Annual Meeting of the Austrian Physiological Society

October 4th 2018

Paracelsus Medical University

Salzburg

Programme
and
Abstracts

Annual Meeting of the Austrian Physiological Society
Jahrestagung Österreichische Physiologische Gesellschaft

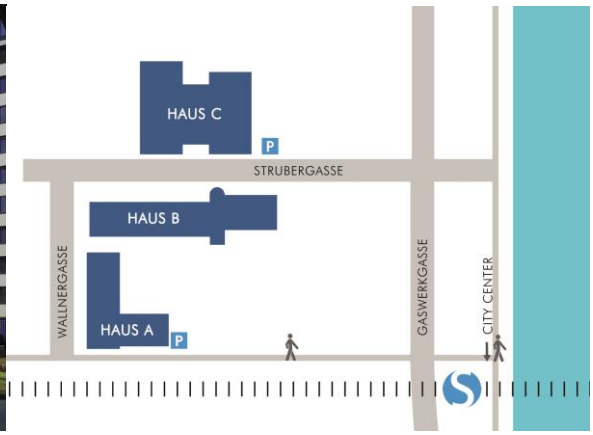
October 4th/4. Oktober 2018

Paracelsus Medical University/Paracelsus Medizinische Privatuniversität Salzburg

Programme/Programm

When	What	Where
09:00 - 09:30	Coffee & Poster mounting	Green Corner
09:30 – 09:50	Welcome	Auditorium Nord
09:50 -10:10	Stozer, Andraz (Maribor): „Beta cell dysfunction in type 2 diabetes“	Auditorium Nord
10:10 – 10:30	Fischer, Michael (Vienna): “A human TRPA1-specific pain model”	Auditorium Nord
10:30 – 10:50	Lechner, Judith (Innsbruck): “Modeling Sex Differences in Vitro”	Auditorium Nord
10:50 – 11:15	Coffee Break	Green Corner
11:15 – 11:35	Kittl, Michael (Salzburg): “Crosstalk between the acid-sensitive outwardly rectifying (ASOR) and the volume-sensitive outwardly rectifying (VSOR) anion channel in microglial cells”	Auditorium Nord
11:35 – 11:55	Goswami, Nandu (Graz): “Lower body Negative pressure: Physiological Effects and Applications”	Auditorium Nord
11:55 – 12:25	Macknight, Tony (ADInstruments): "What do we need for online learning?”	Auditorium Nord
12:25 – 14:00	Postersession & Lunch	Foyer, Green Corner
13:00 – 14:00	Board Meeting/Vorstandssitzung ÖPG	Stiegl Lounge, 3rd floor, House A
14:00 – 15:00	General Assembly/Generalversammlung ÖPG	Auditorium Nord
15:00 – open end	Poster Awards	Auditorium Nord

Venue/Veranstaltungsort:
Paracelsus Medizinische Privatuniversität, Strubergasse 22, 5020 Salzburg
Gebäude/Building: Auditorium Nord, Erdgeschoß, Haus C
Kontakt: Tel.: +43 662/2420-80501, E-mail: physiology@pmu.ac.at



Anreise: Die PMU ist vom Bahnhof sowie vom Zentrum aus zu Fuß in 15 Minuten erreichbar. Sonst mit folgenden öffentlichen Verkehrsmitteln.
Bus: 4 (Haltestelle "Stadtwerk Lehen"), 7 (Haltestelle "Strubergasse"), 24 (Haltestelle "Stadtwerk Lehen"); Flughafen und Salzburg Hauptbahnhof: 2 (Haltestelle "Gaswerkgasse"), 27 – Haltestelle (Landeskrankenhaus (Lindhofstraße)
Train/S-Bahn: S-Bahn-Linien S2 und S3 (Haltestelle “Salzburg-Mülln-Altstadt")

Beta Cell Dysfunction in Type 2 Diabetes

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Objective

Enhanced intracellular signaling at different levels in the stimulus-secretion coupling cascade has been described in states of insulin resistance and it is believed that this is the mechanistic explanation for the hyperinsulinemic compensation and normoglycemia during early stages of development of type 2 diabetes mellitus (Gonzalez et al. 2013). When insulin resistance worsens, even stronger intracellular signals are present in beta cells, thus failure in the stimulus-secretion coupling cascade is probably not fully responsible for early decompensation *en route* to frank diabetes. It has been reported recently that intercellular coupling is reduced in the genetically induced obesity and insulin resistance in *ob/ob* mice (Irles et al. 2015) and also under diabetogenic gluco(lipo)toxicity (Johnston et al. 2016). In our study, we wanted to find out whether disrupted intercellular synchronization of calcium signaling in beta cells in islets from Langerhans plays a pathophysiological role in development of type 2 diabetes.

Methods

Confocal live cell calcium imaging in beta cells in islets of Langerhans from acute pancreas tissue slices of mice of either sex fed a western diet for 8 weeks (between the age of 12 and 20 weeks). Additionally, we performed calcium imaging on isolated human islets from normal and diabetic donors (Stožer et al. 2013a). Long time series of calcium responses to stimulatory glucose concentrations were analyzed off-line, filtered, exported, and similarity of signals between every pair of beta cells in mouse islets and between every two chosen regions in human islets assessed. Based on signal similarity, we constructed graphs and quantified the functional connectivity patterns in islets from normal and diabetic mouse and human islets (Stožer et al. 2013b).

Results

In islets from both western-diet induced obese mice and diabetic human donors, the correlation between calcium oscillations in different cells was lower than in islets from control animals and non-diabetic donors. This was also reflected in functional connectivity patterns that showed a significantly reduced number of connections between cells compared with normal islets.

Conclusions

Intercellular connectivity seems to be affected early in the pathogenesis of type 2 diabetes mellitus and is a promising new target for preventive and therapeutic interventions.

Accompanying posters

Korošak D, Slak Rupnik M. 2018. Collective sensing of beta cells generates the metabolic code.
Dolenšek J, Skelin Klemen M, Gosak M, Križančić Bombek L, Pohorec V, Slak Rupnik M, Stožer A. 2018. Glucose-stimulated calcium dynamics in murine beta cells: concentration dependence.
Skelin Klemen M, Gosak M, Paradiž E, Pohorec V, Dolenšek J, Križančić Bombek L, Slak Rupnik M, Stožer A. 2018. The role of cyclic AMP and Epac2 in beta cell calcium dynamics.
Markovič R, Stožer A, Dolenšek J, Perc M, Slak Rupnik M, Marhl M, Gosak M. 2018. Phenomenological modelling and numerical simulations as a key towards understanding of complex spatio-temporal responses of pancreatic beta cells to glucose stimulation.
Dolenšek J, Valladolid-Acebes I, Paradiž E, Gosak M, Skelin Klemen M, Križančić Bombek L, Pohorec V, Marjan Slak Rupnik M, Brismar K, Stožer A. 2018. Beta cell calcium dynamics and electrophysiological responses in a western diet-induced mouse model of Diabesity.

