CANAUX IONIQUES

31st Ion Channels meeting

11th-14th September 2022

Domaine du Lazaret, Sète, France

Ion channels and tumour microenvironment
Maike Glitsch (Hamburg, DE)
Elena Adinolfi (Ferrara, IT)
Charles Thodeti (Toledo, OH, USA)

Ion channels in motor neuron physiopathology
Gareth Miles (St Andrews, UK)
Daniel Zytnicki (Paris, FR)
Frederic Brocard (Marseille, FR)

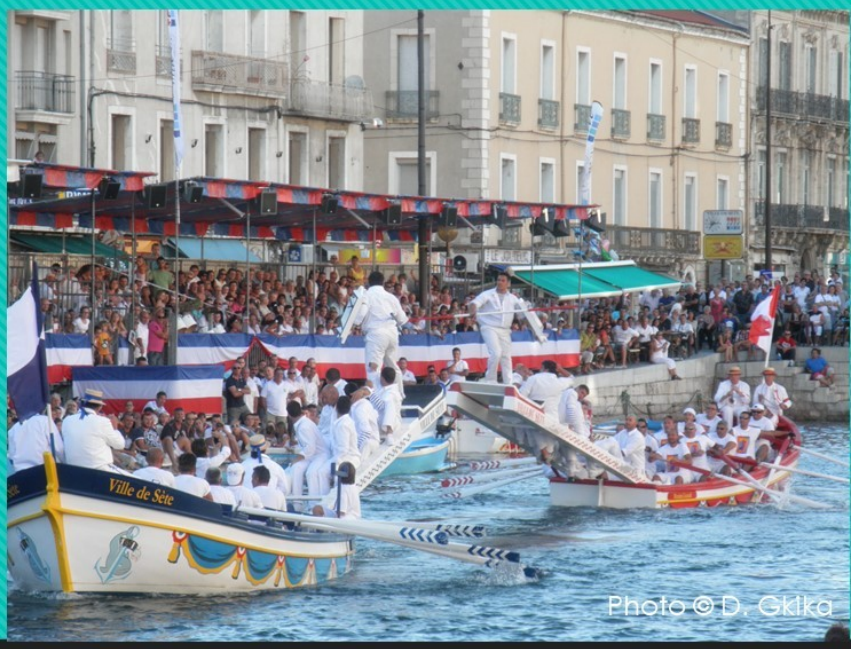
Ion channels and pain processing
Reza Sharif Naeini (Québec, CA)
Emmanuel Deval (Nice, FR)
Pierre F Mery (Montpellier, FR)

Mechanosensitive ion channels: from bacteria to mammalians
Jean-Marie Frachisse (Paris, FR)
Boris Martinac (Darlinghurst, AU)
Bertrand Coste (Marseille, FR)

Ion channels & nanodomains
Julia Gorelik (London, UK)
Elise Balse (Paris, FR)
Joahnnes Hell (Davis, CA, USA)

Plenary Lecture

Albrecht Schwab (Münster, DE)



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Registration from April 2022 at

https://www.canaux-ioniques.fr/colloque-annuel/home.html

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Cécile Hilaire (Montpellier), Perrine Inquimbert (Strasbourg),
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FOREWORD / AVANT-PROPOS

Dear Colleagues,

We are delighted to meet again in Sète after a two-year-long break forced upon us by the Covid-19 pandemic. During this time, much progress was made in the ion channel field, which drew the spotlight even more on these amazing molecular sensors with the recent Nobel laureates David Julius and Ardem Patapoutian.

The French Ion Channel Association is proud of its 31 years of annual meetings and thankful to you for your participation, which reached 120 registrations this year. This year's program includes remarkable speakers presenting the latest ion channel research. The talks will focus on the function and regulation of mechanosensation, motor neuron physiology, nociception, and tumor microenvironment.

This meeting wouldn’t be possible without the support of our sponsors, as well as the resilience and hard work of all the members of the organizing committee, Anna Rita, Claire, Lise, Perrine, Cécile, Fabien, Aubin, and Pietro, not forgetting Arnaud and Caroline. We hope that our scientific exchanges will be even more fertile after this long pause and that the Mediterranean environment of the Lazaret domain will be the perfect setting to strengthen and promote new collaborations within our network.

Enjoy the meeting!

Dimitra and Sylvie
Co-presidents of the 31st Ion Channels Meeting

PROGRAM

Sunday, September 11th 2022

16:00 – 19:00 Welcome of the meeting attendees

19:00 ***Welcome drink and Dinner***

21:00 **Plenary lecture**

Albrecht Schwab (Münster, Germany)

The role of KCa3.1 channels in non-small cell lung cancer

Monday, September 12th 2022

08:15 Opening session

08:30 Symposium 1: "Ion channels and tumor microenvironment"

*Organized by **Anna Rita Cantelmo** (Lille, France)*

Maike Glitsch (Hamburg, Germany): OGR1 and TRPC4 – partners in crime?

Elena Adinolfi (Ferrara, Italy): The P2X7 receptor: an ATP gated ion channel with multifaced roles in cancer

Charles Thodeti (Toledo, Ohio, USA): A "TRP" to tumor angiogenesis

Selected speaker: Raphael Rapetti-Mauss (Nice, France): A potassium channel set a signaling hub bolstering CAF-triggered metastatic process in pancreatic cancer

10:15 ***Coffee break***

10:30 Oral communication session 1: "Biophysical Aspects of Ion Channels"

*Organized by **Pietro Mesirca** (Montpellier, France)*

Guénaëlle Lizot (Poitiers, France): Electrophysiological characterization of mouse intracardiac neurons

Camille Blandin (Paris, France): CASK is a key organizer of cardiomyocyte electrical and structural polarity

Andreas Zoumpoulakis (Paris, France): Electrophysiology and biophysical studies on conformational changes of a human potassium channel, Kir2.1

Thibault Jouen-Tachoire (Oxford, UK): Gain-of-function mutations in KCNK3 cause a developmental disorder with sleep apnea

11:40 One-minute oral presentation for posters (even numbers)

12:30 *Lunch*

14:00 Poster session 1 - Even numbers

15:45 *Coffee break*

16:00 Symposium 2: "Ion channels in motor neuron pathophysiology"

*Organized by **Cécile Hilaire** (Montpellier, France)*

Gareth B Miles (St Andrews, UK): Deciphering novel modulatory components of spinal motor circuits

Frederic Brocard (Marseille, France): Calpain-mediated cleavage of Nav1.6 and Kv7.2 channels fosters the hyperexcitability of motoneurons after spinal cord injury and leads to spasticity

Daniel Zytnicki (Paris, France): Early reversible structural and functional impairments of excitatory synapses on spinal motoneurons from ALS mice

Selected speaker: Ana Sofia Eria Oliveira (Grenoble, France): Viral Rhodopsins: novel function and novel optogenetic tools

17:45 Oral communication session 2: "Atypical ion channel function/regulation"

*Organized by **Aubin Penna** (Poitiers, France)*

Camille Berenguier (Nice, France): Cancer-related mutations of KCNQ1 promote Wnt/β-catenin pathway activity

Giorgia Chinigò (Lille, France / Turin, Italy): The protective role of TRPM8 in prostate cancer progression

Florian Beignon (Angers, France): The TRPV1 channel, a thermostat to regulate mitochondrial thermogenesis?

Léa Réthoré (Angers, France): Is NaVβ3 a new actor of mechanotransduction in endothelial cells?

18:45-19:15 Sponsors presentations

19:30 ***Apéritif dinatoire Sétois***

Tuesday, September 13th 2022

08:30 Symposium 3: "Ion channel and pain processing"

Organized by **Perrine Inquimbert** *(Strasbourg, France)*

Reza Sharif Naeini (Québec, Canada): How we feel: molecular sensors underlying our senses of touch and pain

Emmanuel Deval (Valbonne, France): Acid-Sensing Ion Channels (ASICs) in peripheral and spinal pain processes

Pierre F. Mery (Montpellier, France): The T-type calcium channel Cav3.2 is a "pain" in the spinal cord

Selected speaker: Clémence Gieré (Strasbourg, France): Toward a central origin of nociceptive hypersensitivity seen in adult rats after a neonatal maternal separation

10:15 ***Coffee break***

10:30 ***One-minute oral presentation for posters (Odd numbers)***

11:00 Poster session 2 - Odd numbers

12:30 ***Lunch***

13:30 ***Social event: Boat excursion***

17:15 Symposium 4: "Mechanosensitive Ion channels: from bacteria to mammalians"

Organized by ***Claire Legendre*** (Angers, France)

Jean-Marie Frachisse (Gif sur Yvette, France): Sensing forces in plants with mechanosensitive channels

Boris Martinac (Darlinghurst, Australia): What have we learned about mechanotransduction from studies of bacterial mechanosensitive channels

Bertrand Coste (Marseille, France) : Somatosensory mechanotransduction

Selected speaker: Sarah Rougé (Paris, France): Stomatin, a major modulator of mechanosensitive ion channels in human Red Blood Cells?

19:00 Annual meeting of the Association

20:00 *Dinner*

21:30 Mort Subite &Evening Party

Wednesday, September 14th 2022

09:30 Symposium 5: "Ion channels & nanodomains"

*Organized by **Fabien Brette** (Montpellier, France)*

Joahnnes Hell (Davis, California, USA): How phosphorylation of the calcium channel CaV1.2 captures your attention

Julia Gorelik (London, UK): Differential L-Type calcium channel nanodomain signaling in Heart Failure

Elise Balse (Paris, France): Spatiotemporal regulation of ion channels in the cardiomyocyte: source of electrical plasticity or risk of arrhythmia?

Selected speaker: Shihab Shah (Leeds, UK): Deciphering molecular architecture of ano1-containing multiprotein signaling complexes in mammalian sensory neurons

11:15 *Coffee break*

11:40 Prizes and Meeting closure

12:00 *Lunch*

14:00 Airport shuttle departure

SYMPOSIA AND ORAL COMMUNICATIONS ABSTRACTS

Sunday, September 8th 2019

21:00 **Plenary lecture**

The role of KCa3.1 channels in non-small cell lung cancer

Albrecht Schwab; Luca matteo TODESCA; Etmar BULK.
Institut für Physiologie II, Universität Münster, Germany

KCa3.1 channels play an important pathophysiological role in non-small cell lung cancer (NSCLC). Their overexpression and/or the hypomethylation of their promoter predict a poor prognosis of NSCLC patients. Since cancer patients usually die of the sequelae of cancer metastasis we reasoned that KCa3.1 channels must contribute to steps of the metastatic cascade. Using a combination of patch clamp, live-cell imaging and atomic force microscopy, we could show that KCa3.1 channels promote NSCLC cell aggressiveness by modulating processes such as migration, proliferation and tumor cell extravasation. Recent data show that KCa3.1 channels are not only expressed in the plasma membrane, but that they are also found in the inner membrane of mitochondria. MitoKCa3.1 channels regulate the potential of the inner mitochondrial membrane and thereby modulate ROS production. We will discuss which functional properties of NSCLC cells are regulated by this mechanism. Taken together, our findings lend support to viewing KCa3.1 channels as potential therapeutic target in NSCLC.

