Dear Young Physiologists,

We warmly welcome you to our first *Young Physiologists Online Symposium*. We arranged a one-day program that, around a core of oral and poster presentations from different fields of physiology, also features a session on teaching as well as a keynote lecture on career development given by Matt Gillum (University of Copenhagen).

Even more than real conferences, virtual symposia are only as good as their participants, so we strongly encourage you to commit yourself to this very special format and get the best out of it. Finally, we hope to physically welcome you again in spring next year in Marburg.

We wish you a fruitful scientific symposium as well as a vivid social exchange.

Yours,

Andreas, Gustavo and Dominik

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ORGANISATION

PLATFORM

We use two platforms: On **ILIAS.online** we have a "course" in which you are automatically enrolled by creating an account using the code we sent you. In this course you will find everything you need. For the actual conference, we use **BigBlueButton** (BBB), an open-source platform with a good privacy policy.

When you enter a BBB room, you have to click "with microphone" in order to be able to talk to us ("head phone only" only enables listening and writing). Then you have to allow your browser to access the microphone you use. By clicking the camera symbol in the bottom line you start the video function of your webcam (yes, it demands multiple clicking, no worries!). If you deny access to either microphone or camera, you have to leave the BBB room by logging out in the top right corner and rejoin, now granting access.

There were issues with using BBB via the Safari browser of Mac devices. Those problems are mostly solved, but we still recommend either using a Windows PC or Mac with another browser (Chrome, Firefox etc.). To get used to BBB, we highly encourage you to check out this room.

General advice: connecting via LAN is better than via Wifi; try not to get distracted by mails or your mobile during the meeting; use the coffee breaks actively for chatting with your peers or for physical activity etc. to avoid getting tired!

ORAL PRESENTATIONS

The oral presentations are scheduled for 15 minutes talk + 5 minutes of discussion. We will have one BBB room for all talks sessions. Please be aware that BBB only allows static presentation format, so no PowerPoint animations or videos. We recommend to test quality of both conversions before. To simplify switching between the presentations, we recommend you to send us the files until October 6th, 2 p.m., so we can upload and manage them ahead on the BBB platform.

POSTER SESSIONS

We will have one poster session, with one BBB room per poster. The session chairs will be the administrators of the rooms. It is possible to be a member of different BBB rooms at the same time, so you do not have to be technically present in "your" poster room permanently and you may also roam around and have a look at other posters.

Please prepare your posters in landscape format. Data density as on a normal DIN A0 poster may be okay on a full screen, but if it's possible we recommend to reduce data density. As mentioned above for the talks, you can upload the .pdf file and test visibility in the test room. For the actual presentation, please send us the .pdf until October 6th, 2 p.m. We will upload the posters when we start the poster session.

To draw attention to your poster, we will have a short "posterblitz": 30-60 seconds chance for you to give a rapid overview about your poster, right before the actual poster session.

DETAILED PROGRAM

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09:30 Oral Session I: Neurophysiology

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Expression and localization of Kcne2 in the vertebrate retina

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Vesicle recruitment is not accelerated during LTP at neocortical layer 5 pyramidal neurons

O-03 Laura Lindner (Marburg)

Phosphoinositide effector proteins in hair cells of the mouse organ of Corti

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Non-Neuronal Acetylcholine Released by Tracheal Solitary Cholinergic Chemosensory Cells Activates Mucocililary Clearance in the Murine Trachea

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Direct optogenetic stimulation of smooth muscle cells to control gastric contractility

11:50 Coffee Break

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The last shall be first: A case for physiologists as educational researchers

O-08 Tobias Heinrich (Hamburg)

Interdisciplinary course on peripheral vascular physiology: oscillatory pulse wave diagnostics and impedance venous occlusion plethysmography

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16:15 Oral Session IV: Inflammation and Cancer

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O-09 Micol Rugi (Münster)

Modulation of mechano-sensing of pancreatic stellate cells by pancreatic pH: focus on K2P2.1 channel

O-10 Elena Czyrnik (Essen)

Analysis of stromal-epithelial interactions via soluble factors in prostatic cell cultures

O-11 Karolina Najder-Nalepa (Münster)

Neutrophil response is governed by ions

17:15 Closing Remarks

ABSTRACTS – ORAL SESSIONS

Oral Session I NEUROPHYSIOLOGY

O-01 Expression and localization of Kcne2 in the vertebrate retina

Moritz Lindner[1,2,3,*]; Michael J. Gilhooley[2,3,4]; Teele Palumaa[2]; A. Jennifer Morton[5]; Steven Hughes[2]; Mark W. Hankins[2,*]
(1) Institute of Physiology and Pathophysiology, Department of Neurophysiology, Philipps University, Marburg, Germany; (2) The Nuffield Laboratory of Ophthalmology, Sleep and Circadian Neuroscience Institute, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom; (3) Oxford Eye Hospital, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; (4) Moorfields Eye Hospital, 162 City Road, London, UK; (5) Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, CB2 3DY, United Kingdom; * equal contribution

Purpose: To characterize the retinal expression and localization of Kcne2, an ancillary (β) ion-channel subunit with an important role in fine-tuning cellular excitability.
Methods: We analyzed available single-cell transcriptome data from tens of thousands of murine retinal cells (Macosko et al.) for cell-type specific expression of *Kcne2* using state-of-the-art bioinformatics techniques. This evidence at the transcriptome level was complemented with a comprehensive immunohistochemical characterization of mouse retinae (C57BL/6, aged 8-12) employing co-labelling techniques and cell-type specific antibody markers. We furthermore examined how conserved the Kcne2 localization pattern in the retina was across species performing immunostaining on zebrafish, cowbird, sheep, mice, and macaque.
Results: *Kcne2* is selectively expressed in cone photoreceptors and rod bipolar cells. At a subcellular level, Kcne2 arranges in such a way that in both cell types it encloses the photoreceptor-bipolar cell synapse. Thus, the vast majority of Kcne2 immunoreactivity is observed in a thin band in the outer plexiform layer. In addition to this, faint Kcne2 immunoreactivity can also be observed in cone inner segments and the somata of a small subset of cone ON-bipolar cells. Strikingly, the localization of Kcne2 in the outer plexiform layer is preserved among all species studied, spanning at least 300 million years of evolution of the vertebrate kingdom.