References

Gonzalez A, Merino B, Marroqui L, Neco P, Alonso-Magdalena P, Caballero-Garrido E, Vieira E, Soriano S, Gomis R, Nadal A, Quesada I. 2013. Insulin hypersecretion in islets from diet-induced hyperinsulinemic obese female mice is associated with several functional adaptations in individual beta-cells. *Endocrinology* **154:** 3515-3524.
Irles E, Ñeco P, Lluesma M, Villar-Pazos S, Santos-Silva JC, Vettorazzi JF, Alonso-Magdalena P, Carneiro EM, Boschero AC, Nadal Á, Quesada I. 2015. Enhanced glucose-induced intracellular signaling promotes insulin hypersecretion: Pancreatic beta-cell functional adaptations in a model of genetic obesity and prediabetes. *Molecular and Cellular Endocrinology* **404:** 46-55.
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Stožer A, Dolenšek J, Rupnik MS. 2013a. Glucose-Stimulated Calcium Dynamics in Islets of Langerhans in Acute Mouse Pancreas Tissue Slices. *PLoS ONE* **8:** e54638.
Stožer A, Gosak M, Dolenšek J, Perc M, Marhl M, Rupnik MS, Korošak D. 2013b. Functional Connectivity in Islets of Langerhans from Mouse Pancreas Tissue Slices. *PLoS Comput Biol* **9:** e1002923.

A human TRPA1-specific pain model

Stefan Heber[1], Michael Fischer[1]

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Objective

The cation channel TRPA1 plays an important role in the perception of pain. One of its major functions is to act as a sensor for irritating and potentially hazardous electrophilic substances. TRPA1 involvement has been demonstrated for several diseases. However, for TRPA1 in particular, species differences are pronounced and limit translational research. Therefore, a human TRPA1-specific pain model was established. Our own previous study used carvacrol as TRPA1-agonist. While there is no doubt about TRPA1 activation, the required concentration was high, and a specific antagonist only allowed for a partial (31%) inhibition (Schwarz et al., 2017). JT010 is the most potent published and commercially available agonist, a half-maximal activation in the nanomolar range was reported (Takaya et al., 2015). Using this substance and the so far most potent available antagonist A-967079 we established the first TRPA1-specific pain model.

Methods

With approval of the ethics committee, 16 subjects were tested in two sessions in a double-blind manner. The first session introduced to the experimental paradigm and contained an in injection of phosphate buffered synthetic interstitial fluid, without addition as control and with capsaicin as a painful reference. Intracutaneous injections into the volar forearm were rated every five seconds. In a second session, multiple concentrations of JT010 with and without antagonist were applied in a double-blind manner with a pre-randomized sequence.

Results

JT010 caused a higher pain rating than control injections ($p < 0.001$, t-test). Pain ratings peaked after 30 s and fully subsided in less than five minutes. The JT010-induced pain was concentration-dependently inhibited by the TRPA1 antagonist, almost complete inhibition (98%) could be achieved. Special handling of the substance is required due to the loss observed when in contact to polypropylene.

Conclusions

This specific model provides a new tool to validate new drug candidates with respect to their efficacy in humans.

Modeling sex differences in vitro

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Women are not small men. Every cell in the body is female or male depending on its chromosomal make-up. Sex and sex hormones affect all the cells in the body resulting in different susceptibility to cellular stressors and disease development. Since women and men were found to potentially respond differently to therapeutics, inclusion and analysis of women in clinical trials is mandatory to prevent unpredicted effects. Researchers engaged in basic research and preclinical studies are increasingly challenged by regulatory bodies, major funding agencies, and high ranked journals to also include sex as a biological variable into their study designs [1]. At present, these requirements are mostly met – if at all – through animal experimentation. *In vitro* models suffer from cells losing their sex memory with time in culture as shown for established cell lines.

Human induced pluripotent stem cells (iPSC) generated from human somatic cells present a new trajectory for modeling sex differences. In order to introduce hiPSC as novel *in vitro* models for integrating sex as a biological variable into basic and preclinical research designs a thorough validation and comparison to cells in vivo is required.

Sex differences originate from male and female specific gonad development giving rise to lifelong exposure with sex hormones, which display organizational (persistent) and activational (transient) effects as detailed in [2]. In addition, XX and XY cells are distinct regarding their epigenomic imprinting, transcriptome, and proteome, thus being different independent of sex hormone exposure, but also responding differently to sex hormones.

Developmental processes and hormonal cycles in the different life stages of women and men need to be taken into consideration when developing novel *in vitro* models. For this purpose, cell culture conditions during reprogramming, stem cell propagation and differentiation need to be adapted in order to establish valid models for researching sex as a biological variable *in vitro* by using hiPSC. Once established, these models are expected to provide improved preclinical data allowing to account for sex differences and genetic variability avoiding problems arising from species differences between animals and humans.

1. Clayton, JA: Studying both sexes: a guiding principle for biomedicine. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* 30**:** 519-524, 2016.
2. Arnold, AP: The organizational–activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Hormones and Behavior,* 55**:** 570-578, 2009.

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

Michael Kittl[1,2], Katharina Helm[1,4], Marlena Beyreis[1], Markus Ritter[1,3,4], Martin Jakab[1]

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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. Mechanism regulating pH are especially important in the brain because predominant high electrical activity elicits rapid H^+ movements to acidify or alkalise intra- or extracellular compartments. In case of ischemic brain damage or any neuronal damage, pH regulating mechanism fail to maintain a constant pH and consequently acidosis in these regions occurs. Acidification has been shown to influence the electrical behaviour of numerous cell types, including microglial cells in the brain. *In vitro*-induced extracellular acidosis revealed an outwardly rectifying current, which has been later identified as a chloride (Cl^-) current (ASOR – acid-sensitive outwardly rectifying). This acid- sensitive current displays many biophysical similarities to the well investigated volume-dependent Cl^- current (VSOR – volume- sensitive outwardly rectifying); i.e outward rectification, sensitivity to Cl^- blockers and ion permeability sequence. Accordingly, in the literature there is an upcoming controversial discussion about whether the same or different channel entities underlie both, ASOR and VSOR currents.