Monday, September 9th 2019

08:15 Opening session

08:30 Symposium 1: "Ion channels and tumor microenvironment"

Organized by ***Anna Rita Cantelmo*** *(Lille, France)*

OGR1 AND TRPC4 – PARTNERS IN CRIME?
Maike GLITSCH
MSH; Am Kaiserkai 1; 20457 Hamburg, Germany

OGR1 is a G protein coupled receptor that acts as a coincidence detector for extracellular fluid acidity and cytoskeletal polymerisation. This means that both a sufficiently polymerised cytoskeleton and extracellular proton concentration need to be present simultaneously for the receptor to be active. Cytoskeletal polymerisation happens in response to cell stretch which in turn reflects either a complex cell morphology, cell migration or a stiff extracellular matrix to which cells adhere. Acidification of interstitial fluid as well as changes in tissue stiffness accompany most if not all pathologies, meaning that OGR1 is perfectly placed to report pathological transformations in tissues.
As a Gq-coupled receptor, OGR1 can release Ca2+ from intracellular Ca2+ stores and also has the potential to activate ion channels. We find that OGR1 interacts on two levels with Canonical Transient Receptor Channel 4 (TRPC4) subunits. OGR1 stimulation promotes opening of Ca2+-permeable TRPC4-containing channels as well as enhances TRPC4 subunit expression in a number of cells. Intriguingly, the permeability of TRPC4-containing channels has been shown to be potentiated by extracellular protons in a bell-shaped manner, meaning that for mild to moderate acidosis, TRPC4-containing channels activated by OGR1 will contribute to increases in intracellular Ca2+ concentration alongside Ca2+ release from intracellular stores.
A number of members of the superfamily of Transient Receptor Potential channels have been identified as mechanosensitive. Whilst TRPC4 is not thought to be one of the mechanosensitive TRP family members, experiments investigating the mechanosensing ability of ion channels usually involves studying of cells seeded on stiff matrices, i.e. under conditions of high cytoskeletal polymerisation.
We have investigated whether TRPC4 is mechanosensitive in terms of sensing matrix/environmental stiffness and what the physiological consequences of OGR1 and/or TRPC4 stimulation are in cerebellar granule cells and their transformed counterparts.

THE P2X7 RECEPTOR: AN ATP GATED ION CHANNEL WITH MULTIFACED ROLES IN CANCER
Elena ADINOLFI
Department of Medical Sciences, University of Ferrara, Via L. Borsari, 46, 44121, Ferrara, Italy

The P2X7 is an ATP gated ion channel known to be the main responsible for the activity of this nucleotide in the tumour microenvironment (TME). Tumour cells P2X7

favours tumour growth, neovascularization and metastatic dissemination. However, immune cells expressing P2X7 also trigger antitumoral immune responses. Here I will summarize recent advancements emerging from the work of our research group, identifying P2X7 as a central regulator of ATP levels and the purinergic /adenosinergic axis in the TME. These data demonstrate an essential role of the receptor in moulding tumour eradicating and immunosuppressive responses by acting at T effector and regulatory cells and immune checkpoints expression. Moreover, I will describe the role of two receptor splice variants, both active as ion channels (P2X7A and P2X7B) in chemo and radiotherapy resistance. Finally, I will show you evidence of the role played by P2X7 in the release of miRNA bearing microvesicles and exosomes from cancer cells.

A"TRP" TO TUMOR ANGIOGENESIS

Charles THODETI
Department of Physiology and Pharmacology, College of Medical and Life Sciences,University of Toledo, Toledo OH,USA

Solid tumors require angiogenesis for the growth and metastasis. Unlike normal vessels, tumor vessels are characterized by abnormal morphology and patterning that cause vascular hyper-permeability and inefficient delivery of anti-cancer agents. Although most work on tumor angiogenesis focused on soluble angiogenic factors such as vascular endothelial growth factor (VEGF), these conventional anti-angiogenic strategies showed only modest success in clinical trials due to the development of resistance as tumor endothelial cells (TEC) became refractory to anti-VEGF therapy over time. Here, we present evidence that the mechanosensitive ion channel, transient receptor potential vanilloid 4 (TRPV4), regulates tumor angiogenesis and tumor vessel maturation via modulation of EC mechanosensitivity and crosstalk with VEGFR2 signaling. We found that TRPV4 is a mechanosensor in EC in vitro and in vivo and TRPV4 expression and function is reduced in TEC that is correlated with aberrant mechanosensitivity towards extracellular matrix stiffness, increased migration, and abnormal angiogenesis by TEC. Further, we demonstrate that extracellular vesicles from tumor microenvironment transforms normal EC into TEC via downregulation of TRPV4. In vivo, syngeneic Lewis lung carcinoma (LLC) tumor experiments revealed that the absence of TRPV4 induced increased tumor angiogenesis and vascular leakage resulting in enhanced tumor growth and metastasis in global and endothelial-specific-TRPV4 knockout mice. Mechanistically, absence/knockdown of TRPV4 induced abnormal angiogenesis via Rho/YAP/VEGFR2 pathway. Taken together, our findings demonstrate that endothelial TRPV4 is a critical modulator of vascular integrity and tumor angiogenesis and that deletion of TRPV4 promotes tumor angiogenesis, growth, and metastasis. We propose that targeting endothelial TRPV4 could offer a potential novel growth factor-independent strategy for vascular normalization and cancer therapy.

A POTASSIUM CHANNEL SET A SIGNALING HUB BOLSTERING CAF-TRIGGERED METASTATIC PROCESS IN PANCREATIC CANCER

Raphael RAPETTI-MAUSS[1]; Jérémy NIGRI[2]; Camille BERENGUIER[1]; Sarah-simha TUBIANA[2]; Pascal FINETTI[2]; Hélène GUIZOUARN[1]; Patricia MELNYK[3]; Franck BORGESE[1]; Richard TOMASINI[2]; Olivier SORIANI[1];
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[2]: Institut Paoli-Calmettes, Cancer Research Center of Marseille (CRCM), INSERM U1068, CNRS UMR7258, Aix-Marseille University, 13009 Marseille, France
[3]: Univ. Lille, Inserm, CHU Lille, UMR-S1172 – LilNCog – Lille Neurosciences & Cognition, 59000 Lille, France

Intercellular communication within pancreatic ductal adenocarcinoma (PDAC) contributes to metastatic processes. Yet the underlying mechanisms are poorly understood, resulting in a lack of targeted therapy to counteract stromal-induced cancer cell aggressiveness. Here we investigated whether ion channels, which remain understudied in cancer biology, contribute to intercellular communication in PDAC.
Using a conditioned media from patient-derived cancer-associated-fibroblasts (CAF) we evaluated the effect of secreted stromal cues on electrical features of pancreatic cancer cells (PCC). The molecular mechanisms were deciphered using electrophysiology, molecular and biochemistry techniques in cell lines and human samples. An orthotropic mouse model where CAF and PCC were co-injected was used to evaluate tumor growth and metastasis dissemination. Pharmacological studies were carried out in the KICpdx1 mouse model.
We report that a potassium channel (KCh) is stimulated by secreted cues from CAF leading to the activation of an Integrin-EGFR-AKT signaling axis which participates in the acquisition of pro-metastatic features. We also found that an ion channel chaperone (βKCh), is required for the dynamic and spatial formation of functional membrane complexes associating β1-integrin subunit, EGFR, KCh and AKT upon CAF-induced stimulation of cancer cells. Interestingly, the pharmacological targeting of KCh-dependent signaling using a βKCh ligand reduced cancer cell invasive potency in vitro and abrogated CAF-induced metastatic spreading in vivo.
Our results establish a new paradigm in which an ion channel shifts the activation-level of a signaling pathway in response to stromal-cues, opening a new therapeutic window targeting the formation of ion channel-dependent signaling hubs.

10:30 Oral communication session 1: « Biophysical Aspects of Ion Channels»

*Organized by **Pietro Mesirca** (Montpellier, France)*

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF MOUSE INTRACARDIAC NEURONS

Guénaëlle LIZOT; Jocelyn BESCOND; Patrick BOIS; Jean-françois FAIVRE; Aurélien CHATELIER;
Laboratoire PRéTI UR 24184 - Université de Poitiers - 1 Rue Georges Bonnet 86073 POITIERS France

Neural control of the heart involves central and peripheral neurons that act interdependently to modulate cardiac parameters such as heart rate or contractility. Within this cardiac neuronal regulation, the intracardiac nervous system, which correspond to clusters of neurons found on the dorsal atrial surface of the heart, is receiving growing attention. Indeed, whereas they were initially considered as simple

parasympathetic postganglionic neurons, studies conducted over the past 30 years suggested a more complex organization, involving the existence of sensory, local regulatory and motor neurons within intracardiac ganglia. Moreover, growing evidence suggest the implication of this neural network in the initiation and maintenance of cardiac arrhythmias. However, the functional organization of this intracardiac neural network, as well as its involvement in cardiac diseases have not been fully elucidated. Therefore, this study aims to decipher the complexity of this mouse cardiac nervous system by examining the electrophysiological properties of intracardiac neurons. The characterization of passive and active electrical membrane properties of these neurons gave rise to the identification of two distinct firing profiles. The first group was classified as phasic due to its limited firing activity while the second was defined as adapting. In addition, intracardiac neurons could also be distinguished by the presence or absence of an afterhyperpolarization. By using cre-transgenic mice and targeted viral transduction strategy, we identified calbindin-expressing neurons as a population of neurons with a distinct electrophysiological signature. This could be explained by the differential expression of several ionic channels including the N-type voltage-gated calcium channel.

CASK IS A KEY ORGANIZER OF CARDIOMYOCYTE ELECTRICAL AND STRUCTURAL POLARITY

Camille BLANDIN[1]; Gilles DILANIAN[1]; Nathalie MOUGENOT[2]; Basile GRAVEZ[1]; Solenne CHARDONNET[3]; Elise BALSE[1];
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Although the organization of the cardiomyocyte membrane into functional microdomains directly influences the anisotropic propagation of the action potential, the mechanisms underlying the rod-like structural organization of cardiomyocytes during post-natal development are poorly understood. We have previously shown in the adult cardiomyocyte that CASK regulates sodium channel expression at the lateral membrane in interaction with focal adhesion complexes. In addition, preliminary results have shown that the expression level of CASK evolves during postnatal development.
CASK viral constructs were designed to inhibit the expression of CASK in both neonatal cardiomyocytes, in vitro (Ad-shCASK) and in vivo (AAV-shCASK). Proteomic analysis showed that CASK invalidation strongly modify immature cardiomyocyte proteome, notably the acting cytoskeleton signaling. Immunostaining experiments on neonatal cardiomyocytes confirmed that CASK is required for the correct organization of the cytoskeleton and impacted both mechanical and electrical junction formation of the intercalated disc. Neonatal cardiomyocytes invalidated for CASK displayed abnormal contractility, focal adhesion fragility, and anoikis. Echocardiography phenotyping of CASK-invalidated rats at the neonatal stage reveals eccentric ventricular remodeling, decreased systolic performance but a preserved contractile reserve. In addition, CASK silencing was associated to QT duration modifications and increased ventricular arrhythmia incidence after myocardial infarction, combined with reduced Nav1.5/Kir2.1 expression level.
This study demonstrates that CASK, a partner of lateral membrane Nav1.5, confers the ability to orchestrate both cardiomyocyte molecular organization and tissue cohesion. CASK appears to be a key modulator in molecular arrangement of electrical and structural polarity of cardiomyocytes during postnatal development.