Conclusion: The data we present here suggest an important and specific role for Kcne2 in the highly specialized photoreceptor-bipolar cell synapse.

O-02 Vesicle recruitment is not accelerated during LTP at neocortical layer 5 pyramidal neurons

Iron Weichard, Stefan Hallermann, and Jana Nerlich
Carl-Ludwig-Institute for Physiology, Faculty of Medicine, Leipzig University, Leipzig, Germany

Long-term potentiation (LTP) is a potential mechanism for experience-dependent learning and memory. LTP can be expressed both pre- and postsynaptically. The mechanisms of presynaptic transmitter release and its regulation during plasticity remain poorly understood. Particularly, the speed of vesicle recruitment is an important parameter determining synaptic efficacy but difficult to quantify. It has not been studied carefully whether vesicle recruitment can be accelerated during LTP. To address this question we focused on LTP at synapses of layer 5 pyramidal neurons, which have been shown to exhibit presynaptic LTP referred to as redistribution of the synaptic efficacy. We performed whole cell recordings from thick-tufted layer 5 pyramidal cells with extracellular stimulation of local excitatory inputs and measured paired-pulse ratio, short-term plasticity during high-frequency transmission and recovery from depression before and after the induction of LTP. After LTP the EPSC amplitude and the depression during high-frequency transmission was increased, concomitant with a decrease in the paired pulse ratio. Interestingly, the steady state level was potentiated in addition, which would be consistent with an increase in vesicle recruitment kinetics. However, further mechanistic analyses including the investigation of the recovery from synaptic depression revealed that LTP increased the vesicular release probability and the number of readily releasable vesicle but not the speed of vesicle recruitment. Our data therefore indicate that the speed of vesicle recruitment is not regulated during LTP at neocortical layer 5 neurons suggesting a biophysically maximized recruitment speed which cannot be further accelerated during plasticity.

O-03 Phosphoinositide effector proteins in hair cells of the mouse organ of Corti

Laura Lindner[1], Boris A. Stuck[2], Katrin Reimann[3], Dominik Oliver[1]
[1]Institut für Physiologie und Pathophysiologie, Dept. für Neurophysiologie, Philipps-Universität Marburg; [2]Klinik für Hals-, Nasen- und Ohrenheilkunde, UKGM, Standort Marburg; [3]Cochlea-Implant-Centrum Marburg, UKGM, Standort Marburg

Phosphoinositides are phospholipid components of all cell membranes that regulate many different cellular key functions like membrane trafficking, ion channel and transporter activity, cytoskeletal dynamics, as well as signal transduction. Even though these components are essential for hearing, to date the role of phosphoinositides in the organ of Corti is understudied. The purpose of this study was to examine the possible roles phosphoinositides play in hearing function. Therefore, we took a look at known phosphoinositide effector proteins: *Tubby-like protein 3* (TULP3), a phosphoinositide- dependent regulator of G-protein coupled receptors; *tubby*, a putative transcription factor affected by hydrolysis of the phosphoinositide $PI(4,5)P_2$ and *SWAP70*, a cytoskeletal regulator, dependent on the production of $PI(3,4)P_2$. Using immunohistochemistry on whole-mount preparations of the mouse organ of Corti, we were able to detect the protein expression and cellular localization of these effectors.
Here we report, that TULP3 is mainly expressed in the apical parts of the inner and outer pillar cells, as well as in the phalangeal processes of the Deiters cells. Both structures are known to be filled with microtubules, which have also been shown to be regulated by phosphoinositides. We further detected TULP3 in the cell body of the inner and outer hair cells. While we did not find expression of SWAP70, we show that tubby localizes in the distal segments of the stereocilia of the outer hair cells, where previously prominent $PI(4,5)P_2$ labelling was described, and in the nuclei of the Deiters cells. Together this shows that these effectors distribute throughout the organ of Corti, which suggests that phosphoinositides manage a variety of important functions in hearing.

Oral Session II TISSUE PHYSIOLOGY

O-04 The (patho)physiology of CatSper Ca^{2+} channels in human sperm

Samuel Young[1], Schiffer C[1], Brenker C[1], Tüttelmann F[2], Röpke A[2], Hamzeh H[3], Wachten D[3,4], Kaupp U B[3], Kliesch S[1] and Strünker T[1]
[1]Centre of Reproductive Medicine and Andrology, Münster, Germany; [2]Institute of Human Genetics, Münster, Germany; [3]Center of Advanced European Studies and Research, Bonn, Germany; [4]Institute of Innate Immunity, University Hospital, University of Bonn, Bonn, Germany

In human sperm, the sperm-specific Ca^{2+} channel CatSper serves as a polymodal chemosensor that registers ligands released by the oviductal epithelium and cells

surrounding the oocyte. Thereby, CatSper translates the chemical code of the oviductal microenvironment into changes of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which controls the flagellar beat and swimming behavior. We have identified seven infertile patients that suffer from the so called deafness-infertility syndrome (DIS). DIS patients lack the genes encoding for stereocilin (STRC) and CatSper 2; STRC is expressed in cochlear hair cells. The phenotype of the CATSPER2-deficient sperm was thoroughly characterized by standard semen analysis, electrophysiology, Ca^{2+}-fluorimetry, motility analysis, and 3D-STORM. We show that CatSper-mediated Ca^{2+} influx and membrane currents are abolished in sperm from DIS patients, demonstrating that the homozygous deletion of CATSPER2 abrogates the expression of functional CatSper channels. Though, in the absence of CatSper 2, CatSper 3 and CatSper 4 assemble into non-functional protein complexes, whose sub-cellular arrangement is similar to that of the functional CatSper-channel complex. Moreover, according to standard semen analysis, the DIS patients are normozoospermic, indicating that male infertility caused by the lack of functional CatSper channels escapes current andrological methods used to assess sperm function and male fertility. Finally, we demonstrate the utility of CatSper-deficient human sperm as a model to gain insight into the function of CatSper.