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 (outward current) and smaller values and an initial peak at negative potentials (inward current). Long-term measurements revealed that the outward current remains constant, whereas the inward current increases over time. This current can be elicited repeatedly in the same cell by alternating neutral and acidic salines and can be inhibited by Cl^- channel blockers like DCPIB, NPPB and DIDS. Moreover, the application of an external acidic solution depolarizes V_{mem} to the reversal potential of Cl^- and increases the MCV. The increase in MCV is augmented by the Cl^- channels blockers DCPIB and DIDS. Regarding the relation between ASOR and VSOR current, we could show several similarities, including the pharmacological profiles, the ion permeability sequence (I^- > Cl^- > gluconate) and outward current rectification. Both are unaffected by substitution of Na^+, depolarize V_{mem} upon activation and affect cell volume regulation. Current activation over time at constant positive holding potentials of the ASOR current is, however, clearly different from VSOR, which inactivates over time. Concurrent exposure to hypotonicity and acidic conditions does not cause simultaneous activity of both currents. Rather, after a short time frame in which both currents apparently coexist, the current rapidly 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 but also distinct differences to the volume-sensitive Cl^- current (VSOR). If both currents are mediated by the same or different channel entities, which give rise to ASOR or VSOR phenotypes depending on modulating factors like pH or osmolality, needs to be further investigated.

Lower Body Negative Pressure: Physiological Effects, Applications and Implementation

Nandu Goswami[1], Andrew Phillip Blaber[2], Helmut Hinghofer-Szalkay[1], Victor A. Convertino[3]

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3. *Battlefield Health & Trauma Center for Human Integrative Physiology, Combat Casualty Care Research Program, US Army Institute of Surgical Research, JBSA Fort Sam Houston, USA.*

This talk presents lower body negative pressure (LBNP) as a unique tool to investigate the physiology of integrated systemic compensatory responses to altered hemodynamic patterns during conditions of central hypovolemia in humans. An early review published in Physiological Reviews over 40 years ago focused on the use of LBNP as a tool to study effects of central hypovolemia, while more than a decade ago a review appeared which focused on LBNP as a model of hemorrhagic shock. Since then there has been a great deal of new research which has applied LBNP to investigate complex physiological responses to a variety of challenges including orthostasis, hemorrhage and other important stressors seen in humans such as microgravity encountered during spaceflight.

The LBNP stimulus has provided novel insights into the physiology underlying areas such as intolerance to reduced central blood volume, gender differences concerning blood pressure regulation, autonomic dysfunctions, adaptations to exercise training, and effects of space flight. Furthermore, approaching cardiovascular assessment using prediction models for orthostatic capacity in healthy populations - derived from LBNP tolerance protocols - has provided important insights into the mechanisms of orthostatic hypotension and central hypovolemia, especially in some patient populations as well as in healthy subjects. This talk also presents a concise discussion of mathematical modeling regarding compensatory responses induced by LBNP.

Given the diverse applications of LBNP, it is to be expected that new and innovative applications of LBNP will be developed to explore the complex physiological mechanisms that underline health and disease.

Keywords: Central hypovolemia; LBNP tolerance; Cerebral blood flow; Hormones; Hemorrhage; Gender; Autonomic responses.

Postural Hemodynamic Parameters in Older Persons Have a Season-Dependency

Irhad Trozic[1*], Dieter Platzer[2*], Franz Fazekas[3], Alexander I. Bondarenko[4], Bianca Brix[1], Andreas Rössler[1], Nandu Goswami[1]

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2. *Section of Biophysics, Medical University of Graz, Austria*
3. *Department of Neurology, Medical University of Graz, Austria*
4. *Bogomoletz Institute of Physiology of NAS, Kiev, Ukraine*

Objectives: The regulation of blood pressure (BP) during upright standing depends on many factors. It is well known that the effects of temperature on the cardiovascular system and BP values are higher in winter months. But there is a lack of studies that have analysed how the cardiovascular system in post-stroke patients responds to postural changes during cold and warm months. The aim of this study was to examine how temperature in two seasons (cold months and warm months) affects the responses of hemodynamic mechanisms and heart rate variability during a sit-to-stand test in stroke patients and non-stroke participants.
Methods: We investigated 41 participants: stroke (n= 16) and age-matched non-stroke participants (n=25), age >55 yrs, during a sit-to-stand test (5 min of sitting followed by 5 min of standing) in two different seasons. We used a Task Force Monitor® device to continuously monitor beat to beat systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), stroke index (SI) and cardiac index (CI), and power spectral analysis of the heart rate variability (HRV).
Results: Mean values of baseline DBP (stroke: 90.624 ± 4.522; non-stroke participants: 84.846 ± 2.018) and MBP (stroke: 107.803 ± 5.084; non-stroke participants: 103.318 ± 4.1825) were significantly higher during colder months compared to warmer months in both groups. Mean values of baseline SI (stroke: 26.820 ± 1.552; non-stroke participants: 28.781 ± 1.4382), and CI in non-stroke participants (2.142 ± 0.1052) were found to be significantly lower during colder months. After standing, there was a significant decrease in SBP, and MBP values in non-stroke participants only and stroke patients in warmer months only.
Conclusions: Our study of the effects of seasonal variations in postural blood pressure changes and differences in postural blood pressure change between stroke and non-stroke participants shows that BP values depend on season, being higher in colder months in both groups. Furthermore, standing up in warmer months in non-stroke participants leads to a significant decrease in BP but not in stroke patients. Since postural instability and falls are common in older persons, the results of our study point for a season-dependent reduction in blood pressure during standing up in older persons that could predispose such persons to orthostatic intolerance and falls in warmer months.

Key terms: Stroke; seasons, postural changes, orthostatic intolerance; ageing.

Phenomenological modelling and numerical simulations as a key towards understanding of complex spatio-temporal responses of pancreatic beta cells to glucose stimulation

Rene Markovič [1, 2, 3], Andraž Stožer [4], Jurij Dolenšek [1, 4], Matjaž Perc [1, 5, 6], Marjan S. Rupnik [4, 7], Marko Marhl [1, 2, 4], and Marko Gosak [1, 4]

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Objective

Insulin producing beta cells in pancreatic islets of Langerhans form a complex syncytium that responds to stimulation by glucose with a heterogeneous and non-trivial spatio-temporal activity pattern. To gain a deeper insight into this complex experimentally observed dynamical behavior, we build up a phenomenological model of coupled excitable cells and explore empirically the model's necessities that ensure a good overlap between computational and experimental results.

Methods

We employed functional high-speed multicellular calcium imaging of fluorescently labelled acute mouse pancreas tissue slices to record calcium signals in a large number of cells simultaneously. To mathematically describe the dynamics of beta cell population we utilize a phenomenological model of coupled electrically excitable cells. We additionally introduce delayed feedbacks and heterogeneity to some model parameters.

Results

Our results reveal that the responses of beta cells to stimulation with glucose are biphasic and glucose-dependent. Under physiological as well as under supraphysiological levels of stimulation the initial activation phase is followed by the plateau phase with a high number of global intercellular calcium waves. However, the activation phase under lower stimulation levels exhibits a very heterogeneous dynamical behavior with a progressive recruitment of cells, whereas the activation phase provoked by high stimulatory conditions is considerably different: its more rapid and less continuous. Our mathematical model can firmly reproduce the experimentally observed complex dynamical patterns if a combination of heterogeneous and stimulus-dependent time lags, variability in excitability levels and a heterogeneous coupling are included into the model.