ELECTROPHYSIOLOGY AND BIOPHYSICAL STUDIES ON CONFORMATIONAL CHANGES OF A HUMAN POTASSIUM CHANNEL, KIR2.1

Andreas ZOUMPOULAKIS[1]; Dania ZUNIGA[1]; Carlos FERNANDES[1]; Laurie PEVERINI[2]; Sophie SHI[2]; Pierre-jean CORRINGER[2]; Catherine VENIEN-BRYAN[1];
[1]: Sorbonne University - Institut de minéralogie, de physique des matériaux et de cosmochimie (IMPMC)
[2]: Institut Pasteur - Channel Receptors

The inward rectifier potassium (Kir) channels belong to a family of integral membrane proteins that selectively control the permeation of K+ ions at the cell membranes in a variety of tissues. The objective of my PhD project is to understand the structural, dynamic and functional characteristics of Kir2.1, a subfamily-member that is highly expressed in the heart and is involved in the regulation of the resting membrane potential.
Having recently resolved the structure of Kir2.1 in n-Dodecyl-B-D-maltoside detergent (DDM) with cryo-electron microscopy at 4.3 Å, the goal now is to decipher the gating mechanism of the channel which, for the moment, is poorly understood. For that, our strategy is to deploy voltage-clamp fluorometry, an electrophysiology technique that allows for the simultaneous detection of ionic currents across the membrane and the conformational rearrangements associated with channel gating.
Two-electrode voltage-clamp experiments, important for the establishment of the biophysical properties of the channel, and preliminary results on voltage-clamp fluorometry will be shown on Kir2.1 WT, as well as two pathological mutants associated with channelopathies, such as Anderson-Tawil syndrome : i) one located in a putative intracellular binding site for PIP2 (phosphatidylinositol 4,5-bisphosphate), a membrane phospholipid essential for the modulation of the gating of the channel and ii) one involved in a disulfide bond in the extracellular domain of Kir2.1, important for the preservation of the structure and proper folding of the protein.
Future experiments include the use of Förster resonance energy transfer (FRET) in order to unravel the crucial conformational changes involved in the gating of this channel.

GAIN-OF-FUNCTION MUTATIONS IN KCNK3 CAUSE A DEVELOPMENTAL DISORDER WITH SLEEP APNEA

Thibault JOUEN-TACHOIRE; Janina SÖRMANN; Peter PROKS; Stephen TUCKER;
University of Oxford

Sleep apnea is a common disorder that represents a global public health burden. KCNK3 encodes TASK-1, a K+ channel implicated in the control of breathing, but its link with sleep apnea remains poorly understood. Here we describe a novel developmental disorder with associated sleep apnea (DDSA) caused by rare de novo gain-of-function mutations in KCNK3. The mutations cluster around the ‘X-gate’, a gating motif which controls channel opening, and produce overactive channels that no longer respond to inhibition by GqPCR signalling. However, despite their defective X-gating, these mutant channels can still be inhibited by a range of known TASK channel inhibitors. These results not only highlight an important new role for TASK-1 K+ channels and their link with sleep apnea, but also identify possible therapeutic strategies. In particular, the physiological mechanism of regulation of the ‘X-gate’ is unknown. Despite differences in their biophysical properties relevant to both their

pathogenicity and functional behaviour, a common feature of all these DDSA mutations is the lack of GqPCR inhibition. This is currently being investigated using a range of structural, functional and computational approaches in heterologous and physiologically-relevant cell types.

16:00 Symposium 2: "Ion channels in motor neuron pathophysiology"

*Organized by **Cécile Hilaire** (Montpellier, France)*

DECIPHERING NOVEL MODULATORY COMPONENTS OF SPINAL MOTOR CIRCUITS

Gareth MILES;
School of Psychology & Neuroscience, University of St Andrews, St Andrews, Fife, UK

Networks of neurons within the spinal cord are sufficient to generate the complex neural output required to control rhythmic movements such as locomotion. These neural networks must adjust their output to suit ever-changing environmental and organismal demands. Our research aims to decipher the cellular mechanisms, including target ion channels, that enable motor control systems to function in an independent and adaptable manner. We have used a combination of molecular-genetic, live imaging and electrophysiological techniques, applied to isolated rodent spinal cord preparations, to reveal the mechanisms by which neuromodulatory pathways adjust the pattern and strength of locomotor-related motor network output. Our recent work has focussed on neuromodulatory pathways originating from spinal, cholinergic interneurons and spinal astrocytes. In parallel, we have investigated how changes in these neuromodulatory pathways can tip the scales towards neurodegeneration, as seen in devastating conditions such as Amyotrophic Lateral Sclerosis (ALS).

CALPAIN-MEDIATED CLEAVAGE OF NAV1.6 AND KV7.2 CHANNELS FOSTERS THE HYPEREXCITABILITY OF MOTONEURONS AND LEADS TO SPASTICITY AFTER SPINAL CORD INJURY.

Jeremy VERNEUIL; Cecile BROCARD; Julie PEYRONNET-ROUX; Frederic BROCARD;
Marseille, France

The main clinical symptoms of spasticity, hyperreflexia and spasms, develop after spinal cord injury (SCI) when an excitatory-inhibitory imbalance occurs in motoneurons. Understanding the cellular pathophysiological processes underlying this imbalance might offer new therapeutic perspectives for SCI-induced motor deficits. The SCI enhances the intrinsic excitability of motoneurons by upregulating their persistent sodium (INaP) currents pointed out as major contributors of hyperreflexia in rats and humans. Remarkable advances have been made on the

molecular mechanisms involved in alterations of INaP. We demonstrated that the calpain-mediated proteolysis of Nav1.6 channels upregulates INaP (Brocard et al. 2016) Nature Medicine. In addition to INaP, we recently showed that neurons of the spinal locomotor network ubiquitously express a slowly activating K+ current (IM) acting in opposition to INaP to dampen neuronal excitability (Verneuil et al., 2020) PlosBiol. In other words, INaP is increased when IM is decreased and vice versa. This interplay between the two opposing conductances questions about the role of IM in the increase of INaP after SCI. We identified Kv7.2 channels as the main molecular correlate of IM in the spinal locomotor network. In neuronal cultures, indirect evidence suggests that Kv7.2 channels are sensitive to calpains that downregulate their membrane expression (Benned-Jensen et al., 2016). Therefore, we posit that a decrease of IM, subsequent to a calpain-mediated cleavage of Kv7.2 channels after SCI, may contribute to release INaP leading to spasticity. Based on new experimental data, we showed that calpain-mediated cleavage of Nav1.6 and Kv7.2 after SCI fosters the IM/INaP imbalance in motoneurons leading to spasticity.

EARLY REVERSIBLE STRUCTURAL AND FUNCTIONAL IMPAIRMENTS OF EXCITATORY SYNAPSES ON ALS MOTONEURONS

Marcin BąCZYK[1]; Najwa OUALI ALAMI [2]; Kamil GRYCZ[1]; Nicolas DELESTREE[3]; Clémence MARTINOT[3]; Linyun TANG[2]; Barbara COMMISSO[2]; Marin MANUEL[3]; Francesco ROSELLI[2]; Daniel ZYTNICKI[3];
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Excessive excitation is hypothesized to cause motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), but actual proof of hyperexcitation in vivo is missing: how are synaptic inputs to MN affected by the disease, and are they increased or decreased? We demonstrate, by in vivo intracellular MN electrophysiology, that, contrary to expectations, excitatory post-synaptic potentials evoked by electrical or mechanical stimulation of Ia sensory fibers are reduced in MNs of adult presymptomatic mutSOD1 mice. This synaptic impairment correlates with disrupted postsynaptic clustering of Homer1b, Shank, and GluR4 subunits. Moreover, this impairment has a deep impact on the whole MN biology since mechanically-induced Ia inputs translate in a reduced phosphorylation of the CREB transcription factor in MNs. Interestingly, a similar functional impairment is observed in synapses on MN originating from the brainstem descending medial longitudinal fasciculus, indicating a widespread phenomenon. Restoration of excitatory synapses can be achieved by activation of the cAMP/PKA pathway, by either intracellular injection of cAMP or DREADD-Gs stimulation. Furthermore, we reveal, through independent control of signaling and excitability in MN allowed by multiplexed DREADD/PSAM chemogenetics, that PKA-induced restoration of synapses triggers an excitation-dependent decrease in misfolded SOD1 burden and autophagy overload. In turn, increased MN excitability contributes to restoring synaptic structures. Thus, the decrease of excitation to MN is an early but reversible event in ALS. Failure of the postsynaptic site, rather than hyperexcitation, drives disease pathobiochemistry at this stage of the disease evolution.

VIRAL RHODOPSINS: NOVEL FUNCTION AND NOVEL OPTOGENETIC TOOLS

Ana sofia ERIA OLIVEIRA[1]; Mathilde FOLACCI[1]; Amandine CHASSOT[2]; Kirill KOVALEV[1]; Valentin GORDELYI[1]; Guillaume SANDOZ[2]; Michel VIVAUDOU[1];

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Rhodopsins are membrane proteins that harvest light for diverse functions such as ion pumping, ion channeling, sensory or kinase activities. Widely distributed, they are also found in the genomes of giant viruses infecting phytoplankton. While several viral rhodopsins (VRs) structures have been solved, the function and biological role of these viral rhodopsins remain elusive.
We initially used Xenopus oocytes as a heterologous expression system for VRs. We performed surface expression measurements with the newly-developed XenoGlo technique and electrophysiological recordings with two-electrode voltage clamp. We found that VR accumulate almost exclusively intracellularly. Their activation by light induced dose-dependent calcium release from intracellular stores as evidenced by activation of endogenous Ca2+-activated Cl- currents and block by internal BAPTA and pharmacological Ca2+ stores depletion. These findings were confirmed in mammalian HEK-293 cells using coexpressed Ca2+-activated K+ and Cl- channels.
To record the direct channel activity of VRs we resorted to protein engineering. Ultimately, we were able to redirect one VR towards the cell surface by fusing it to a high-expressing GPCR. Preliminary results suggest that the VR is a channel permeable to monovalent cations.
The precise release of calcium from intracellular stores mediates a large panoply of cellular processes such as gene expression, neurotransmitter release or muscle contraction. The propensity of VRs to accumulate in internal compartments and modulate calcium release make them great candidates for novel optogenetics tools, with potential applications in basic research and medicine.

17:45 Oral communication session 2: "Atypical ion channel function/regulation"

*Organized by **Aubin Penna** (Poitiers, France)*

CANCER-RELATED MUTATIONS OF KCNQ1 PROMOTE WNT/β-CATENIN PATHWAY ACTIVITY

Camille BERENGUIER; Benoit ALLEGRINI; Franck BORGESE; Hélène GUIZOUARN; Bernard PELLISSIER; Olivier SORIANI; Raphael RAPETTI-MAUSS;
Inserm, CNRS, iBV, Université Côte d'Azur, Nice, France

While the role of ion channels in cancer biology has emerged in the past years, the impact of ion channel mutations in cancer remains unexplored. We showed that the potassium channel KCNQ1 acts as a gatekeeper of epithelial integrity through the restriction of the Wnt/β-catenin signalling pathway (Rapetti-Mauss et al., PNAS 2017). We have identified several mutations carried by the KCNQ1 gene in colorectal cancer patients. This project aims to understand how the cancer-related mutations carried by KCNQ1 alter the Wnt/β-catenin pathway and thus promote tumorigenesis. Our results show that most of the mutations are loss-of-function on the KCNQ1 current. These loss-of-function mutations are associated to an increased Wnt/β-catenin activity and a loss of the epithelial phenotype in vitro in colorectal cancer cell lines. On a multicellular level, in mice colon organoids, the loss-of-function mutation alter the morphology of mice colon organoids. We further hypothesize that these loss-of-function mutations participate to the epithelial-to-mesenchymal transition

through the activity of the Wnt/β-catenin pathway and thus promote the progression of colorectal cancer. We expect that the project will highlight that cancer-associated KCNQ1 mutations enhance Wnt/β-catenin pathway activity and participate in colorectal cancer physiopathology.

