37. Leibl, Victoria (P-25)
38. Lenke, Livia (P-26)
39. Lenz, Dominik (O-13)
40. Mahorivska, Iryna (P-27)
41. Mayr, Christian (P-28, P-29, P-30)
42. Meents, Jannis (O-01)
43. Mertens, Bettina (O-12)
44. Najder, Karolina (O-23)
45. Obendorf, Janine (P-31)
46. Ojha, Navin K. (O-22)
47. Pethö, Zoltan (O-18)
48. Rauh, Oliver (P-32)
49. Rieke, Marius (P-33)
50. Riel, Elena (P-34)
51. Ritzau-Jost, Andreas (O-24)
52. Rugi, Micol (O-16)
53. Schewe, Markus (O-11)
54. Schröder, Indra (O-20)
55. Schulze, Tobias (P-35)
56. Seidel, Thomas (O-02)
57. Sierra Marques, Juan (P-36)
58. Stölting, Gabriel (P-37)
59. Thull, Sarah (O-21)
60. Wenzl, Anja (O-03)
61. Wrobeln, Anna (P-38)
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