Conclusions

The proposed phenomenological modelling approach that involves very few parameters is not only beneficial for getting insights into basic mechanisms that shape the cellular responses, but also points out the various attributes that should be considered by building up comprehensive and realistic beta cell models.

Glucose-stimulated calcium dynamics in murine beta cells: concentration dependence

Jurij Dolenšek [1,2], Maša Skelin Klemen[1], Marko Gosak[1,2], Lidija Križančić Bombek[1], Viljem Pohorec[1], Slak Rupnik M. [1,3], Stožer A[1].

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Objective

Glucose is a well known and potent secretagogue for beta cells, triggering calcium oscillations, and ultimately resulting in insulin release. Quantity and dynamics of the insulin release depends on the stimulus intensity, however coding properties of beta cells underlying insulin release remain only partially described

Methods

We used fresh murine tissue slice approach to assess calcium dynamics in a large population of beta cells simultaneously. We systematically assessed beta cell response to near-threshold and up to supraphysiological glucose concentrations, with unprecedented spatial and temporal cellular resolution.

Results

Following stimulation, activation of cells displayed dose-dependent and relatively high heterogeneity, and activation profile displayed dose-dependent size of clusters. Deactivation, on the other hand, displayed inverse glucose dependence. Following initial response, the plateau phase of the response expressed dual coding properties: physiological concentrations affected predominantly the frequency of oscillations whereas supraphysiological concentrations affected predominantly the duration of oscillations. Combined effect resulted in linear increase in active time over a wide concentration range, a good predictor of concentration range described for insulin secretion. Moreover, beta cells formed functional networks that became increasingly efficient with increasing glucose concentration. We also demonstrate that calcium waves originate from distinct and separate locations. While some cells serve as hubs with most connections to remaining cells, these cells are not acting as wave initiators in either glucose concentration.

Conclusions

Novel understanding of coding properties in beta cells is presented, and its deciphering may have repercussions on understanding the normal physiology of glucose homeostasis as well as disturbances of glucose homeostasis such as diabetes mellitus.

The role of cyclic AMP and Epac2 in beta cell calcium dynamics

Maša Skelin Klemen[1], Marko Gosak[1,2], Eva Paradiž[1], Viljem Pohorec[1], Jurij Dolenšek[1], Lidija Križančić Bombek[1], Marjan Slak Rupnik[1,3], Andraž Stožer[1]

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Objective

Insulin secretion in beta cells is mainly stimulated by nutrients such as glucose, but various hormones and neurotransmitters are also important for optimal beta cell function. They alter the production of intracellular second messengers, among which cyclic adenosine monophosphate (cAMP) is the most important for amplification of insulin secretion. Cyclic AMP augment insulin secretion by activation of PKA- and Epac2- dependent pathways which are still not fully understood. One of the many proposed mechanisms is the enhancement of Ca^{2+} signals either by voltage-dependent Ca^{2+} entry or intracellular Ca^{2+} mobilization.

Methods

To clarify the role of cAMP in Ca^{2+} dynamics we analysed the impact of forskolin on beta cells from C57BL/6 mice. More specifically, we assessed the $[Ca^{2+}]_i$ dynamics in beta cell populations with electro- and opto-physiological approaches combined with the acute tissue slice technique, supported by network-based analyses.

Results

In the absence of forskolin, substimulatory glucose concentration (6 mM) failed to increase intracellular Ca^{2+}, while stimulatory glucose concentration (12 mM) evoked synchronized high frequency Ca^{2+} oscillations superimposed on slower basal oscillations. Addition of forskolin to 6 mM glucose triggered high frequency Ca^{2+} oscillations, but with a delay of several minutes. When forskolin was added to 12 mM glucose, the frequency of Ca^{2+} oscillations increased significantly compared to glucose only. Despite a modest decrease in durations of individual oscillations, the relative active time increased by more than 50 %. Furthermore, the beta cell functional networks become denser in the forskoline regime, thereby pointing out a higher degree of synchronicity. To determine which of the two cAMP-dependent pathways was responsible for augmented Ca^{2+} oscillations, the same sets of experiments were performed on the pancreatic slices from mice lacking the Epac2 protein. In this case, qualitatively very similar behaviour was observed.

Conclusions

Cyclic AMP augments Ca^{2+} dynamics in mouse pancreatic beta cells independently of Epac2. These results corroborate previously published data describing that phosphorylation of several targets by PKA is responsible for cAMP-augmented Ca^{2+} oscillations in pancreatic beta cells.

Beta cell calcium dynamics and electrophysiological responses in a western diet-induced mouse model of diabesity

Jurij Dolenšek[1], Ismael Valladolid-Acebes[2], Eva Paradiž[1], Marko Gosak[1], Maša Skelin Klemen[1], Lidija Križančić Bombek[1], Viljem Pohorec[1], Marjan Slak Rupnik[1,3], Kerstin Brismar[2], Andraž Stožer[1]

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Objective

We have recently introduced the acute mouse pancreas tissue slice preparation in conjunction with electrophysiological, optophysiological, and novel complex network approaches to study stimulation-secretion coupling *in situ*. We extend the methodology to the study of the calcium signaling and calcium-secretion coupling, as well as coupling between different cells at different glucose concentrations in a mouse model of western diet-induced obesity, characterized by adult onset type 2 diabetes.

Methods

Western-diet (WD) containing 40 % fat was fed to C57BL/6 male mice, starting at 12 weeks of age. We characterized the model by measuring body weight, blood glucose, insulin and triglycerides and performing *in vivo* ipGTT and ipITT. From the same animals we obtained acute pancreatic tissue slices that were further subdued to confocal imaging of intracellular calcium dynamics and electrophysiological assessment.

Results

8 weeks of WD induced profound diabesity that resulted in increase in body weight (38+-1g vs. 30+-1g), hyperglycemia, hyperinsulinemia, hypertriglyceridemia, glucose intolerance, and insulin resistance. The calcium response of beta cells was qualitatively typical for glucose stimulation, displaying high frequency oscillations superimposed on slower basal oscillations. The only parameter that seemed to be changed was enhancement, i.e. change in frequency and duration in response to increasing glucose concentration. Importantly, the coupling between beta cells at medium glucose concentrations (9 mM) were lower in WD-fed mice. The sensitivity of calcium-secretion coupling was also not affected by WD, as the concentration triggering the first phase of slow-photolysis-induced exocytosis was about 5 μM (EC50) for both WD-fed and control mice.

Conclusions

In sum, we found evidence that already after 8 weeks of WD, early dysfunction of beta cells is detectable. Developing relevant mouse model of western-diet induced obesity may serves as a platform to gain novel knowledge on the development and function of human diabetic diseases.