THE PROTECTIVE ROLE OF TRPM8 IN PROSTATE CANCER PROGRESSION

Giorgia CHINIGò[1]; Guillaume GROLEZ[2]; Jerome DE-RUYCK[2]; Séverine MARIONNEAU-LAMBOT[3]; Stéphanie LERONDEL[4]; Sébastien ROGER[5]; Albrecht SCHWAB[6]; Anna rita CANTELMO[2]; Alessandra FIORIO PLA[7]; Dimitra GKIKA[8];
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Prostate cancer (PCa) is the second most lethal tumor among men and its mortality is mainly due to metastasis. Thus, it is critical to understand the mechanisms by which tumors grow and how metastases can diffuse throughout the body. Several Transient Receptor Potential (TRP) channels are deregulated in cancer cells and have been suggested as valuable markers in predicting cancer progression as well as potential targets for pharmaceutical therapy.
In this context, a protective role of TRPM8 in PCa progression consistent with its strong down-regulation during late metastatic stages has been suggested. By using a prostate orthotopic xenograft mouse model, we confirmed in vivo the protective role of TRPM8 in PCa showing that its overexpression in PC3 cells not only reduces tumor growth through cell cycle arrest in G0/G1 and reduced clone forming capacity but also inhibits metastatic dissemination by impairing the motility of these cancer cells. In particular, investigating the molecular mechanism underlying this biological effect, we found that, as previously described for endothelial cells, TRPM8 inhibits PCa cell migration and adhesion independently from its channel function. More specifically, TRPM8 intracellularly traps the small GTPase Rap1A in its inactive form, thus avoiding its translocation to the plasma membrane and the subsequent activation of adhesion signaling pathways. Moreover, we identified and validated the residues involved in the interaction between TRPM8 and Rap1A which are residues E207 and Y240 in the sequence of TRPM8 and Y32 in that of Rap1A.
Overall, these data deepen our knowledge of the role of TRPM8 in PCa progression, providing new insight into its possible use as new therapeutic target in PCa treatment.

THE TRPV1 CHANNEL, A THERMOSTAT TO REGULATE MITOCHONDRIAL THERMOGENESIS?

Florian BEIGNON[1]; Sylvie DUCREUX[2]; Naig GUEGUEN[1]; Léa TUIFUA[1]; Arnaud CHEVROLLIER[1]; Salim KHIATI[1]; Yannick LE DANTEC[1]; Hélène TRICOIRE-LEIGNEL[3]; Cesar MATTEI[3]; Guy LENAERS[1];
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During evolution, the mitochondrial energetic metabolism has been linked to thermogenesis in endothermic species. This heat production is caused by the uncoupling of biochemical reactions related to transformation, storage and use of energy sources, which subsequently evolved in homeotherms to maintain a constant body temperature.
The systemic physiological mechanisms involved in thermoregulation have been extensively studied. However, the cellular and molecular pathways of thermoregulation are still incomplete. Mitochondria are known to be the center of energy metabolism and cellular thermogenesis, and recent estimates of the temperature of active mitochondria suggest that it ranges between 45°C to 50°C, rather than 37°C. This new data upsets our current knowledge about the regulation of thermogenesis at the cellular level.
In this context, we have identified a mitochondrial isoform of TRPV1, a cation channel sensitive to high temperatures (>43°C), with a mitochondrial targeting sequence. Its potential involvement in mitochondrial calcium homeostasis associated with its thermosensitivity fits with the concept of a mitochondrial thermostat. In addition, 4 TRPV1 variants have recently been associated with syndromes related to dysregulation of body thermoregulation, namely malignant hyperthermia and exertional heat stroke. The identification and characterization of this mitochondrial TRPV1 isoform open new perspectives on the metabolic contribution of mitochondria in physiological and pathological regulations of thermogenesis.

IS NAVβ3 A NEW ACTOR OF MECHANOTRANSDUCTION IN ENDOTHELIAL CELLS?

Léa RETHORE; Anne-laure GUILBAUD - GUIHOT; Linda GRIMAUD; Coralyne PROUX; Daniel HENRION; Christian LEGROS; Claire LEGENDRE;
Laboratoire MitoVasc, UMR CNRS 6015 - INSERM U1083-Université d'Angers

The endothelial cell (EC) is the central actor of vascular tone and homeostasis and as such endothelial dysfunction promote cardiovascular diseases including atherosclerosis. This endothelial function is mainly controlled by shear stress generated by blood flow. This physical force is detected by many mechanosensors and mechanotransductors in EC, which trigger intracellular signalling pathways influencing endothelial behaviour. Among the mechanoproteins, several ion channels have been identified.
Despite the fact that the voltage-gated Na+ (NaV) channels have been shown to be mechanosensitive in cardiac and intestinal cells, these ion channels also expressed in vascular cells, have not been extensively studied in EC. Here, the aim of this study is to explore their contribution in the endothelium mechanotransduction.
Human Umbilical Vein EC (HUVEC) and Human Aorta EC (TeloHAEC) have been exposed either to a physiological laminar shear stress (LSS) at 20 dynes/cm² or to a pathological oscillatory shear stress (OSS) at 20 dynes/cm² with 1 Hz frequency using the Ibidi pump system. Only LSS induced cell orientation in the direction of flow (quantified by local gradient orientation method) and activated the atheroprotective signalling pathway (KLF2/KLF4, eNOS). Interestingly, this physiological LSS lead to

an important modification of the expression of the NaV channel subunit transcripts. Notably, SCN3B expression, encoding NaVβ3 protein, belonging to Ig-CAM superfamily, was increased with a 2.1-fold at 24 hours, 3.9 at 4 days and 12.6 at 7 days in HUVEC and 2.9 at 4 days in TeloHAEC, whereas OSS did not lead to a significant increase of SCN3B expression. Moreover, the atheroprotective KLF2 transcription factor might be involved in SCN3B regulation since trans-resveratrol-induced KLF2 expression and KLF2 plasmid transfection led to increase SCN3B expression in EC. Furthermore, extinction of SCN3B by siRNA seems to impaire EC alignment in response to LSS. Finally, immunoprecipitation of NaVβ3 and mass spectrometry analysis revealed that integrin α5 could be a novel protein partner of NaVβ3.

Taken together, our results suggest that the Ig-CAM NaVβ3 could be a novel actor of the endothelial mechanotransduction involved in cell alignment in response to atheroprotective flow through the KLF2 pathway activation.

Tuesday, September 13th 2022

08:30 Symposium 3: "Ion channel and pain processing"

Organized by ***Perrine Inquimbert*** *(Strasbourg, France)*

"HOW WE FEEL: MOLECULAR SENSORS UNDERLYING OUR SENSES OF TOUCH AND PAIN"

Reza SHARIF NAEINI
Department of Physiology and Cell Information Systems group, McGill University

Arthritis pain is a debilitating condition affecting millions of people worldwide. Although pain upon movement or palpation of the joint is a cardinal symptom of this disease, it remains poorly managed. We have previously demonstrated that mechanosensitive ion channels expressed at the nerve terminal of pain sensing neurons in the joints play a key role in arthritis pain. Yet efforts are still underway to determine the molecular identity of these channels. Recently, we identified an ion channel whose expression is necessary for detecting painful mechanical stimuli. Our present experiments examine the role of this channel is joint pain experienced in a mouse model of osteoarthritis pain.

ACID-SENSING ION CHANNELS (ASICS) IN PERIPHERAL AND SPINAL PAIN PROCESSES

Emmanuel DEVAL
Institut de Pharmacologie Moléculaire et Cellulaire, UMR 7275 CNRS/Université Côte d'Azur, Valbonne, France

ASICs are depolarizing ion channels mainly selective for sodium ions and gated by extracellular protons. They are widely expressed in the pain neuraxis, including peripheral, spinal and supra-spinal neurons. There are different ASIC channel subtypes with different sensitivities to extracellular pH and biophysical properties, reflecting the great diversity of neuron responses to extracellular pH variations. These channels are made of the trimeric association of different subunits, including ASIC1a, ASIC1b, ASIC2a, ASIC2b and ASIC3. ASIC1b and ASIC3 are essentially expressed in peripheral neurons, whereas ASIC1 and ASIC2 are found both in peripheral and central neurons, including neurons of the dorsal spinal cord.
We identified a family of endogenous lipids, namely the lysophosphatidyl-choline (LPC) family, in the synovial fluids of patients with painful joint diseases, as activators/potentiators of ASIC3 channel. We found elevated levels of LPC, and more particularly the LPC16:0 species, in synovial fluids from two independent cohorts of patients with rheumatic diseases. Moreover, the levels of LPC16:0 correlated with pain outcomes in the cohort of patients with osteoarthritis. We next developed a pathology-derived rodent model by performing intra-articular injections of LPC16:0 in mice. Both male and female mice developed persistent pain and anxiety-like behaviors following LPC16:0 injections, and these effects were dependent on peripheral ASIC3 channels, which drive sufficient peripheral inputs to generate spinal sensitization processes.

We also demonstrated a particular participation of ASIC1a channels in dorsal spinal cord neurons to the facilitation/sensitization process called windup. To do so, we combined both ex vivo and in vivo electrophysiological recordings of spinal neurons with computational modeling.
These data highlights the potential of ASICs, both in peripheral and spinal neurons, to participate to pain sensitization processes that lead to chronic pain.

T-TYPE CALCIUM CURRENT IN PKCγ NEURONS GATING CHRONIC PAIN IN THE DORSAL SPINAL CORD IN MICE

Pierre-François MERY[1], Célia Cuculière[1], Antoine Fruquière[1], Vanessa Soubeyre[1], Enola Mainvis[1], Miriam Candelas[1], Coline Ruhle[1], Jean Chemin[1], Emmanuel Deval[2], Eric Lingueglia[2], Amaury François[1], Sophie Laffray[1], Emmanuel Bourinet[1].
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Pain management is not always successful, and 20% of the population suffer chronic pain. In the quest for new analgesic targets, our team found that the genetic ablation of CaV3.2, a T-type calcium channel, could alleviate chronic mechanical allodynia (François et al 2015; Fruquière et al. submitted). The data also suggested that the dorsal horn (DH) of the spinal cord was the locus of this analgesic effect (Candelas et al. 2019; Fruquière et al. Submitted). Cav3.2 is expressed in excitatory as well as inhibitory interneurons in the DH, and does not segregate with a selective firing pattern. Thus, understanding the analgesic effect of CaV3.2 ablation requires a dissection of its roles in identified, tagged, subpopulations of DH interneurons. The ongoing study of a protein kinase C-γ (PKCγ)-Cre ERT2 mouse line is providing first insights since 1) Cav3.2 is homogeneously expressed in these PKCγ interneurons, and 2) PKCγ interneurons are the key component of the gate control of pain. Combining viral-directed transgene expression, electrophysiology, immunofluorescence, and clarification techniques, we are addressing the functional role of CaV3.2 in these neurons and the neuronal network gating neuropathic chronic pain. Our results suggest multiple roles for Cav3.2 in the intrinsic properties and in the synaptic transmission of PKCγ-expressing neurons. Surprisingly, other T-type channels are also involved in these mechanisms. The significance of these redundancies will be discussed.

TOWARD A CENTRAL ORIGIN OF NOCICEPTIVE HYPERSENSITIVITY SEEN IN ADULT RATS AFTER A NEONATAL MATERNAL SEPARATION

Clémence GIERE; Yannick MENGER; Vincent LELIEVRE; Meggane MELCHIOR; Pierrick POISBEAU ;
8 Avenue du Général Rouvillois 67000 Strasbourg

Early life adversities alter the development of a still maturing nervous system and have long-term consequences on its function at adult age. As such, adult rats with a history of neonatal maternal separation (NMS) show a visceral and a general somatic nociceptive hypersensitivity to mechanical and thermal nociceptive stimulation.
In this study, we have characterized the integration of nociceptive heat and cold as well as mechanical stimuli by convergent Wide Dynamic Range neurons situated in the deep layer of the spinal cord through in vivo electrophysiological extracellular recordings.