O-05 Non-Neuronal Acetylcholine Released by Tracheal Solitary Cholinergic Chemosensory Cells Activates Mucocililary Clearance in the Murine Trachea

Alexander Perniss (1), Liu S. (2), Boonen B (3). Keshavarz M. (1), Ruppert A.L. (4), Timm T. (5), Pfeil U. (1), Soultanova A. (1), Kusumakshi S.(6), Delventhal L. (1), Aydin Ö. (1), Pyrski M. (1), Deckmann K. (1), Hain T. (7), Schmidt N. (8), Ewers C. (8), Günther A. (9,10), Lochnit G (5), Chubanov V. (11), Gudermann T. (11), Oberwinkler J (12), Klein J. (13), Mikoshiba K. (14), Leinders-Zufall T (3), Offermanns S. (2), Schütz B. (4), Boehm U. (6), Zufall F. (3), Bufe B. (3, 15), Kummer W. (1)

(1) Institute for Anatomy and Cell Biology, German Center for Lung Research, Justus Liebig University, Giessen, Germany; (2) Department of Pharmacology, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany; (3) Center for Integrative Physiology and Molecular Medicine, Saarland University, Homburg, Germany, (4) Institute of Anatomy and Cell Biology, Philipps-University, Marburg, Germany; (5) Protein Analytics, Institute of Biochemistry, Faculty of Medicine, Justus Liebig University, Giessen, Germany; (6) Experimental Pharmacology, Center for Molecular Signaling (PZMS), Saarland University, Homburg, Germany; (7) Institute of Medical Microbiology, German Center for Infection Research, Partner Site Giessen-Marburg-Langen, Justus Liebig University, Giessen, Germany;

(8) Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University, Giessen, Germany; (9) Center for Interstitial and Rare Lung Diseases, Dept. of Internal Medicine, German Center for Lung Research, Justus Liebig University, Giessen, Germany; (10) Agaplesion Lung Clinic Waldhof-Elgershausen, Greifenstein, Germany; (11) Walther-Straub Institute of Pharmacology and Toxicology, German Center for Lung Research, Ludwig-Maximilians-Universität München, Munich, Germany; (12) Insitute of Physiology and Pathophysiology, Philipps.University, Marburg, Germany; (13) Department of Pharmacology and Clinical Pharmacy, FB14, Goethe University Frankfurt, Frankfurt am Main, Germany; (14) Laboratory of Cell Calcium Signaling, Shanghai Institute for Advanced Immunochemical Studies (SIAIS), ShanghaiTech University, Shanghai, China; (15) Department of Informatics and Microsystems Technology, University of Applied Sciences, Kaiserslautern, Germany

Introduction: Acetlycholine (ACh) is a powerful stimulator of mucociliary clearance (MC) which is driven by the coordinated beating of ciliated cells. Solitary cholinergic chemosensory cells (SCCC), a subpopulation of epithelial cells, express the ACh producing enzyme, choline acetyltransferase (ChAT), and components of the taste transduction cascade. We here asked whether formylated bacterial signal peptides modulate MC and if SCCC release ACh upon stimulation, thereby activating MC.

Materials and Methods: Particle transport speed (PTS) and ciliary beat frequency (CBF) were assessed on the mucosal surface of explanted murine tracheas. Changes in $[Ca^{2+}]_i$ were measured using tracheal whole-mount preparations of TRPM5-GCaMP6-mice. ACh release in supernatants was quantified by HPLC and by measurement of changes in $[Ca^{2+}]_i$ of muscarinic m3 reporter cells. Stimuli were either bacterial peptides in C57BL/6J-mice or LED stimulation (8 Hz) in ChAT-ChR2(H134R)-YFP-mice. Channelrhodopsin-2-YFP (ChR2) expression in SCCC was analysed by (immuno)fluorescence microscopy. Presence of formylated peptides in sputum samples of COPD patient samples was analyzed using HPLC.

Results: The N-formylated bacterial signal peptide f-MKKFRW, produced by pathogens such as S*almonella typhimurium*, increased PTS. FPR1/2 inhibitors cyclosporine-H and t-BOC2 did not reduce the effect. The effect was reduced by more than 90 % in *TRPM5*$^{-/-}$, PLCβ2$^{-/-}$, ITPR3$^{-/-}$, TRPM5-DTA and Pou2f3$^{-/-}$ mice. The effect was nearly abolished by the muscarinic antagonists atropine (reduced by ~74) and 4-DAMP (reduced by ~84), but persisted after blocking nicotinic receptors (mecamylamine) or voltage gated sodium channels (TTX). Application of f-MKKFRW lead to sharp but not long lasting increase in $[Ca^{2+}]_i$ in a subset of SCCC (21%; 19/92 cells analyzed). ACh levels increased after stimulation with f-MKKFRW measured by HPLC in wildtype but not in TRPM5$^{-/-}$ mice (component of the taste transduction cascade), in which an decrease in ACh was observed. The same outcome was observed using m3 reporter cells and changes in $[Ca^{2+}]_i$ as a readout. ACh was

elevated in supernatants of tracheas after stimulation by LED in ChAT-ChR2-YFP mice but not in WT mice or without LED stimulation of ChAT-ChR2-YFP mice measured by HPLC. PTS was increased after LED stimulation in ChAT-ChR2-YFP mice but not in WT mice. The formylated peptides f-MKKFR and f-MKKFRW (produced by pulmonary pathogens, e.g. *Klebsiella pneumoniae* and *Pseudomonas aueruginosa*) were found in sputum samples of hospitalized COPD Patients.

Conclusion: Solitary chemosensory cells in the trachea release ACh upon stimulation with formylated peptides which activates mucociliary clearance and probably modulates other innate immune mechanisms.