Cerebello-cortical inhibition changes in response to peripheral somatosensory input

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Objective
Monitoring, processing and integration of sensory inputs by the cerebellar circuitry is important for learning and adaptation in order to anticipate sensory events. The aim of this transcranial magnetic stimulation (TMS) study is to investigate the cerebellar involvement in the formation of cortical plasticity in response of vibrotactile hand stimulation.

Methods
The activation of the cerebello-thalamo-cortical networks in response to vibrotactile hand stimulation was assessed using TMS and twin coil for conditioning stimulation of the ipsilateral cerebellum and test stimulation of the contralateral motor cortex. Motor evoked potentials (MEP) were recorded from the right first dorsal interosseus muscle. The cerebellocortical inhibition (CBI) was tested before and after hand stimulation at 25Hz for 20min (HANDSTIM) versus SHAM in 17 healthy subjects in within subject design. Additionally vibration threshold (using a bone conduction probe of a clinical audiometer) and motor performance (Pegboard test) were evaluated.

Results
Following HANDSTIM the normalized MEP amplitude was increased which shows reduced effect of CBI. The vibration threshold was decreased for 30min while the Pegboard test motor performance remained unchanged. No effects were found in response to SHAM stimulation.

Conclusions
The reduction of CBI in response to hand vibrotactile stimulation confirms the cerebellar involvement in mediating the sensory information to the cortex and will be compared in a follow-up study to patients with an infarct on the territory of the posterior inferior cerebellar artery. These patients often show sensorimotor/learning deficits, whereby the processes staying behind these effects are still not clarified.

Abstract Nr. 12

Epidemiology of the Antiphospholipid syndrome – a retrospective study at the University Hospital Graz, Austria

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Established epidemiologic data suggest gynaecotropism of the antiphospholipid syndrome (APS). In this study an exploratory, retrospective study with participation of six clinical departments of the University Hospital Graz was performed. Epidemiological and disease-related data were collected on the topic to compare the epidemiology of the antiphospholipid syndrome in the catchment area of the University Hospital Graz with data from the literature.

Design: 120 of a total of 311 patients fullfilled the „Sidney Criteria“ for definite antiphospholipid syndrome and were included in the study, 72 females, 48 males.

Results: Females were diagnosed at the age of 40.5 ± 17.3 years, males at 50.5 ± 17.2 years. 64.2% suffered from primary antiphospholipid syndrome (PAPS), 33.3% from secondary antiphospholipid syndrome (SAPS) and 2.5% had a catastrophic antiphospholipid syndrome (CAPS).

Conclusion: Our study confirmed the data from the literature. The antiphospholipid syndrome at our clinic showed the known and expected gynaecotropism. The gender dimorphism based on the age at diagnosis was significant as males were about 10 years older when APS was diagnosed. However, thrombotic events and presence of the different autoantibodies did not show any significant gender differences. From our results we conclude that women are diagnosed more likely for APS than men and they also suffer from it much earlier. However, the clinical and laboratory severity of APS seems to be similar or at least not significantly different for both sexes.

Effects of Complete Decongestive Therapy in Lymphedema Patients: a Pilot Study

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Objective

Lymphedema is manifested in a chronic swelling that arises due to stasis in the lymphatic flow. No cure is currently available. A non-invasive treatment of this chronic disease is a three week complete decongestive therapy (CDT), consisting mainly of bandaging and compression to control the swelling. As CDT lead to mobilization of several liters of fluid from the affected body part, we investigated what effect CDT has on lymphatic outflow (assessed using hyaluronic acid levels as surrogate marker), volume regulating hormones (aldosterone, plasma renin activity) and total plasma protein as well as plasma density, osmolality and selected electrolytes (chloride and sodium).

Methods

In this pilot study, we assessed hyaluronic acid levels and volume regulating hormone responses in 9 patients (n=3 males, n= 6 females, aged between 25 and 70 years) with lymphedema stage II-III before and after three weeks of CDT**.**

Results

The main findings of this novel study are that lymphedema patients lost volume as well as weight due to therapy in the affected extremety. Hyaluronic acid decreased almost by half pre- and post-therapy but due to the high variance between patients no significance was seen. Volume regulating hormones reflected partly the fluid shift effects of CDT: aldosterone increased significantly after therapy while plasma renin activity increased, but not significantly. Plasma total protein, density, osmolality and sodium and chloride levels did not show differences after CDT.

Conclusions

To our knowledge, no study has investigated the effects of CDT on volume regulating hormones or electrolytes. To identify the time course of volume regulating hormones as well as lymphatic flow changes induced by CDT, future studies should assess these parameters serially over the three weeks of therapy.

Keywords: hyaluronic acid, lymphatic flow, volume regulation

Collective computation and metabolic code in pancreatic islets

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Objective

Major part of a pancreatic islet is composed of β-cells that constantly communicate with each other using both direct, short-range interactions through gap junctions, and paracrine long-range signalling. When stimulated by specific ligands, primarily glucose, β-cells collectively respond with expression of a series of transient Ca^{2+} changes on several temporal scales. Here, we assess how both direct, short-range interactions, and paracrine long-range signaling shape collective sensing and cell behavior in islets that leads to insulin release.

Methods

We re-analyze a set of Ca^{2+} spike trains recorded in acute rodent pancreatic tissue slice under physiological conditions. We use a simple spin glass-like model for the functional network of a β-cell collective to describe collective response and we argue that Ca^{2+} spike trains produced by collective sensing of β-cells constitute part of the islet metabolic code that regulates insulin release and limits the islet size.

Results

We found strongly correlated states of co-spiking cells coexisting with mostly weak pairwise correlations widespread across the islet. Furthermore, the collective Ca^{2+} spiking activity in islet shows on-off intermittency with scaling of spiking amplitudes, and stimulus dependent auto-associative memory features.

Pregnancy complications – need for lifetime follow-up?

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Objective

It is well established that pregnancy complications such as preeclampsia are associated with increased risk for cardiovascular disease in later life. The later risk of dying from cardiovascular disease is even dramatically enhanced, if the triad of preterm birth, low birthweight of the offspring, and preeclampsia was present. However, pregnancy may also be seen as a window of opportunity to improve long-term maternal health. Therefore, we aimed to investigate whether chronic stress experience and preeclampsia may have additive adverse effects on the cardiac ability to flexibly adapt to challenge, that is, to mount an appropriately vigorous heart rate response to an acute psychological challenge, or whether they may perhaps have synergistic effects (e.g., mutual augmentation of effects). Blunted cardiac responding to challenge has been linked to poor health outcomes in the longer term.

Methods

Women previously affected by preeclampsia and women after uncomplicated pregnancies were tested 15–17 weeks post-partum in a standardized stress-reactivity protocol, while cardiovascular variables were simultaneously recorded. Changes in heart rate and blood pressure in response to the stressor were analyzed with regard to the effects of history of preeclampsia and chronic stress experience.