If thresholds and latencies of response were not affected, we found that neurons of NMS rats have a higher frequency of discharge following a peripheral mechanical or thermal nociceptive stimulation, a higher instantaneous frequency, as well as a higher frequency of post-discharge only in the case of nociceptive heat. We also found a decreased expression of genes coding for thermoreceptors TRPV1, TRPM8 and TRPA1 in the dorsal root ganglia (DRG) of NMS rats while mechanical nociceptor Piezo2 was not affected. In an attempt to explain this hyperexcitability within the spinal cord, we also have measured the gene expression of two chloride co-transporters involved in the GABAergic balance in the dorsal horn and found that in the DRG of NMS rats, exporter KCC2 is underexpressed, while in their spinal cord, the importer NKCC1 is overexpressed.
Together, these results argue in favour of a central disinhibition leading to an hyperexcitability of spinal Wide Dynamic Range neurons which alter the integration of nociceptive mechanical and thermal nociceptive stimuli in NMS rats.

17:15 Symposium 4: "Mechanosensitive Ion channels: from bacteria to mammalians"

*Organized by **Claire Legendre** (Angers, France)*

SENSING FORCES IN PLANTS WITH MECHANOSENSITIVE CHANNELS

Jean-marie FRACHISSE
Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), 91198, Gif-sur-Yvette, France

Plant as all living organisms are subjected to mechanical forces arising either from their environment or from inside their own body. From the environment mechanical cue are present through obstacles in the soil encountered by root or through rain wind and gravity exerting mechanical stimulations on aerial parts. From inside, forces result from the high osmotic pressure encountered in the cell and at shoot scale from the compression of inner tissues whilst outer tissues are under tension. To probe these forces, in order to control their development, organisms have develop very efficient mechanosensors: the mechanosensitive (MS) channels.
MS channels identified and partially characterized in plants belong to MSL, MCA, Piezo, OSCA and TPK families [1]. Inserted in the membrane, they are able to convert, almost instantaneously, mechanical stimuli into electrical or biochemical signals. Here, we will discuss on AtMSL10 from the Arabidopsis model plant, homologous to the E.coli EcMscS (Mechanosensitive channel Small conductance) able to catalyse large flux of anions [2,3]. Involved in cell signaling, AtMSL10 is expressed in aerial organs and its activity is amplified by oscillatory stimulation at frequencies corresponding to wind-driven oscillations of plant stems and leaves. Therefore, AtMSL10 is proposed to represent a molecular component allowing the perception of oscillatory mechanical stimulations by plants [4]. More recently, we characterized in the same plant a calcium MS channel rapidly activated (RMA) associated with the presence of the DEFECTIVE KERNEL1 (DEK1) [5]. We are currently investigating the role of this channel in root mechanosensing.
Finally, we will stress the need to reintegrate plant MS channel in cellulo-contexte. Indeed, MS channels described with the patch-clamp technique, are characterized in

the absence of a cell wall. There is a need to map membrane tension at the cellular scale in order to specifying where and under which conditions MS channels operate.
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[3] Maksaev et al., 2012 doi : doi.org/10.1073/pnas.1213931109
[4] Tran et al., 2021, doi: 10.1073/pnas.1919402118.
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WHAT HAVE WE LEARNED ABOUT MECHANOTRANSDUCTION FROM STUDIES OF BACTERIAL MECHANOSENSITIVE CHANNELS?

Boris MARTINAC
Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia School of Clinical Medicine, UNSW Medicine & Health, St Vincent's Healthcare Clinical Campus, Faculty of Medicine and Health, UNSW Sydney

Over 30 years ago, patch clamp recordings from E. coli giant spheroplasts revealed mechanosensitive (MS) channel activities known today as MscS and MscL [1, 2]. Following on these initial discoveries, MscL was the first MS channel ever been cloned, purified, reconstituted into liposomes [3] and crystallized for 3D structural determination [4]. These pioneering studies of MS channels firmly established the existence of mechanosensitive channels in the late 1980s, which paved the way to the 2021 Nobel Prize for the discovery of the Piezo mechanoreceptor family as duly recognized by the Nobel Assembly [5]. The channel is a homopentamer with each subunit consisting of two transmembrane alpha-helices (TM1 and TM2) and with both the amphipathic N-terminus and alpha-helical C-terminus facing the cytoplasm. There are currently about 2,300 members of the MscL channel subfamily listed in the UniProt database with homologues found in all three domains of life, Bacteria (Gram-negative and Gram-positive), Archaea and Eucarya [6].
MscS was identified several years after MscL [7]. 3D-crystal structure of MscS shows that the channel is assembled as a homoheptamer [8]. Like MscL, MscS can be reconstituted into liposomes and gated by mechanical force according to the Force-From-Lipids (FFL) principle [9]. It requires about a half the membrane tension that gates MscL [10]. MscS-like proteins are found in numerous organisms ranging from bacteria and archaea to plants, fungi, single-celled algae, and a variety of mammalian parasites [6].
MscL and MscS have been important for establishing FFL as the fundamental physico-chemical principle underlying the gating of MS channels from bacteria to humans. Besides MscL and MscS, Piezo1, the two-pore-domain K+ channels TRAAK and TREK as well as OSCA/TMEM63 have been purified and shown to be inherently mechanosensitive in lipid bilayers [11].
In my talk, I will briefly revisit a history of MS channels, including formidable obstacles against interdisciplinary research involving bacterial electrophysiology of MscL and MscS.

References:
[1] Martinac et al., 1987 ; [2] Martinac et al., 2013 ; [3] Sukharev et al., 1994 ; [4] Chang et al., 1998 ; [5] Ernfors et al., 2021 ; [6] Martinac et al., 2014 ; [7] Levina et al., 1999
[8] Bass et al., 2002 ; [9] Teng et al., 2015 ; [10] Nomura et al., 2012 ; [11] Cox et al., 2019

PATCH-SEQ PROFILING OF MECHANOSENSITIVE SOMATOSENSORY NEURONS

Thibaud PARPAITE; Lucie BROSSE; Nina SEJOURNE; Amandine LAUR; Yasmine MECHIOUKHI; Patrick DELMAS; Bertrand COSTE;
LNC-UMR 7291, Marseille, France

A variety of DRG mechanosensory neurons are involved in touch, proprioception, and pain. Physical cues are detected at the sensory nerve endings/auxiliary cells, where specialized mechanosensitive (MS) ion channels convert mechanical stimuli into electrochemical signals.
DRG neurons express a large repertoire of excitatory MS currents classified as rapidly adapting (RA), intermediately adapting (IA), and slowly/ultra slowly adapting currents, according to their inactivation kinetics to sustained mechanical stimulation. It is now well established that PIEZO2 channels sustain RA MS currents. Importantly, IA and slow-type MS currents are largely unaffected in Piezo2 KO DRG neurons, demonstrating that as-yet-unknown MS channels must account for the other MS currents. It is worthwhile to identify these channels since many sensations related to innocuous and noxious mechanical stimuli are independent from PIEZO2.
We combined recordings of mechanosensitive (MS) currents in mechanosensory neurons with single-cell RNA sequencing. Transcriptional profiles of individual neurons are mapped onto previously identified sensory neuron types to identify cell-type correlates between datasets. Correlation of current signatures with single-cell transcriptomes provides a one-to-one correspondence between MS current types and transcriptomically defined neuronal populations.
Gene-expression differential comparison provides a set of candidate genes for mechanotransduction complexes. Piezo2 is expectedly found to be enriched in rapidly adapting MS current-expressing neurons, whereas Tmem120a (Tacan) and Tmem150c (Tentonin 3), thought to mediate slow type MS currents, are uniformly expressed in all mechanosensory neuron subtypes. Further knockdown experiments disqualify them as mediating MS currents in sensory neurons.
Our dataset constitutes an open resource to explore further the cell-type-specific determinants of mechanosensory properties.

STOMATIN, A MAJOR MODULATOR OF MECHANOSENSITIVE ION CHANNELS IN HUMAN RED BLOOD CELLS?

Sarah ROUGE[1]; Sandrine GENETET[1]; Michael DUSSIOT[2]; Benoit ALLEGRINI[3]; Mariano a OSTUNI[1]; Lars KAESTNER[4]; Hélène GUIZOUARN[3]; Isabelle MOURO-CHANTELOUP[1];
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[3]: Institut de Biologie de Valrose, Université de Côte d'Azur, Nice, FRANCE
[4]: Theoretical Medicine and Biosciences & Experimental Physics, Saarland University, Homburg & Saarbrücken, GERMANY

In human Red Blood Cells (RBCs), mechanosensitive ion channels (MICs) have been recently described to regulate hydric content and therefore modify cell morphology, a process that allows erythrocytes passage through microcapillaries. Recently, two MICs have emerged in RBCs: Piezo1 and Pannexin1. The first, by allowing calcium entrance, permits water loss by KCNN4 channel activation and the second, allowing ATP release permits gain of water by purinergic receptor activation.

MICs regulation has been historically described in C. elegans worm as provided by stomatin family protein. Erythroid stomatin (band 7.2b), initially shown to be largely expressed in lipid rafts, is missing in the RBCs of patient with Overhydrated Hereditary Stomatocytosis (OHSt).
The aim of this study was to measure ion fluxes after Pannexin1 and Piezo1 mechanical activation in an erythroid context, with or without stomatin.
Physical proximity between stomatin and the two MICs was assessed by Proximity Ligation Assay, showing 27.09% of PLA+ RBCs for stomatin-Pannexin1, and 32.34% of PLA+ ghosts (haemoglobin depleted RBCs) for stomatin-Piezo1.
After activation by high extracellular K+, Pannexin1 activity was tested by the permeability increase to a fluorescent anionic dye (5(6)-carboxyfluorescein), revealing 11.8% in OHSt RBCs and 34% in control RBCs. Similarly, after activation of Piezo1 by Yoda1, intracellular calcium measurement of Fluo-4-AM preloaded cells using fluorescence imaging led to a relative fluorescence (Ft=30-80sec/F0) of 1.511 for OHSt RBCs versus 2.633 for control RBCs.
In K562 cell line invalidated for stomatin by CRISPR Cas9, the stimulation of endogenous Pannexin1 by a hypotonic shock resulted in an increase of TO-PRO-3 fluorescent dye influx (18.46% in stom- cells and 35.72% in stom+ cells). Similarly, stimulation of transfected-Piezo1 with Yoda-1 resulted in ruthenium-red sensitive amplitude current density of -67pA/pF in stom+ cells versus -55pA/pF in stom- cells, using whole-cell patch clamp.
This study showed a clear decrease of activity for both Pannexin1 and Piezo1 MICs in stimulated stomatin-deficient cells, indicating a loss of mechanosensitivity in absence of stomatin. By destabilizing microdomain structures, stomatin-deficient cells could have partially lost membrane rigidity, suggesting a potential role of lipids and cytoskeletal proteins, still to be identified, in the activity of RBC MICs.

Wednesday, September 14th 2022

09:30 Symposium 5: "Ion channels & nanodomains"

*Organized by **Fabien Brette** (Montpellier, France)*

HOW PHOSPHORYLATION OF THE CALCIUM CHANNEL CAV1.2 CAPTURES YOUR ATTENTION

Kyle E. Ireton[1,2], Timothy Hanks[2,3], and Johannes W. Hell[1,2]
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The β_2 adrenergic receptor (AR) forms a unique signaling complex with the L-type Ca^{2+} channel $Ca_V1.2$ that also contains the trimeric G_s protein, adenylyl cyclase, and the cAMP-dependent protein kinase A (PKA). Phosphorylation of $Ca_V1.2$ by PKA on Ser^{1928} augments Ca^{2+} influx and is required for long-term potentiation that is induced by prolonged theta-tetanus (PTT-LTP). Norepinephrine (NE) is a critical mediator of attention. It acts via AR but the critical downstream mechanisms are largely unknown. Here we show that S1928A knock-in mice show striking deficiencies in attention tasks. Accordingly, $Ca_V1.2$ emerges as a critical molecular locus that governs attention.