O-06 Direct optogenetic stimulation of smooth muscle cells to control gastric contractility

Markus Vogt[1], Benjamin Schulz[2], Ahmed Wagdi[1], Gijsbert J. van Belle[1], Robert Patejdl[2], Tobias Brügmann[1]

[1]Institute of Cardiovascular Physiology, University Medical Center Göttingen, Humboldtallee 23, 37073 Göttingen, Germany; [2] Oscar Langendorff Institute for Physiology, University of Rostock, Germany

Antral peristalsis is responsible for gastric emptying and its failure, gastroparesis, is most often caused by loss of function of the enteric nervous system and interstitial cells of Cajal. Current treatment options are non-satisfying and also gastric electrical stimulation may improve symptoms but fails to restore gastric emptying. Herein, we explore direct optogenetic stimulation of smooth muscle cells (SMC) via the light-gated non-selective cation channel Channelrhodopsin2 (ChR2) to control gastric motor function.

We used a transgenic mouse model expressing ChR2 in fusion with eYFP under the control of the chicken-β-actin promoter. We found ChR2/eYFP expression in the circular and longitudinal layer of the tunica muscularis in SMC but not in interstitial cells of Cajal nor nerve fibres, detected by staining against α smooth muscle actin, cKit or anti-β-III-tubulin respectively. After dissociation, 36±3% of SMC (n = 5) were showing membrane-bound eYFP signals and in these blue light (460 nm) induced inward currents typical for ChR2 with the half-maximal effective light intensity of the peak current of 0.2±0.01 mW/mm^2 (n=20). In isometric force measurements of antral muscle strips, these depolarizing currents led to contractions stronger than supramaximal electrical field stimulation and comparable to global depolarization with high [K^+]. Furthermore, panoramic illumination via three LEDs placed around the intact stomach efficiently increased intragastric pressure achieving 239±46% (n=6) of the pressure induced by electrical field stimulation. Finally, we mimicked

gastroparesis ex vivo via methylene blue photo-oxidization. Gastroparesis was proven by failure of electrical field stimulation but light still efficiently generated pressure waves indicating the direct stimulation of SMC as well as the translational potential.
Taken together, we demonstrate for the first time the use of direct optogenetic stimulation of SMC to control gastric contractility. This completely new approach could allow to restore motility in gastroparesis in the future.

Oral Session III TEACHING

O-07 The last shall be first: A case for physiologists as educational researchers

Robert Patejdl
Oscar Langendorff Institute for Physiology, University of Rostock, Germany

Over the last years, the impact of educational research in medicine is expanding. Traditional concepts of education, teaching and testing are being challenged. While physiology has a long tradition as one of the central subjects within the curriculum, physiologists have not yet managed to establish vivid and effective networks for educational research. Nevertheless, physiologists should be encouraged to design and conduct research projects for the following reasons:

- Good teaching requires feedback that goes far beyond classical multiple choice exams. Only educational research can tell why certain students fail to get familiar with physiological concepts and how to provide efficient support and motivation for every student.
- The institutional context of education is tightly coupled to that of the universities as a whole. In the long term, physiological departments and their staff will need to develop elaborated and “evidence based” concepts of teaching including continuous evaluation and refinement. Otherwise, they might be considered obsolete for educating students and be integrated to general basic science facilities.
- Good education and teaching at universities has long suffered from being non-relevant for scientific careers. The increasing number and impact of journals in the field of medical education, combined with increasing amounts of money provided make it more worthwhile for young scientists to flank their commitment to teaching with innovative and significant research.

This contribution is intended to give an introduction to the landscape of medical education and to invite young researchers to connect and initiate educational research projects in physiology education.

O-08 Interdisciplinary course on peripheral vascular physiology: oscillatory pulse wave diagnostics and impedance venous occlusion plethysmography

Tobias Heinrich[1, 2], Robert Baehring[1], Axel A. Larena-Avellaneda[3], Juergen Querengaesser[4], Olaf Solbrig[4], Alexander P. Schwoerer[1, 2]

[1)] Department of Cellular and Integrative Physiology University Medical Centre Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany; [2)] DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck; [3)] Department of Vascular and Endovascular Surgery, Asklepios Hospital Altona, Hamburg, Germany; [4)] medis. Medizinische Messtechnik GmbH, Werner-von-Siemens-Str. 8, 98693 Ilmenau, Germany

Introduction: Diseases affecting the vascular system of the lower limb (e.g. arteriosclerosis, peripheral arterial disease, deep vein thrombosis) are highly prevalent and considerably affect quality of life and life expectancy. For students, understanding the underlying vascular physiology is central to derive the consequences of vascular malfunctions leading to vascular diseases. Ideally, this translational concept is integrated in individual courses. For laboratory courses addressing vascular (patho)physiology, however, finding experiments which illustrate relevant aspects of both, physiologic vascular function, and clinical dysfunction, is challenging. Activities must be simple enough to be conductible by students, yield parameters which visualise physiologic vascular properties, and as well are sensitive to the impact of pathologies. Most techniques used to investigate vascular physiology do not fulfil these combined requirements. Thus, a translational gap between physiological basics and active clinical practice may regularly be observed in physiology laboratory courses.

Aims: Here, we describe a laboratory activity integrating physiological basics of leg perfusion into a clinical perspective. Moreover, we document learning success as well as student's satisfaction.

Methods: The activity is integrated in the second or third year of our integrative medical curriculum. It is immediately preceded by an advanced physiology seminar on vascular properties and a lecture on vascular medicine.

Results: Using oscillography and impedance venous occlusion plethysmography, the course addresses key aspects of the arterial and venous vascular system: 1) arterial pulse wave properties, 2) regional differences in systolic blood pressure, 3)

changes in arterial perfusion (reactive hyperemia), 4) venous capacity and venous outflow. Following the experiments, the most frequent corresponding diseases, their impact on the recorded parameters, and diagnostic options are discussed.
All experiments are well feasible and provide robust data on physiologically and clinically relevant vascular functions. Overall, the activity was perceived positively by the students and lead to a substantial improvement of knowledge.