Results

Findings indicated blunted cardiac responses in women with higher chronic stress experience ($p = 0.020$) and, independently from that, in women with a history of preeclampsia ($p = 0.018$), pointing to an additive nature of the effects of preeclampsia and chronic stress on impaired cardiovascular functioning. Consequently, if both are present, a history of preeclampsia may add to the already deleterious effects of the experience of chronic stress.

Conclusions

The present study once more supported the notion that some implications of the pregnancy specific disorder preeclampsia extend to at least post-partum and perhaps even beyond that. While is premature to deduce the necessity of early interventions for improving the long-term outcomes of affected mothers from these findings, it seems about time to provide aftercare programs to mothers with previous preeclampsia. These should be targeted at recognizing and treating hypertension, diabetic metabolic dysfunction, and dyslipidemia at an early stage, in order to mitigate or postpone potentially hazardous negative consequences for the vascular system.

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Radon Registry Study

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Objective
Curative Radon (Rn) treatments for patients who suffer from inflammatory and degenerative diseases of the musculoskeletal system or chronic ailments of the skin and respiratory system have a long tradition in the Gastein Valley. Different clinical studies demonstrated that Rn therapy can cause a significant reduction of pain as well as a significant enhancement of functionality.

Methods
The purpose of the Radon Registry Study is to evaluate the modification of health related parameters before and after cure treatment, as well as three, six and nine months later. Those parameters will be collected by quality of life, pain and disease activity questionnaires.
Simultaneously the received physical therapies and Radon treatments will be evaluated. The main target is the identification of correlations between the cure treatments, applied Rn intensity and the improvement of patients health status. Patients that fulfil defined inclusion criteria and suffer from Osteoarthritis, Rheumatoid Arthritis, Ankylosing Spondylitis or Back Pain, can participate in the Radon Registry Study.

Results
The first study participants were included back in March 2016. Until now the analysed data of the questionnaires, reveal that the parameters for quality of life and pain show a significant improvement after cure in all indications. Similar data are illustrated in the disease-specific questionnaires.

Conclusions
These preliminary data exhibit that the Rn cure treatment adduces a positive effect in the investigated parameters. In the long term the comparison of cure effectiveness against duration, type and intensity of treatments should bring an insight into the way Rn acts in patients.

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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, that is, generation of clones. The ability of cells to generate clones is interpreted as a trait of aggressive tumor cells that potentially harbor tumor-initiating. As a consequence, the clonogenic assay is used in cancer research to test primary tumor cells, tumor cell lines and tumor cell subpopulations for their ability to generate clones. The standard protocol uses 6-well or 24-well format for seeding and counting of clones, limiting the number of samples (and proper controls) that can be handled on one plate. Moreover, interpretation of the clonogenic assay is based on endpoint-analysis by colony staining (e.g. with crystal violet). We here describe a new and modified protocol for the clonogenic assay that miniaturizes the method in the 96-well microplate format and utilizes the confluence detection method for non-endpoint and continuous measurement of clonogenic growth. To demonstrate the robustness and applicability of our protocol we used PTC-209 as a model substance to inhibit colony formation.

Methods

Biliary tract cancer cells (HuCCT-1) were seeded in the 96-well microplate format using low seeding numbers (60 cells per well). Clonogenic growth was evaluated after 5, 6 and 7 days using the confluence detection function of a Spark multimode reader (Tecan, Grödig, Austria). The cytotoxic effect of PTC-209 on cell growth of HuCCT-1 cells was measured using the resazurin assay and an appropriate concentration was chosen for the subsequent clonogenic growth assay. The effect of PTC-209 on clonogenic growth was measured using the described setup. For evaluation, confluence pictures were analyzed with the “Analyze Particles” function of ImageJ, using appropriate settings for size and circularity to allow non-endpoint counting of colonies.

Results

By comparing conventional pictures and confluence pictures, we observed that the confluence detection function of the multimode reader was able to reliably detect colonies. Next, using different seeding concentrations, we were able to miniaturize the clonogenic assay in the 96-well microplate format: based on visual assessment and quantification of the number of colonies, a seeding density of 60 cells per well led to reproducible results. Moreover, we were able to demonstrate that non-endpoint measurement using the confluence detection function in the same well allows for resolution of (expected) time-dependent effects. We observed an increase of the colony mean size over time. In contrary, the amount of colonies declined over time, due to merging of individual colonies that showed initial spatial proximity. Importantly, using our new protocol, we were able to resolve the expected cytotoxic effect of PTC-209 on clonogenic growth: at concentrations ≥ 0.60 µM, PTC-209 abolished 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.

Conclusions

In the present project, we developed a new protocol for the clonogenic assay. 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 measurement of clonogenic growth. Moreover, the protocol allows for meaningful time-resolved and non-endpoint measurement and the inclusion of appropriate controls and technical duplicates in the same plate.

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Continuous, label-free, 96-well-based determination of cell migration using confluence measurement

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Abstract based on manuscript submitted to *Cell Adhesion and Migration* (current status: accepted)

Objective
Cellular migration is fundamental in many physiological and pathological processes. Migration and invasion of tumor are prerequisites for formation of secondary malignancies – the leading cause for cancer-related deaths. Unsurprisingly, investigation of cellular migration represents a powerful tool in cancer research. Commonly used assays include the scratch assay and the gap closure assay (cell exclusion assay). Although easy to setup, the scratch assay has several disadvantages as the process of scratching might stress cells at the border and consequently alter their migratory behavior. Moreover, reproducibility is limited, as size and shape of scratches differ between laboratories. The gap closure assays measures cellular migration into a cell-free area, which is defined before cell seeding by a gel spot, thereby avoiding the limitations of the scratch assay. However, monitoring of migration into the cell-free area is often performed at defined time points for practical reasons, thereby limiting the significance of the assay regarding migration kinetics of cells and/or effects of substances on migration. We here present a modified protocol and analysis approach of the gap closure assay using the confluence detection function of a multimode reader to allow continuous measurement of cellular migration for a time period of 48 hours under cell culture conditions. To demonstrate the applicability of our approach we included the Na^+/K^+-ATPase inhibitor ouabain and cytochalasin D as model substances to inhibit migration as well as the epidermal growth factor (EGF) as a model substance to enhance migration.

Methods
A549 lung carcinoma cells were used as a model cell line with high migratory capacity. Possible cytotoxic effects of ouabain, cytochalasin D and EGF treatment were evaluated with the resazurin assay and a Spark multimode reader (Tecan, Grödig, Austria). The Radius™ 96-Well Cell Migration Assay (Cell Biolabs) was used to measure cellular migration on a Spark multimode reader. Confluence pictures were taken every 30 minutes. Serum-free media was used for the experiments to avoid extensive proliferation.