DIFFERENTIAL L-TYPE CALCIUM CHANNEL NANODOMAIN SIGNALING IN HEART FAILURE

Julia Gorelik
National Heart, and Lung Institute (NHLI), Faculty of Medicine, Imperial College London, United Kingdom

Heart failure (HF) is a major contributor to the cardiovascular disease and impacts significantly to global health expenditure. Pathological remodelling is consistently observed in HF patients; that includes the alterations in ion channels, in particular L-type calcium channels (LTCCs), Ca2+-handling proteins, and proteins mediating cell-cell coupling. In HF, a progressive loss of T-tubules (TT) results in molecular remodelling and in the development of triggers of arrhythmia (early and delayed after-depolarizations, EADs and DADs). In cardiac myocytes, receptors are coupled to effector molecules and ion channels in nanodomains. Using novel cutting-edge methodology including scanning ion conductance microscopy (SICM) and scanning patch clamp technique we uniquely resolved surface topography in living myocytes at nanoscale. We examined rat cardiomyocytes from a myocardial infarction model and human cardiomyocytes from patients with ischemic and dilated cardiomyopathies, as well as from patients with left ventricular assist devices (LVAD).
Particularly, we focused on the TT and intermediate crest of the sarcolemma. We studied how the disturbance of these nanodomains affects LTCC function. We found that disruption of cytoarchitecture in failing human ventricular myocytes leads to re-distribution of functional LTCCs from TT to the sarcolemma crest. In ischemic cardiomyopathy, the increase in LTCC activity in the TT depends on the activity by

protein kinase A, whereas in dilated cardiomyopathy, the increased LTCC opening on the sarcolemma crest results from enhanced calcium-calmodulin kinase II modulation. Further, we attempted to restore the normality of nanodomain organisation by means of LVAD implantation to indirectly influence the calcium channels and improve contraction. Implantation restored LTCC activity to normal levels.
This work gives an understanding of how signalling operates at nanoscale resolution in living cells. These findings demonstrate that targeting pathological LTCC rearrangement at an early stage of heart failure could prevent later maladaptive changes. This will translate to new methods of restoring proper organisation of nanodomains for treatment of failing hearts.

SPATIOTEMPORAL REGULATION OF ION CHANNELS IN THE CARDIOMYOCYTE: SOURCE OF ELECTRICAL PLASTICITY OR RISK OF ARRHYTHMIA?

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The intracellular trafficking and membrane expression of ion channels constitute a plasticity sources at the myocardium level, refining electrical activity and contributing to adaptive change of working myocardium. Our group have contributed to the understanding of mechanisms regulating dynamic expression of cardiac ion channels and characterization of molecular determinants for trafficking to specialized nanodomains in cardiac myocytes. We maintain an interest in dissecting spatio-temporal process in relation to 3D architecture of myocardium. Our studies combine cellular biology approaches (cardiac myocytes isolation and culture), biochemistry and molecular biology (viral vectors to specifically target proteins expression), electrophysiology (patch-clamp, microelectrode), immuno-histo/cytochemistry, and molecular and cellular imaging (3D deconvolution, TIRF, light sheet). In addition to this work on ion channels trafficking and targeting mechanisms in cardiac myocytes, a major perspective is to understand the link between electromechanical remodeling and disease heart, in particular atrial fibrillation, and alteration of ion channel trafficking and targeting to sarcolemma.

DECIPHERING MOLECULAR ARCHITECTURE OF ANO1-CONTAINING MULTIPROTEIN SIGNALING COMPLEXES IN MAMMALIAN SENSORY NEURONS

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Intracellular signaling is paramount for efficient and correct functionality of excitable cells. Since neurons receive and integrate multiple external signals, tight and precise control of these signaling mechanisms must be in place. This is particularly true for ‘pain-sensing’ neurons (nociceptors) as incorrect excitation of these cells may lead to pathological pain. One way in which specificity of intracellular signaling can be bestowed is through compartmentalization of signals. Compartmentalization can be achieved by structuring areas of the endoplasmic reticulum and plasma membrane (ER-PM) into junctions where these are in very close proximity (<30nm) to aid local, compact signaling events. ER-PM junctions are important structural components of nociceptors where inflammatory signaling events often occur; ion channel complexes

have been discovered to form at these junctions in nanodomain organization. We report that ANO1 (TMEM16A), a Ca2+-activated Cl- channel, is central to inflammatory complexes found at ER-PM junctions in nociceptors; due to relatively high intracellular Cl- levels in sensory neurons, ANO1 activation produces an excitatory effect. As revealed through super-resolution stochastic optical reconstruction microscopy, proximity ligation assay and molecular biology approaches, ANO1 forms complexes with inositol triphosphate receptors (IP3R), transient receptor potential vanilloid (TRPV1) and G-protein coupled receptors (GPCRs) such as bradykinin-activated B2 receptors (B2R). Activation of TRPV1 or B2R induces Ca2+-release from the ER through IP3R, which has been shown to be in direct contact with ANO1 at the PM. This nanodomain arrangement allows the low Ca2+-sensitivity of ANO1 to be overcome by the high Ca2+ concentration found at the mouth of IP3R. This arrangement at ER-PM junctions is extremely important as GPCRs able to induce IP3R Ca2+-release are also able to activate ANO1 however, other Ca2+ sources such as voltage-gated Ca2+ channels are poorly coupled to ANO1 in these neurons, hence compartmentalizing ANO1 activation. Our current work is focused on discovering other components of ER-PM junctions in sensory neurons; these include scaffolding molecules, Junctophilin-4 and Extended Synaptotagmin 1. In summary, ANO1-containing signaling complexes at ER-PM junctions are important signaling units involved in inflammatory pain signaling. Understanding structure-functional relationships of molecular residents of this complex may uncover new ways to target inflammatory pain.

POSTER ABSTRACTS

P1- CHARACTERIZATION OF HONEYBEE CAV4/DSC1: A CA CHANNEL WITH SURPRISING PROPERTIES

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We have recently cloned a channel homologous to DSC1 (drosophila sodium channel 1) in the honeybee. Despite sequence similarity with voltage-dependent Na+ channel, we demonstrate a strong calcium (Ca2+) permeation, and suggested that AmSC1 may belong to a new type of voltage-dependent calcium channel (VDCC), now named CaV4.
Identification of a particular sequences for the selectivity filter (DEEA), for inactivation domain (MFL, homologous to the IFM of the NaV channel) and a C-terminus sequence containing a calmodulin (Cam)-binding site led us to analyze the singular permeation and the voltage and calcium-dependent inactivation properties of this new channel.
Its voltage-dependence of activation and deactivation suggest an important role in the Ca influx during action potential

P2- CONTROL OF THE CA2+ DEPENDENT K+ CHANNEL, KCNN4, BY CALCIUM PUMP.

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Gain of function mutations in KCNN4, a calcium-activated potassium channel, and in the mechanosensitive non-selective cation channel PIEZO1 have been identified in patients suffering the rare disease hereditary xerocytosis (HX)(1). This condition is an autosomic dominant haemolytic anaemia affecting 1/50 000 people. It is characterized by an abnormal RBC cation permeability correlated to RBC dehydration. The cells are fragilized and easily removed from the circulation. HX is clinically highly heterogeneous, patients can have moderate to severe anaemia, requiring, or not, transfusions. This heterogeneity is yet unexplained and slows the diagnosis. It is observed that with PIEZO1 gain-of-function mutations, RBC are dehydrated due to hyper activation of KCNN4 (2). The increased intracellular Ca2+ concentration mediated by PIEZO1 activates KCNN4 and this activation leads to RBC dehydration due to KCl loss. Strikingly, gain-of-function mutations in KCNN4, effector of the RBC dehydration, might not associate with changes in RBC volume (3). This paradox suggests a complex regulation of KCNN4 activity in RBC.
In this study, we aimed at understanding the regulation of ion channel activity involved in RBC volume homeostasis. We showed that the RBC Ca2+ pump, PCMA4b (ATP2B4), regulates KCNN4 activity. In RBC, PMCA4b controlled KCNN4-mediated dehydration independently of intracellular Ca2+ changes. Using electrophysiology, we showed that PMCA4b expression reduced KCNN4 current induced by PIEZO1 activation in HEK293T cells. Surprisingly, this effect was not

mediated by the Ca2+ extrusion activity of the pump but rather by a molecular interaction between KCNN4 and PMCA4b. We observed PMCA4b as part of KCNN4 interactome at the membrane of RBC using co-immunoprecipation, coupled with mass spectrometry and proximity ligation assay. This interaction was confirmed in HEK293T cells.
Overall, we present PMCA4b as a new regulating partner of KCNN4/PIEZO1 functional complex.

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(2) Rapetti-Mauss R. et al. Haematologica 2017
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P3- HETEROMERIZATION OF ALKALINE-SENSITIVE TWO-PORE DOMAIN POTASSIUM CHANNELS

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Two-pore domain (K2P) potassium channels are active as dimers. They produce inhibitory currents regulated by a variety of stimuli. Among them, TALK1, TALK2 and TASK2 form a subfamily of structurally related K2P channels stimulated by extracellular alkalosis. The human genes encoding them are clustered on chromosomal region 6p21. They are expressed in different tissues including the pancreas. By analyzing single cell transcriptomic data, we show that these channels are co-expressed in insulin-secreting pancreatic $_{L\chi}$ cells. By different approaches we show that they form functional heterodimers. Heteromerization of TALK2 with TALK1 or with TASK2 endorses TALK2 with sensitivity to extracellular alkalosis in the physiological range. The association of TASK2 with TALK1 and TALK2 increases their unitary conductance. These results provide a new example of heteromerization in the K2P channel family.

P4- CHARACTERIZATION OF THE EFFECTS OF THE INSECTICIDE DELTAMETHRIN ON NEURONAL SODIUM CHANNELS AND MUSCULAR CALCIUM CHANNELS FROM THE HONEY BEE APIS MELLIFERA.

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Honey bees are important pollinators and are often described as indicator species for the quality of the environment. Domestic honey bees have suffered significant losses over the last decades and insecticides are clear contributing factors to this decline. Bees intoxicated with deltamethrin, a widely used pyrethroid insecticide, exhibit symptoms of strong neuromuscular alterations. In order to better understand the modes of action of this molecule on bees, we characterized its effects on isolated antennal neurons and skeletal muscle fibers. Using the patch-clamp

electrophysiological method, we monitored the effects of deltamethrin on voltage-gated sodium channels (NaVs) and on voltage-gated calcium channels (CaVs), which are responsible for action potentials in neurons and insect muscle fibers, respectively. Under voltage-clamp, deltamethrin clearly impeded neuronal NaVs function by slowing down their closure. The sodium deactivation current was altered in both a use-dependent and a concentration-dependent manner. Conversely, in muscle fibers, preliminary observations suggest that ionic current amplitudes through CaVs were not altered by similar concentrations of deltamethrin. Further analysis of the CaVs biophysical properties is needed to confirm this latter result and in particular to fully describe their voltage-dependency in the presence of the insecticide.