Oral Session IV INFLAMMATION AND CANCER

O-09 Modulation of mechano-sensing of pancreatic stellate cells by pancreatic pH: focus on K2P2.1 channel

Micol Rugi, Albrecht Schwab
Institüt für Physiologie II, Westfälische Wilhelms-University Münster, Germany

Pancreatic Adeno Carcinoma (PDAC) is one of the most lethal forms of cancer. One of the main characteristics of this type of cancer is its microenvironment which has a unique pH landscape and high stiffness of the fibrotic tumor tissue. Due to the massive fibrosis the pressure in PDAC tissue is known to reach high values (ca. 100 mmHg). In a healthy pancreas, Pancreatic Stellate Cells (PSCs) are usually in their quiescent state. However, in PDAC those cells are strongly activated among others through the mechanical stimulation (high pressure and stiff tissue) of their mechanosensitive ion channels and the shifts in the pH values. Activated PSCs are responsible for the excessive production of extracellular matrix and thereby tumor tissue fibrosis.
The aim of my work is focused on the investigation of the role of the two-pore domain K^+ channel K2P2.1, in PSCs. K2P2.1 is known to be modulated by both pH and mechanical stimuli and to be important for the setting of the resting membrane potential of different cells. We quantified migration as a surrogate of the PSC activation. We first investigated the impact that pH and pressure have on migration of PSCs from wild type mice and K2P2.1 knock-out mice. Migration was evaluated with live-cell imaging for 6h. Images were acquired in 300s intervals and the stacks were analyzed with Amira software. Pressure was applied in a pressure chamber, in which cells were left for 24h under a constant pressure of 100mmHg above ambient pressure. K2P2.1 channel does have an impact on cell pressure detection: in physiological condition wild type PSCs reacted to pressure increasing their speed, while in acidic pH cells decreased it. Interestingly the knock-out cells showed a small

decrease of velocity when pressure was applied in pH 7.4. Many mechano-sensitive ion channels mediate influx of Ca^{2+} into PSCs and they are relevant for many cellular processes (e.g. migration, proliferation). K2P2.1 activation by pressure may counterbalance the depolarizing effect of Ca^{2+} entry via other mechano-sensitive ion channels and thereby allow their response to external stimulation.

O-10 Analysis of stromal-epithelial interactions via soluble factors in prostatic cell cultures

Elena Czyrnik, J. Dankert, G. Wennemuth
Department of Anatomy, University Hospital Essen, Germany

In prostate glands, epithelial and stromal cells function in a close meshwork. This is not only the case in young or adult normal tissue, but also during organogenesis and eventually in prostate cancer. It is assumed that a reactive stroma during the early development of prostate cancer causes an altered tumor microenvironment, which in turn can promote epithelial-mesenchymal transition of cells. The latter is associated with invasion, metastasis and immune escape. The aim of this study is to investigate new genes involved in stromal-epithelial interaction via soluble factors. For this approach, LNCaP prostate cancer cells and primary stromal cells were co-cultivated without contact. After one and after seven days, RNA was isolated and sequenced by RNA sequencing referring to their polyadenylated 3' end. The analysis of four independent experiments revealed differential expression profiles. Targets of interest were validated in three additional independent co-culture experiments by real-time PCR. To further verify the importance of soluble factors during communication between epithelial and stromal cells, cultivation with conditioned medium was performed. Furthermore, the localization within human prostate tissue (fetal, normal, carcinoma) and the *in vitro* function of the genes of interest will be studied to investigate their putative role in prostate cancer.

O-11 Neutrophil response is governed by ions

Karolina Najder-Nalepa[1], Micol Rugi[1,2], Mégane Lebel[3], Julia Schröder[1], Leonie Oster[1], Albrecht Schwab[1]
[1]Institute of Physiology II, University Hospital Münster, Münster, Germany; [2]University of Florence, Florence, Italy; [3]University of Sherbrooke, Quebec, Canada

Neutrophils are key players and the first responders to the tissue injury and inflammation. Efficient and immediate immune reaction is initiated and led by fine-

tuned neutrophil mobilization, chemotaxis and implementation of a potent anti-bacterial armory.

In our research, we study the role of intra- and extracellular ions in neutrophil response. Combining the analysis of $[Ca^{2+}]_i$, $[Na^+]_i$, pH_i and membrane potential with functional assays of chemokine-induced neutrophil chemotaxis, LTB_4 production and release of reactive oxygen species, we aim to explore the ions and respective transport proteins involved in major neutrophil functions.

Using ion-sensitive dyes, we observed that not only $[Ca^{2+}]_i$ and pH_i, but also neutrophil $[Na^+]_i$ alters upon fMLF or C5a stimulation. Increase in $[Na^+]_i$, to some extent, inversely correlates with neutrophil chemotactic response observed in 3D collagen matrix. We showed that both non- and electrogenic, Na^+ gradient-driven exchangers (NCX1, NHE1) regulate neutrophil chemotaxis and therefore, ion homeostasis and membrane potential are determining factors of appropriate neutrophil function.

In ongoing studies, we further explore the interwoven relationship between ions, transport proteins and membrane potential in human and murine neutrophils. This detailed analysis might help us understand complex mechanisms of neutrophil behavior, especially in conditions of ionic imbalance, like inflammation and tumor.