Results
The confluence detection function was able to reliably detect and measure gap closure of A549 cells (untreated, ouabain-treated, EGF-treated) over a time period of 48 hours. EGF promoted cellular migration, whereas ouabain and cytochalasin D inhibited gap closure. Quantification of the confluence measurements was performed in several steps. First, we plotted the increase of the confluent area over time. We next plotted the decrease of the cell-free area over time as well as the increase of the confluence in the cell free area, representing cellular migration. Curve fitting of these results was possible with high quality using a Michaelis-Menten function. Based on this fit, we calculated the required time of untreated, ouabain-treated, cytochalasin D-treated and EGF-treated A549 cells to occupy a certain percentage of the cell-free area. As expected, EGF-treated cells showed enhanced migration into the non-confluent area, whereas ouabain and cytochalasin D treatment resulted in delayed migration.

Conclusions
Continuous measurement of cellular migration is important to gather detailed information about migration kinetics and the effect of enhancers and inhibitors of migration. We here describe a new protocol that uses the confluence detection function of a multimode reader to allow such continuous monitoring. Analysis of the confluence measurement results indicate robustness of our protocol and allow detailed investigation of experimental setups and physiological/pathophysiological questions.

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 dimethylates lysine 9 at histone 3 (H3K9me2) – an epigenetic mark linked with transcriptional repression. Although G9a is required for many physiological processes, its association with cancer development and progression is evident. Overly active G9a is involved in cancer metabolism, metastasis, cell survival and response to hypoxia. Moreover, 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 therefore aimed at investigating a potential role of G9a in BTC for the very first time.

Methods
Sixty-eight (68) cases of formalin-fixed, paraffin-embedded BTC samples archived between 1997 and 2017 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 on a multimode reader using the resazurin assay.

Results
G9a was expressed in about 90% of the BTC samples. Interestingly, G9a was more intensively expressed at the tumor periphery than in the tumor center. G9a expression was higher in intrahepatic tumors compared to perihilar tumors and higher in mass-forming tumors compared to periductal cases. Importantly, 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 migration and 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). Moreover, Cox regression analysis identified G9a expression as an independent prognostic factor in BTC. *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.

Conclusions
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.

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Evaluation of Napabucasin as a direct inhibitor of cancer stem cells in biliary tract cancer

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Objective

The prognosis of biliary tract cancer (BTC) remains unacceptably low – the median survival of advanced BTC is only about one year. A major problem in clinical BTC management is the general aggressiveness and the intrinsic or developed resistance towards common therapeutic approaches. Recent studies suggest an involvement of cancer stem cells (CSCs) in development and progression of BTC. According to the CSC model, CSC reside of the top of the hierarchy of the heterogeneous tumor and are responsible – at least in part – for tumor recurrence, metastasis and resistance. Therefore, direct targeting of CSC represents an attractive approach. In 2015, it was demonstrated that Napabucasin (BBI608) had a cytotoxic effect on cells with CSC properties and these results were confirmed for prostate CSCs. Twenty-four ongoing clinical studies regarding Napabucasin underline the potential clinical relevance of the substance. With the current study we aim to firstly evaluate whether Napabucasin might serve as a potential therapeutic relevant drug for BTC.

Methods

The concentration- and time-dependent cytotoxic effects of Napabucasin were measured using the resazurin assay. Mode of cytotoxicity was evaluated via Annexin staining. The effect of Napabucasin on functional CSC characteristics was analyzed using a sphere formation assay, the aldehyde-dehydrogenase-1 (ALDH-1) assay and the clonogenic assay. The number of EpCAM-positive cells was measured using specific antibodies and flow cytometry. Western blot was used to investigate the effect of Napabucasin on the expression of established CSC markers in BTC cells. A comprehensive gene expression analysis for CSC-related genes will be performed with the RT^2 Profiler™ PCR Array “Human Cancer Stem Cells” Assay (Qiagen).

Results

Napabucasin showed a cell line- and concentration-dependent cytotoxic effect in our BTC *in vitro* model (n = 9 cell lines) with LD_{50} values ranging from 0.19 to 18.39 µM. Time-resolved analysis revealed that, dependent on the used concentration, Napabucasin treatment resulted in either slow-down of proliferation, proliferation stop or cell death. Annexin staining showed that Napabucasin caused apoptosis in BTC cells. Moreover, Napabucasin significantly diminished functional CSC characteristics. Treatment of BTC cells with Napabucasin reduced size and amount of tumor spheres compared to control cells. Interestingly, this effect was also observed when cells were pre-incubated with Napabucasin, suggesting a lasting effect of Napabucasin in this regard. Furthermore, Napabucasin significantly reduced the number of ALDH-1-positive cells and abolished clonogenic growth. CD326 (EpCAM) is a surface protein that was found in BTC cells with stem cell traits. Napabucasin reduced the number of EpCAM-positive cells, indicating effects on this specific cell population. On protein levels, Napabucasin reduced expression of the CSC markers C-MYC, STAT3, EPCAM, NANOG and CD44.

Conclusions

Based on the current data, Napabucasin shows strong cytotoxic effects in BTC cells and reduces functional CSC characteristics. In ongoing and following experiments we will investigate the effect of Napabucasin on the expression levels of CSC-related genes and pathways to get a better understanding of the potential modes of action.

Precontraction strategies for assessment of vasodilative effects of potassium channel openers in porcine coronary arteries.

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Objective

The coronary arteries supply the myocardium with blood to ensure heart function. Dysfunction of arteries is a hallmark feature of coronary artery disease (CAD), which is largely mediated by plaque formation and pathological vasoconstriction. CAD severely impacts prognosis and is a major driver of mortality. Pathophysiological mechanisms of CAD remain incompletely understood. Therapeutically, potassium channel openers like nicorandil and diazoxide, have been suggested to improve symptoms of CAD.

Isolated coronary artery rings are used to analyze vessel response to vasoactive substances. However, they lack spontaneous myogenic tone even after passive stretching to representative wall tension. This complicates the assessment of vasodilative substances while necessitating the employment of precontraction.

Methods

We employed a porcine coronary artery ring myograph system. Pig hearts were obtained from a local slaughter house. The right coronary artery was explanted for generation of ring specimens with a length of 5 mm. Rings were mounted onto two roughly L-shaped metal hooks, which were then immersed in a perfusion organ bath. The upper metal hook was, via a silk thread, connected to a force transducer. Contraction of coronary rings, leading to downward force, was translated into an electrical signal and recorded in real time. After equilibration and adjustment of passive stretch, rings were precontracted using either 20 mM potassium chloride (KCl, depolarizing agent) or 100 nM U46619, a thromboxane A2 analogue. Then, 200 µM diazoxide, a potent ATP-sensitive potassium (K+ATP) channel opener, was added.

Results

Both application of KCl and U46619 induced vasoconstriction; the effect of U46619 exhibited a slower and less accentuated increase. Upon addition of diazoxide, rings reacted with vasodilation.

Conclusions

We observed substantial differences in precontraction regarding the used substance. Since potassium-induced depolarization only occurs in pathological states (e.g. severe kidney failure or rhabdomyolysis), U46619 more closely reflects physiological membrane potential changes and is therefore recommended to study the effect of potential vasodilators like diazoxide.