P5- ELECTRO-PHARMACOLOGICAL PROPERTIES OF CAV3.1 CALCIUM CHANNEL CARRYING GAIN-OF-FUNCTION MUTATIONS CAUSING NEURODEVELOPMENTAL DISORDERS

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T-type voltage-gated calcium channels (Cav3) play important roles in neuronal excitability and calcium signaling because of their particular electrophysiological properties. Mutations in Cav3 channels are mainly associated with neurological diseases, such as Childhood Cerebellar Atrophy (ChCA) also called early-onset severe spinocerebellar ataxia 42 (SCA42ND). ChCA is a serious and complex neurodevelopmental disease caused by de novo, gain-of-function mutations in CACNA1G, the gene encoding the channel Cav3.1. Here we describe novel missense mutations causing ChCA lining the inner S6 pore region. These mutations lead to defects in Cav3.1 gating, in particular a slowing of the inactivation and deactivation kinetics as well as an increase of the window current. In addition, neuronal modeling reveals an impact of these mutations on neuronal excitability. Towards identifying a therapeutical strategy for ChCA, we now evaluate the efficiency of T-type calcium channel blockers (e.g. TTA-P2 and Z944) to alleviate the aberrant gating properties of Cav3.1 mutant channels. Overall, this study describes further the functional consequences of the ChCA gain-of-function mutations of the Cav3.1 channel and provides a first pharmacological investigation of these mutant channels.

P6- IDENTIFICATION AND VALIDATION OF TRPV2 AS NEW DRUGGABLE TARGET IN METASTATIC MELANOMA

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Melanoma is a highly aggressive cancer endowed with a unique capacity of rapidly metastasizing, which is fundamentally driven by aberrant cell motility behaviors. Discovering “migrastatics” targets, specifically controlling invasion and dissemination of melanoma cells during metastasis, is therefore of primary importance. Here, we uncover the prominent expression of the plasma membrane TRPV2 calcium channel as a distinctive feature of melanoma tumors, directly related to melanoma metastatic dissemination. In vitro as well as in vivo, TRPV2 activity was sufficient to confer both migratory and invasive potentials, while conversely TRPV2 silencing or pharmacological inhibition in highly metastatic melanoma cells prevented aggressive behavior. In invasive melanoma cells, TRPV2 channel localizes at the leading edge, in dynamic nascent adhesions, and regulates calcium-mediated activation of calpain and the ensuing cleavage of the adhesive protein talin, along with F-actin organization. In human melanoma tissues, TRPV2 overexpression correlates with advanced malignancy and poor prognosis, evoking a biomarker potential. Hence, by regulating adhesion and motility, the mechanosensitive TRPV2 channel controls melanoma cells invasiveness, highlighting a new therapeutic option for migrastatics in the treatment of metastatic melanoma.

P7- MECHANICAL AND PHARMACOLOGICAL ACTIVATION OF PIEZO1 CHANNELS CHARACTERIZED BY HIGH THROUGHPUT ELECTROPHYSIOLOGY

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PIEZO channels are mechanically-activated cation channels that play important roles in biological functions including touch, proprioception, shear stress and stretch sensation as well as blood pressure regulation. Mutations in Homo sapiens PIEZO1 channels are associated with anemia, malarial resistance, lymphatic dysplasia and varicose vein disease, suggesting important red blood cells and vascular roles in humans. The pharmacology of the PIEZO1 channels is in its infancy. Here we sought high throughout methodology for investigating small-molecule modulation in combination with mechanical stimulation. A bottleneck in PIEZO drug development has been the lack of mechanical stimulation in automated patch clamp. Here we show how the optimization of pipetting parameters of the SyncroPatch 384 lead to the possibility to mechanically stimulate PIEZO1 channels using high throughput electrophysiology. Data of mouse and human PIEZO1 channels expressed in HEK T-REx™ 293 cells activated by either mechanical or chemical stimuli will be shown as well as the combination of both methods. Under voltage-clamp we were able to show reliable quantification of PIEZO1 activation by fluid flow, Yoda1 (a small-molecule PIEZO1 agonist) and a Yoda1 analogue. To our knowledge, this is the first time that mechanical stimulation of PIEZO channels in a high throughput planar patch clamp system has been shown. The possibility of comparing and combining mechanical and chemical stimulation in a high throughput electrophysiological assay facilitates the biophysical and pharmacological studies of PIEZO channels.

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P8- TRPV4 CHANNEL ACTIVITY IN RESPONSE TO HYPOXIA IS AFFECTED BY CELL DENSITY

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Transient receptor potential vanilloid 4 (TRPV4) is a polymodal Ca2+-permeable channel involved in various hypoxia-sensitive pathophysiological phenomena. TRPV4 is activated by stretch and might thus be affected by change in cell rigidity. Indeed, modification of cell density used in experimental conditions can induce such a change, and lead to modified TRPV4 activation. The aim of this study was to evaluate the effect of cell density on TRPV4 activity in response to hypoxia. Transiently TRPV4-transfected HEK293T cells were seeded at low (1x104 cells/cm²) or high (3x105 cells/cm²) densities corresponding to non-confluent or confluent cells, respectively, on the day of experiments. Cells were then cultured under in vitro normoxia (21% O2) or hypoxia (1% O2) for 48 h. First, TRPV4 activation was assessed in response to GSK1016790A (100 nM), a specific TRPV4 agonist, and channel activity was measured with patch-clamp in cell-attached mode, calcium imaging thanks to the ratiometric Fura2-LR-AM fluorescent probe and Bioluminescence Resonance Energy Transfer (BRET) techniques. Then, TRPV4 localisation to membrane was evaluated using confocal microscopy imaging, cell surface biotinylation and BRET. Our results show that hypoxia exposure has a differential effect on TRPV4 activation depending on cell confluence. At low confluence level, TRPV4 response is increased in hypoxia, whereas at high confluence level, TRPV4 response is strongly inhibited. This diminution of TRPV4 activation can be explained by an internalization of the channel observed in hypoxia at high cell density. Thus, this highlights the importance of specific culture condition, namely cell confluence, which can influence many cellular processes, especially regarding TRPV4 channel activity in response to hypoxia.

P9- ELECTRICAL PROPERTIES AND INTRACELLULAR CALCIUM SIGNALING IN CARDIOMYOCYTES FROM THE HONEY BEE HEART

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In insects, circulation of haemolymph is driven by the autorhythmic contraction of the dorsal heart vessel located in the abdomen. In a previous study, a recently authorized class of insecticides was found highly toxic when honey bees were exposed through the dorsal abdomen, suggesting direct cardiotoxic effects. In order to explore cardiotoxicity, we developed enzymatical isolation of heart cells from bees. Their function was explored with traditional electrophysiological methods (current-clamp, voltage-clamp, intracellular microelectrode) and calcium imaging. Cell parameters were consistent with the in situ morphology observed in intact heart. Action potentials obtained under current-clamp with a patch pipette resembled action potentials recorded with intracellular electrodes in intact heart. Under voltage-clamp, ionic currents were recorded and their basic pharmacology was studied. Dynamic confocal microscopy was used to assess calcium homeostasis. Intact cardiomyocytes from honey bee may help better understand the excitability of insect heart and cardiotoxicity of insecticides.

P10- THE PROTECTIVE ROLE OF THE CA2+ CHANNEL TRPV6 IN PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is a major cause of cancer-associated mortality in Western countries. Prevention is exceedingly difficult and currently no specific tumor markers are available for early diagnosis at a curable stage. Ca2+ signals are emerging to be particularly relevant in PDAC. They mediate interactions between tumor cells and the tumor microenvironment to drive different aspects of cancer progression. Increasing evidence suggests that the overexpression of the Ca2+ channel TRPV6 is a common event in cancers of epithelial origin. Thus, our work aims at studying the role of TRPV6 channel in PDAC cell lines. After analyzing the channel expression in several PDAC cell lines, we prepared a stable clone model from Panc-1 cells. These clones either under- or overexpress TRPV6. Our work seeks to elucidate the potential role of TRPV6 in cell proliferation, death, and cycle progression, both at basal conditions and treated with common drugs used in PDAC: gemcitabine, cisplatin, and 5-fluorouracil. We demonstrate that cancer cells deficient in TRPV6 have a less aggressive phenotype which is characterized by reduced cell proliferation and intracellular ATP levels. They are also more prone to exhibit a pro-apoptotic phenotype, which was studied using cell cycle assay with focus on the cell cycle arrest in the sub-G1 phase. Contrasting with these results, overexpression of the TRPV6 channel leads to increased cell proliferation. Using both ATP assay and anexin V assay, we can also verify the higher vulnerability to the common chemo-treatments demonstrated by cells deficient in TRPV6. Overall, our results suggest that TRPV6 plays a protective role in PDAC cells, by modulating their phenotype on proliferation, cell survival and chemotherapy resistance.

P11- TARGETING THE CA^{2+}-ACTIVATED K^+ CHANNEL KCA3.1 IN THE PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) is a major cause of cancer-related deaths in Western countries. The knowledge about molecular mechanisms that give rise to PDAC have not yet delivered efficient therapies. PDAC is linked to the physiology and microenvironment of the exocrine pancreas. Ca2+ signals are particularly relevant in cancer. The cellular "Ca2+ toolkit" includes ion channels that indirectly affect Ca2+ signaling such as the Ca2+-dependent K+ channel KCa3.1. In PDAC, its elevated expression indicates a poor prognosis. Despite some in vitro information on the role of KCa3.1 channels, its role is still poorly characterized, and in vivo data are missing. The current project is aimed at reducing this gap of knowledge. In vivo experiments were conducted by using KrasLSL−G12D/+Trp53fl/flPdx1Cre/+ (KPfC) mice, a PDAC model. Mice were treated with vehicle, gemcitabine, the KCa3.1 inhibitor TRAM-34 or a combination of gemcitabine and TRAM-34. Tissue samples retrieved from mouse pancreata were stained and analyzed to assess the size of the tumors, the extent of fibrosis as well as the infiltration of immune cells into the tumor tissue. This was complemented through Western blot analysis. The therapeutic potential of KCa3.1 on migration/invasion was assessed in a spheroid model of PDAC composed of pancreatic tumor cells and pancreatic stellate cells. The inhibition of KCa3.1 in combination with gemcitabine leads to a moderate decrease in tumor size and an increase in the extent of fibrosis in tumors. This change of fibrosis is similar to the one observed in mice treated with only gemcitabine. It could be hypothesized that a combined effect of the two treatments leads to a change in cell type within the tumor tissue towards a more fibroblastic phenotype. This could cause a decreased expression of KCa3.1 and thus an inhibition of tumor growth. In mixed spheroids, treatment with TRAM-34 increases the invasive potential of the cells. This is not observed in the combination treatment, gemcitabine seemingly negating this effect of TRAM-34. These results suggest a function of KCa3.1 channels in complex tumor models that outdo the well described roles of the channels in individual cells types from the tumor microenvironment.

P12- B-LYMPHOCYTES EXPRESS NAV1.3 VOLTAGE-GATED SODIUM CHANNEL

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While the role of voltage-gated sodium channels (Nav) in excitable cells is well known, the presence and the physiological role of such channels in B-lymphocytes is much less investigated. Using patch-clamp and RT PCR, we investigated the presence of Nav channels in immortalized mature B-lymphocytes (EBV-B). In the whole-cell configuration, with a holding potential of -90 mV, depolarization induced an inward current with a threshold around -30 mV and a maximum current amplitude around 10 mV. This current is biphasic with a rapid peak (tau inactivation: 10 ms) followed by a sustained component (tau inactivation around 100 ms). This current was absent after substitution of sodium by NMDG, and it was inhibited by tetrodotoxin (1µM) as well as by ICA121431 (1µM) an inhibitor of Nav1.3. RT-PCR experiments confirmed the presence of Nav1.3 transcript. In conclusion, EBV-B lymphocytes express a Nav 1.3 voltage-gated sodium channel whose physiological role remains to be resolved.