ABSTRACTS – POSTER SESSION

Poster Session A NEUROPHYSIOLOGY

P-01 Direct evidence for voltage-induced conformational changes of Prestin, the electromechanical transducer in outer hair cells

Makoto F. Kuwabara[1], Piersilvio Longo[2], Thomas Berger[1], Dominik Lenz[1], Jan-Philipp Machtens[2], Dominik Oliver [1]

[1]Institute for Physiology and Pathophysiology, Dept. of Neurophysiology, Philipps-University Marburg; [2]Forschungszentrum Jülich, Institute for Cellular Biophysics (ICS-4), Jülich, Germany

Our sense of hearing is remarkable: We are able to discriminate auditory stimuli that differ only minimally in intensity or frequency. Sound waves are efficiently transduced to the cochlea and then actively amplified by the outer hair cells. The key molecular player in cochlear amplification is prestin, a transmembrane protein that belongs to the SLC26/SulP anion transporter family. In mammals, voltage-dependent activation of prestin changes the length of outer hair cells. This "electromotility" is crucial for amplification and frequency selectivity of the mammalian ear. Interestingly, mammalian prestin shows electromotility but no transport activity, while zebrafish prestin shows transport activity without electromotility. It has been proposed that electromotility arises from an incomplete anion transport cycle. Yet, conformational changes of prestin were never observed directly. Here, we apply the voltage-clamp fluorometry technique to identify voltage-induced conformational changes of prestin. We heterologously express zebrafish prestin in Xenopus oocytes. An environmentally sensitive fluorophore (TAMRA) is covalently attached via a thiol-reactive linker to different singly introduced cysteine residues between the third transmembrane helix and the long extracellular loop L1 of prestin according to a homology-model based on hSLC26A9. We identified a cysteine mutant that, when labeled with TAMRA, displays voltage-induced fluorescence changes. This result suggests that a voltage-induced conformational rearrangement of prestin occurs at or in proximity of the attachment site of the TAMRA fluorophore. We will next study whether the conformational changes, the anion transport, and prestin-induced capacitance changes have the same voltage dependence. Our study shows that voltage-clamp

fluorometry is a useful technique to study the molecular mechanism underlying the electromotility of prestin.

P-02 Mapping the ligand binding site on a peptide-gated ion channel

Michèle Bachmann, Lilia Leisle, Stefan Gründer
Institute of Physiology, RWTH Aachen University, Pauwelsstrasse 30, D-52074 Aachen, Germany

The ligand-gated cation channels from the freshwater polyp Hydra magnipapillata, termed Hydra Na+ channels (HyNaCs), belong to the DEG/ENaC gene family. They are activated by endogenous neuropeptides RFamide I and II and contribute to tentacle contraction of Hydra. In total, 11 different HyNaC isoforms have been cloned to date, most of which form heterotrimeric channels eliciting inward currents upon ligand binding. Yet, the ligand binding site remains undefined. Here, we aim to map the binding site of RFamide II on the heterotrimer comprising HyNaC2, HyNaC3 and HyNaC5. First, to improve the expression levels of Hydra proteins in HEK293 cells we performed codon optimisation of the genes. Western Blot analysis of whole protein lysates showed dramatically increased protein levels. Second, to map the ligand binding site we successfully incorporated the photo-cross linking unnatural amino acid 4-azido-L-phenylalanine (AziF) at 20 different positions in the extracellular domain of HyNaC subunits. The site selection was based on in silico molecular docking results of the ligand to a HyNaC2/3/5 homology model. Upon UV irradiation, AziF forms covalent bonds with molecules at distances characteristic for specific interaction, thus providing details on the interaction interface. To detect the crosslinking products via Western Blot, biotinylated RFamide II variants were tested, which showed a strongly decreased affinity compared to the wildtype peptide. However, we were able to detect the wildtype peptide using a monoclonal antibody. Currently we identify the residues that contribute to the binding site. Once the binding site of RFamide II on the HyNaC2/3/5 heterotrimer is mapped, our data will also help elucidate the evolutionary relations of HyNaCs as a precursor to other DEG/ENaC channels in terms of ligand binding.

P-03 The Role of Octopamine during Reward Processing in Drosophila

Ines Köhler[1], Katrin Vogt[2], Mareike Selcho[1], Andreas S[1]. Thum, Dennis Pauls[1]
[1]University of Leipzig, Life Science, Institute for Biology; [2]Harvard University, Department of Physics, Center for Brain Science

Octopamine serves as neurotransmitter, neurohormone and neuromodulator in insects and is functionally analogue to the vertebrate noradrenaline. In Drosophila, octopamine acts in various physiological processes and behaviours like metabolism, locomotion, sleep, feeding and learning. In a fist model, octopamine has been suggested to exclusively play a role in the formation of an appetitive memory which takes place within the mushroom bodies (MBs). However, recent studies challenge this model: First, dopaminergic neurons were identified to be essential in mediating reward information within the brain. Second, contradictory data now exists on the role of octopaminergic neurons in reward processing: while data exist suggesting these neurons indeed mediate rewarding sugar information within the brain, other data proposes that they only respond to the sweetness of a sugar. Third, recent data even indicate, that octopaminergic neurons may function in aversive olfactory learning as well.
In the present study, we revisited the larval octopaminergic system and its function in memory formation and focus on the following questions: (1) What does the octopaminergic system really do during reward processing? Do these neurons mediate any rewarding information to the MBs or do they rather function in the orchestration of a specific action? (2) Which octopaminergic neurons are involved in reward processing? Do octopaminergic neurons respond to aversive stimuli as well? First data of microfluidic-based imaging experiments suggests, that specific octopaminergic neurons respond to sugars that differ in their nutritional value and sweetness. Further, MB neurons – that harbour olfactory memories – respond to octopamine with increasing intracellular Ca^{2+}-levels in a compartment-specific manner. This is in particular interesting as only 3 of around 80 octopaminergic neurons innervate the MBs. In line with our behavioural experiments, these first experiments suggest a function of octopaminergic neurons in mediating various stimuli in the periphery-to-MB axis.