Glycine induces migration of microglial BV-2 cells via SNAT-mediated cell swelling

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Objective

The neutral, non-essential amino acid glycine has manifold functions and effects under physiological and pathophysiological conditions. Besides its function as a neurotransmitter in the central nervous system, glycine also exerts immunomodulatory effects and as an osmolyte it participates in cell volume regulation. During phagocytosis, glycine contributes to (local) cell volume-dependent processes like lamellipodium formation. Similar to the expansion of the lamellipodium we assume that glycine also affects the migration of microglial cells in a cell volume-dependent manner.

Methods

The mean cell volume (MCV) was measured by flow cytometry using the Coulter method. Cell migration was quantified by trans-well migration assays. Electrophysiological recordings of the cell membrane potential (V_{mem}) and chloride (Cl^-) currents were performed using the whole-cell patch clamp technique.

Results

In the murine microglial cell line BV-2 application of glycine (5 mM) increased the MCV by ~9%. The glycine-dependent increase in MCV was suppressed by the partial sodium (Na^+)-dependent neutral amino acid transporter (SNAT) antagonist MeAIB and augmented by the Cl^- current blocker DCPIB. Electrophysiological recordings showed that addition of glycine activated a Cl^- current under isotonic conditions ($ICl_{glycine}$) with biophysical and pharmacological characteristics of the hypotonicity (swelling)-activated Cl^- current (ICl_{swell}, ICl_{vol}, VRAC, VSOR). V_{mem} displayed a distinctive time course after glycine application; initially, glycine evoked a rapid depolarization mediated by Na^+-coupled glycine uptake *via* SNAT, followed by a further gradual depolarization, which was fully suppressed by DCPIB. Furthermore, glycine significantly increased migration of BV-2 cells, which was counteracted by MeAIB, suggesting that SNAT is involved in the migration process of microglial cells.

Conclusions

We conclude that glycine acts as a chemoattractant for microglial cells presumably by a cell volume-dependent mechanism involving SNAT-mediated cell swelling.

The acid-sensitive outwardly rectifying (ASOR) vs. the volume-sensitive outwardly rectifying (VSOR) anion channel in microglial cells: activation characteristics and temperature-sensitivity

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Objective

In different cell types extracellular acidification activates a voltage-dependent channel, the acid-sensitive outwardly rectifying (ASOR) anion channel/current. Acidosis is a hallmark of pathophysiological events like inflammation, stroke, neurodegenerative diseases, or ischemia. Therefore, ASOR activation could play a role in the response of immune cells to these environmental conditions. Up to date, there is not much known about its function, molecular identity and activation characteristics as opposed to the volume-sensitive outwardly rectifying (VSOR) anion channel, which is activated under hypotonic conditions to prevent excessive cell swelling. Both currents are characterized by outward rectification, but while the ASOR current activates over time at constant positive holding potentials, the VSOR anion current inactivates. In our study we investigated ASOR activation in BV-2 microglial cells –immune cells in the central nervous system – in response to the extracellular pH and evaluated its $Na^+$and Cl^--dependency as well as its $Ca^{2+}$and temperature sensitivity.

Methods

Whole cell Cl^--currents were recorded using patch clamp (perforated and ruptured configuration) with varying physiological bath solutions to adjust the extracellular milieu regarding pH (7.2–3.0), temperature (4, 22, 37°C) and ion compositions/concentrations. VSOR currents were activated by exposing cells to a hypotonic extracellular solution.

Results

ASOR currents are inactive at an extracellular pH of 7.2. Extracellular acidification activated the current in a 'dose-dependent' manner under isotonic conditions starting at pH 5.0. Maximum current amplitudes were reached at pH 4.0 and 3.0. Current activation was dependent on extracellular Cl^- but not Na^+, was unaffected by intracellular application of the Ca^{2+} scavenger BAPTA and could not be elicited by extracellular application of the Ca^{2+} ionophore ionomycin. ASOR channels were insensitive to Zn^{2+}, an inhibitor of proton (H^+) currents. Both ASOR and VSOR currents were responsive to temperature changes of the extracellular solution; increasing/decreasing the temperature from 22°C to 37°C or 4°C augmented or diminished the currents, respectively. ASOR currents could be elicited in both the perforated patch clamp configuration, where the intracellular compartment remains intact, and the ruptured configuration, where the cytoplasmic compartment is dialyzed by the pipette solution.

Conclusions

Here we show that the ASOR current in BV-2 microglial cells is a Cl^-- and not a H^+-current, that it is independent of Na^+ and that the conductance is not mediated by Ca^{2+}-sensitive Cl-channels (CaCCs). We also show that ASOR activation does not require an intact cytoplasmic compartment and that both ASOR and VSOR channels are temperature-sensitive. Our data add to a better understanding of the electrophysiological properties of microglial cells and the activation behaviour of ASOR channels. These acid-sensitive channels might play a role in the immune response of microglia cells under acidotoxic conditions associated with necrotic cell injury and inflammatory events in the brain.

pH dependent activation of TALK1 channel

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Objective
TALK1 channel belongs to the two pore K^+ (K_{2P}) channel family and as other members of the K_{2P} channels it functions as a dimer, the individual subunit of which is composed of four transmembrane segments and two pore forming domains. It has been shown that TALK1 channel is pH sensitive, but until now it was not elaborated how TALK1 senses the changes of extracellular pH. Based on the predicted structure of TALK1 channel we hypothesize that positively charged arginine 233 or lysine 84, located near the selective pore, and are required for pH sensing of the channel.

Methods
We have mutated R233 of human TALK1 channel in pcDNA3.1 to neutral, valine or to the negatively charged glutamic acid. In the case of K84 we have mutated it to neutral alanine.
The wild type TALK1 or one of the mutated plasmids were transfected to COS-7 cells.
Activation of the TALK1 channels was measured by patch-clamp technique in a whole-cell mode. In order to test the influence of different pH on the activation of TALK1 or TALK1 mutated channels during recordings we exchanged the extracellular solution in the range between pH 5 to pH 11.

Results
Our results show that the wild type TALK1 channel is sensing the changes in the extracellular pH, exhibiting strongly high-pH activated outwardly rectifying currents. In the R233V the pH dependency was greatly diminished. Even more interestingly R233E shows reverse pH dependent activation with strong acidic activated outwardly rectifying currents.
Mutating K84 to neutral alanine did not change the pH sensing in comparison to the wild type.

Conclusions
We propose that sensing of the extracellular pH changes is accomplished by positively charged arginine 233 located near the selective pore. Positively charged lysine 84 is not involved in the pH sensing. Since TALK1 channels have a substantial expression in beta cells of pancreatic islets, we will next test the hypothesis that TALK1 channel is involved in the fine tuning of beta cell excitability as a resting or a background channel.