Poster Session B TISSUE PHYSIOLOGY

P-04 Calcium Signals in Murine Glomerulosa Cells of *Cacna1h* Knock-out Mice

Hoang An Dinh, Ute Scholl and Gabriel Stölting
Charité – Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, BCRT, Augustenburger Platz 1,13353 Berlin, Germany

The steroid hormone-producing cortex of the adrenal glands consists of the inner zona reticularis, zona fasciculata and the outer zona glomerulosa (ZG), producing androgens, cortisol and aldosterone, respectively. Aldosterone plays a crucial role for the maintenance of serum electrolytes and blood pressure. The two main stimuli of aldosterone synthesis are angiotensin II (Ang II) and hyperkalemia. Stimulation of ZG cells leads to oscillatory membrane depolarizations assumed to be linked to the influx of calcium through calcium channels in addition to the release from IP3-sensitive calcium stores. The increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) provides the signal for aldosterone production. It has been reported that voltage oscillations require Cav3.2 (Gene: *Cacna1h*) but it is unclear what the importance of this channel for the influx of calcium is. Loading of acute slice preparations of adrenal glands from *Cacna1h* knockout (KO) and wild-type mice with Fura-2 AM allowed for measurements of $[Ca^{2+}]_i$. ZG cells from WT and KO mice exhibited fast oscillatory changes of $[Ca^{2+}]_i$ clustered in bursts. On average, $[Ca^{2+}]_i$ was up to 21% lower in cells from KO compared to WT mice but exhibits a positive relationship to the extracellular $[K^+]$ and [Ang II] in both genotypes. The spiking frequency was higher in cells from KO than WT mice (1.38 ± 0.98 Hz versus 0.47 ± 0.16 Hz, mean ± SD). Removal of extracellular Ca^{2+} led to the absence of signals in both WT and KO mice and baseline $[Ca^{2+}]_i$ values were lowered. Our results show that Cav3.2 contributes to overall $[Ca^{2+}]_i$. However, cells from KO mice still exhibited oscillatory activity, indicating that additional calcium channels play a role in ZG calcium signal generation.

P-05 The Role of *Clcn2* in the Murine Zona Glomerulosa

Marina Volkert, Ute Scholl and Gabriel Stölting
Charité – Universitätsmedizin Berlin, Department of Nephrology and Medical Intensive Care, BCRT, Augustenburger Platz 1,13353 Berlin, Germany

The zona glomerulosa (ZG) comprises the outer layer of the adrenal cortex, which synthesizes aldosterone. This steroid hormone regulates the volume and electrolyte balance of the body as well as the blood pressure. The serum concentrations of potassium and angiotensin II (AT-II) are the most important stimuli for aldosterone synthesis in the ZG. They lead to oscillatory depolarizations and cause calcium influx into ZG cells, which is the signal for aldosterone production. Aldosterone synthesis can also be increased by other means like changes in serum osmolality. Heterozygous gain-of-function mutations in *CLCN2*, which encodes the chloride ion channel ClC-2, lead to familial hyperaldosteronism through increased calcium signaling of the ZG. The role of ClC-2 in the healthy ZG, however, is still unclear. In order to determine the role of ClC-2 in the ZG, *Clcn2* knockout (KO) and wild type mice were compared. In acute slice preparations of murine adrenal glands, the cytosolic calcium concentration was examined with fluorescent calcium indicators. We confirmed that calcium oscillations in ZG increased with higher extracellular potassium and AT-II concentrations. After an acute change to a hypoosmolar solution, the wild type showed a higher activity than the KO mouse. Following a prolonged exposure to an extracellular hypoosmolar condition, however, the activity in the ZG of the KO mouse was more than 1.4 times higher compared to the wild type. A rapid increase in extracellular osmolality led to an acute decrease in activity by up to 35 % for the wild type and 72 % for the KO. Our results thus suggest that ClC-2 plays a role in the response of the ZG to changes in extracellular osmolality.

P-06 Influence of short chain fatty acids on the redox potential of ovine ruminal fluid *in vitro*

Stefan Rietmann, G. Gäbel, F. Dengler
Institute of Veterinary Physiology, University of Leipzig

Like the colon of monogastric herbivores, the forestomach of ruminants serves as fermentation chamber for the degradation of crude fiber to short chain fatty acids (SCFA; mainly acetate, propionate and butyrate). These SCFA are produced in large amounts and constitute the major energy source for ruminants. In order to guarantee an optimal fermentation, the intraruminal redox potential (RP) has to be kept at a constant negative level to prevent oxidative degradation to CO_2 and H_2O and to steer the microbial fermentation in favor of the production of the SCFA. We were wondering, if these SCFA might influence the RP as well to balance the intraruminal homeostasis.

To investigate this, we collected ruminal fluid from three male cannulated East Friesian Sheep and divided it to six different setups of 80 ml each that were placed in a 39°C water bath. After an equilibration period of 30 minutes, the basal RP, temperature, and pH were measured before either Na-acetate, Na-propionate, Na-butyrate, NaCl, glucose or mannitol were added at a final concentration of 60 mM (pH 6.5). The fluid was mixed regularly, and the measurements were continued for 30 minutes.

The average basal values in all samples were: RP -316 ± 38 mV, pH 6.5 ± 0.07 and 38.4 ± 0.4°C. The temperature increased by approximately 0.5-1 °C in all setups during the measuring period. In all setups, we observed a time-dependent decrease of the RP, i.e. the RP became more negative. The addition of the SCFA had no effect no RP compared to the control group (NaCl). The addition of glucose led to a slightly different time course of RP compared to its control group (mannitol), but there was no significant difference. The pH remained constant in all setups except for the one with glucose addition, which led to a significant drop in pH ($p < 0.001$; $N = 6$).

Summing up, the presence or absence of SCFA does not influence RP or pH in ruminal fluid. However, glucose, which is fermented quickly, affects ruminal pH and may thereby alter the course of RP indirectly. Still, it was kept at similar levels like in the other setups, hinting at potent mechanisms to stave sudden changes of RP. The effect of pH on intraruminal RP will be investigated in further studies.

Poster Session C CELL PHYSIOLOGY AND CANCER

P-07 Expression of acid-sensing ion channels (ASICs) and ASIC-dependent migration in glioblastoma multiforme (GBM)

Klaus-Daniel Cortés Franco, Yuemin Tian, Stefan Gründer
Institute of Physiology, Uniklinik RWTH Aachen, Germany

Glioblastoma multiforme (GBM) is the most common brain tumor in adults, and, incidentally, with the worst survival rate among brain cancers. Acid-sensing ion channels (ASICs) are ligand-gated cation channels, expressed throughout the central nervous system, that respond to extracellular protons, such as an acidic microenvironment, which is common in tumor tissues.

We previously showed that functional ASIC1a and ASIC3 are also expressed in primary GBM stem cell lines. Here, we characterized the expression of ASIC subtypes 1a, 1b, 2, 3 and 4, under physiological and acidic conditions. We were able

not able to detect ASIC2 at all in our primary GBM stem cell lines. Published mRNA level data from patients suggest that ASIC2 expression in biopsies decreases in correlation with the tumor grade, corroborating our findings.
With the interest of characterizing the functional role of ASICs in GBM, we established a method of evaluating sphere migration in vitro to explore ASIC-dependent migration in GBM cell lines. Migration of GBM cells increased drastically in acidic pH, but ASIC1 and ASIC3 did not appear to be a strong factor for GBM migration, making a different pathway likely for activating proton dependent migration. We then studied ASIC2 overexpressed cell lines, showing strong induction of migration in some cell lines, however, not all cell lines show an increase of migration compared to control, further experiments are necessary to determine whether this is a specific ASIC2 dependent effect. Our next steps will be to investigate the role of ASIC1 and ASIC3 knockout and overexpression of ASIC2 in proliferation and migration of GBM cell lines.

P-08 Pancreatic stellate cell activation is coordinated by environmental pH and mechanical stress

Zoltan Pethö[1], Benedikt Fels[1,2], Karolina Najder-Nalepa[1], Albrecht Schwab[1]
[1]Institute of Physiology II, University of Münster, Robert-Koch Str. 27B, 48147 Münster, Germany; [2]Institute of Physiology, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany

Pancreatic stellate cells (PSCs) are stromal cells of the pancreas that become activated in various diseases, notably in pancreatic cancer. Pancreatic cancer is coupled to changes in the extracellular environment of the stroma, including mechanical stiffness and interstitial pH. 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 model and elucidate how changes in extracellular pH (pH_e) and mechanical stress affect the intracellular pH (pH_i) homeostasis and activation of pancreatic stellate cells.
Following isolation, we cultured murine 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-dependent sensors, regulators and transporters of PSCs, we performed RT-qPCR, Western blot and pH_i measurements.

Alkaline pH_e substantially activates PSC 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.
In summary, alkaline pH_e is a potent activator of PSCs that may enable cell cycle progression on stiff substrates through Yap/Taz signaling.
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P-09 Studying the Recruitment of Synaptojanin-2 to Invadosomes, Actin-Based Protrusions

N. Raymond Thata, D. Oliver
Institut für Physiologie und Pathophysiology, Philips Universität Marburg

Podosomes (or invadopodia as described in malignant cells) are cell protrusions found on the ventral side of the cell. They contain an actin rich core and, once matured, are capable of digesting the extracellular matrix. A crucial protein in this maturation process TKS5. It specifically binds the lipid $PI(3,4)P_2$ at the plasma membrane and upon phosphorylation it functions as an adapter protein, capable of recruiting actin modulators like N-Wasp or Cortactin to the structure.
Several lipid-phosphatases are known to be capable of changing the pool of this Phosphoinositide. Some like PTEN (a 3'-Phosphatase capable of depleting the $PI(3,4)P_2$ pool) and SHIP2 (a 5'-Phosphatase capable of increasing the $PI(3,4)P_2$ pool) are well known and understood have been shown to be important for invadosome dynamics. Others like INPP4B (a 4'-Phosphatase also capable of depleting the pool) are not well understood at this time. Initial experiments in NIH 3t3 and MDA cells show that Synaptojanin-2 localizes to the invadosome. Using truncated versions of Synaptojanin2 in co-transfection with Cortactin (as podosome marker) indicates, that the Proline Rich Region is necessary for localization to the podosome.
This project will aim to characterize and indentify how Synaptojanin-2 (capable through its 5'-phosphatase activity of increasing the $PI(3,4)P_2$ pool) becomes recruited to the invadosome as well as indentifying potential binding partners capable of recruiting it into these structures.

LIST OF PARTICIPANTS

1. Bachmann, Michèle – Aachen (P-02)
2. Chaves, Gustavo – Nuremberg
3. Chugunow, Lena – Salzburg
4. Cortes, Klaus-Daniel – Aachen (P-07)
5. Czyrnik, Elena – Essen (O-10)
6. Dengler, Franziska – Leipzig (P-06)
7. Dinh, Hoang An – Berlin (P-04)
8. Fels, Benedikt – Lübeck (P-08)
9. Gillum, Matthew – Kopenhagen (Career Talk)
10. Heinrich, Tobias – Hamburg (O-08)
11. Köhler, Ines – Leipzig (P-03)
12. Kuwabara, Makoto – Marburg (P-01)
13. Lenz, Dominik – Marburg (P-01)
14. Lindner, Laura – Marburg (O-03)
15. Lindner, Moritz – Marburg (O-01)
16. Najder-Nalepa, Karolina – Münster (O-11, P-08)
17. Patejdl, Robert – Rostock (O-07, O-06)
18. Perniß, Alexander – Gießen (O-05)
19. Pethö, Zoltan – Münster (P-08)
20. Rietmann, Stefan – Leipzig (P-06)
21. Ritzau-Jost, Andreas – Leipzig
22. Rugi, Micol – Münster (O-09, O-11)
23. Stölting, Gabriel – Berlin (P-04, P-05)
24. Thata, Raymond – Marburg (P-09)
25. Vogt, Markus – Göttingen (O-06, P-03)
26. Volkert, Marina – Berlin (P-05)
27. Weichard, Iron – Leipzig (O-02)
28. Wrobeln, Anna – Essen
29. Young, Samuel – Münster (O-04)
30. Zhao, Wencai – Marburg