P13- REGULATION OF CARDIAC NAV1.5 CHANNELS BY FHF2 PHOSPHORYLATION

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The voltage-gated Na+ channels NaV1.5 are responsible for the upstroke of cardiac action potentials, and dysregulations of this channel underlie inherited or acquired cardiac disease. While phosphorylation of NaV1.5 is extensive, the regulation of NaV1.5 by phosphorylation of its accessory proteins remains unexplored. Phosphoproteomics of mouse ventricular NaV1.5 channel complexes showed phosphorylation of the FHF2 accessory protein at 9 specific sites. To determine the functional roles of these sites, two cardiac cellular models were developed. Patch-clamp analyses demonstrated that FHF2 knockdown accelerates the rates of closed-state and open-state inactivation of NaV channels in both neonatal and adult ventricular cardiomyocytes, and shifts the voltage-dependence of NaV channel activation towards hyperpolarized potentials in neonatal cardiomyocytes. Although the rescue of FHF2 with the WT FHF2-VY isoform restored the inactivation properties of NaV channels in both neoanatal and adult cells, no restoration of the activation properties was obtained in neonatal cells, suggesting involvement of another FHF2 isoform in regulating NaV channel activation in neonatal cells. Similar to WT FHF2-VY, however, each of the analyzed FHF2-VY phosphomutants restored the inactivation properties of NaV channels in both models, preventing to identify roles for FHF2 phosphosites. FHF2 knockdown also increased the late Na+ current in adult cardiomyocytes, which was restored similarly by WT and phosphosilent FHF2-VY. Together, our results demonstrate that ventricular FHF2 is highly phosphorylated, implicate differential roles for FHF2 in regulating NaV1.5 channels in neonatal and adult cardiomyocytes, and suggest that the regulation of NaV1.5 by FHF2 phosphorylation is highly complex.

P14- PIEZO1 AND PIEZO2 FOSTER MECHANICAL GATING OF K2P CHANNELS

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Mechanoelectrical transduction is mediated by the opening of different types of force-sensitive ion channels, including Piezo1/2 and the TREK/TRAAK K2P channels. Piezo1 curves the membrane locally into an inverted dome that reversibly flattens in response to force application. Moreover, Piezo1 forms numerous preferential interactions with various membrane lipids, including cholesterol. Whether this structural architecture influences the functionality of neighboring membrane proteins is unknown. Here, we show that Piezo1/2 increase TREK/TRAAK current amplitude, slow down activation/deactivation, and remove inactivation upon mechanical stimulation. These findings are consistent with a mechanism whereby Piezo1/2 cause a local depletion of membrane cholesterol associated with a prestress of TREK/TRAAK channels. This regulation occurs in mouse fibroblasts between endogenous Piezo1 and TREK-1/2, both channel types acting in concert to delay wound healing. In conclusion, we demonstrate a community effect between different structural and functional classes of mechanosensitive ion channels.

P15- KV10.1 CHANNEL INVOLVEMENT IN BREAST TUMOR MICROENVIRONMENT SENSING

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Breast tumors are an heterogenous group of cancers. The most aggressive phenotypes are responsible of the largest part of cancer-related deaths due to the development of metastasis. Tumor microenvironment is one of the main promoters of this aggressivity. Among the different parameters, hypoxia has been shown to promote resistance, migration or metastasis development through the HIF-1α signaling pathway.
Otherwise, ion channels are main drivers of the different cancer cell properties as migration, proliferation or invasion. We previously demonstrated that Kv10.1 potassium channel regulates proliferation or motility of different breast cancer cell models in normoxia condition. We also found that Kv10.1 favors breast cancer survival induced by collagen-1 suggesting its capability to sensing the environment. However, at the best of our knowledge, few information is available about the role of Kv10.1 in response to hypoxia in cancer context.
Here, we present new results on the involvement of Kv10.1 potassium channel in MDA-MB-231 cells in severe hypoxia. Firstly, Kv10.1 channel is expressed in hypoxic sections of breast cancer tissue? samples. Moreover, both silencing Kv10.1 and its pharmacological inhibition reduced the epithelial-to-mesenchymal transition (EMT) markers expression (Vimentin and N-Cadherin). It also regulated migration by affecting different actors like integrin. In addition, the Kv10.1-dependent secretome favors angiogenesis. Finally, we compared our results on EMT, migration and angiogenesis obtained on MDA-MB-231 parental cells with a derived cell line presenting an improved brain metastasis tropism (MDA-MB-231-BrM2).
Kv10.1 could thus become a valuable target to slow the progression of aggressive breast cancers presenting hypoxic area.

P16- IDENTIFICATION OF THE RETICULO-MEMBRANE COMPLEX ORAI3/STIM1/IP3R (OSIR) IN THE FORMATION OF BREAST METASTASES

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Breast cancer is the most common and deadliest cancer in women. Metastasis of cancer cells to distant organs remain the leading cause of death. At the cellular level, dysregulation of calcium homeostasis is one of processes involved in metastasis. Among the proteins modulating this calcium homeostasis, three of them, ORAI3 (a plasma membrane Ca2+ channel), STIM1 (a reticular and membrane protein) and IP3R3 (a reticular Ca2+ channel receptor), acting close to each other, could form a functional complex ORAI/STIM/IP3R (OSIR). Many studies have shown an involvement of these proteins individually in cancer cell behaviors such as migration, invasion, proliferation and angiogenesis. However, the presence of this complex has

never been established in cancer cells. In that context, the aim of our study was to establish the presence and activity of the OSIR complex in two highly metastatic breast cancer cell lines (MDA-MB-231 and MDA-MB-231-BrM2). By the Proximity Ligation Assay technique, we succeeded in showing a proximity of each two actors simultaneously. The interaction between the 3 actors was confirmed with the Co-Immunoprecipitation technique. Ca2+ imaging protocols attest to the functionality of this complex in our breast cancer model. We are now focusing on the validation of the sequential or combined extinction of these three players to modulate their implication in metastasis process, and optimizing the preparation of our cellular samples for localization of the OSIR complex by high-resolution electron microscopy assays. Our aim is to provide proof of concept that by modulating the migratory and secretory capacities of breast cancer cells, the reticulo-membrane complex Orai3/STIM1/IP_3R3 could also promote the occurrence of metastases, making it possible to assign a predictive character of tumor aggressiveness in case of strong expression of these actors in the primary tumor.

P17- CHARACTERIZATION OF TGR5-INDUCED CA2+ SIGNALING IN CHOLANGIOCYTES

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The Bile acid (BA) receptor TGR5 is known to be coupled with cyclic-AMP signaling in cholangiocytes, allowing Cl- and bicarbonate secretion in parallel with water, making a fluid and alkalinized bile. Our aim was to decipher TGR5-dependent bile acid-induced Ca2+ mobilization in cholangiocytes, a previously overlooked pathway.
Using spectrofluorimetry, we measured cytosolic calcium concentration variations in normal human cholangiocytes and cholangiocarcinoma cell lines loaded with the Ca2+ dye Indo1, using the BA taurolithocholate (TLCA) and RO5527239 (RO) as TGR5 agonists. TGR5 expression was also suppressed using shRNAs.
Results: TLCA and RO5527239 (100 and 300 µM respectively for cell lines and normal cholangiocytes) induced dose dependent Ca2+ signals in both normal human cholangiocytes and cholangiocarcinoma cell lines. TGR5-dependent cytosolic Ca2+ mobilization occurred through a complex pathway involving: $G_{\neg\lambda}q/11$ engagement, Ca2+ release from the endoplasmic reticulum and a strong Ca2+ influx from the extracellular medium. Furthermore, TGR5-dependent Ca2+ increase was dependent on an ATP release and on the activation of P2Y2 receptors (which are also able to induce Ca2+ release and Ca2+ influx), as revealed by using P2Y2-specific inhibitors and apyrase. In the lack of TGR5 expression (TGR5 shRNA treatment), BA-induced Ca2+ mobilization was totally impaired.
Conclusion: Beside the well-known cAMP pathway that stimulates CFTR-dependent Cl- ions secretion, TGR5 stimulation also induces Ca2+ signals through a pathway involving ATP release and purinergic P2Y2 stimulation. This new signaling pathway in cholangiocyte may have potential CFTR-independent impact on Cl- ions secretion in bile, and possibly other pathophysiological consequences to be further explored.

P18- XENOGLO: NON-DESTRUCTIVE SINGLE-CELL SURFACE EXPRESSION MEASUREMENTS IN XENOPUS OOCYTES

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We developed a new technique to measure protein expression in Xenopus oocytes, which we named XenoGlo.
The assessment of expression levels is often desirable when studying ion channels. In particular, when using Xenopus oocytes as an heterologous system, variability in expression is considerable. The techniques available (western blotting, fluorescent microscopy of GFP-fused proteins, and chemiluminescent detection of external epitopes) are time-consuming multi-step protocols, associated with problems of reproducibility and the destruction of the cell. We sought to optimize a protein expression detection system for oocytes that overcomes these disadvantages.
XenoGlo relies on the functional complementation of a nanoluciferase with an 11-amino acid peptide fused to the protein of interest. Protein levels of single oocytes dispensed in 96-well plates can be assessed in less than 20 minutes by measuring luminescence upon the addition of the luciferase and its substrate. Remarkably, functional tests (e.g., two-electrode voltage clamp) can be performed on the same oocytes posterior to the surface expression measurements.
Using this method we surveyed multiple aspects related to protein expression, including differences in surface expression of polymorphic proteins and differences in intracellular vs. surface expression. Furthermore, we confirmed the functionality of tagged proteins from distinct families, including ion channels, rhodopsins, and membrane receptors.
The XenoGlo assay is a simple, reproducible, versatile, and non-destructive alternative to evaluate surface protein expression levels in single oocytes enabling prior or subsequent functional testing.

P19- OSMOTIC OR NON-OSMOTIC ACTIVATION OF THE UBIQUITOUS LRRC8 ANION CHANNEL MEDIATES EXTRACELLULAR ATP EXPORT

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Volume regulated anion channel (VRAC) is characterized for its contribution to the cell volume regulation following a decrease of the extracellular osmolarity. Beside its role in cell volume homeostasis, it also contributes to the membrane potential regulation, cell proliferation, migration and numerous other processes. The molecular mechanisms underlying these VRAC-mediated processes are not fully understood but in 2014 two independent studies showed that LRRC8A protein is the mandatory subunit that forms heterohexameric VRAC channels in combination with at least one of the four other family homologs (LRRC8B-E). The different LRRC8 subunits combinations have been shown to changes the VRAC channel permeabilities allowing the transport of a variety of substrates (Cl-, I-, Br-, taurine, lactate, glutamate and glutathione). In this work, we explored Cl- and ATP permeabilities of the LRRC8/VRAC channel during a hypotonic challenge or after pharmacological stimulation by zinc pyrithione (ZPT, a VRAC channel activator).
In HEK293WT cells, using patch-clamp technique, we measured a significant activation of Cl- currents following hypo-osmotic challenge (270mOsm, Imax at 3

min) or ZTP exposure (30µM, Imax at 5min). The amplitude of the ZPT-evoked currents represented only 40% of that measured with the hypotonic challenge. The ZPT-induced Cl- currents were inhibited by DCPIB, the selective inhibitor of LRRC8 and were not recorded in HEK293 LRRC8A knock-down (KD) cells. Next, we studied VRAC-mediated ATP release by measuring extracellular ATP concentration (bioassay) following hypo-osmotic challenge or ZPT exposure. We showed that activation of VRAC channels increases extracellular ATP concentration by 20 times following hypotonic challenge (2µmol/g prot), and by 2 times following ZPT exposure (0,2µmol/g) compared to basal condition (0,1µmol/g). This increase was inhibited by DCPIB and was not observed in LRRC8A-KD cells. Next, we explored the relative permeability of LRRC8 to ATP versus Cl- in whole-cell configuration. We recorded DCPIB-sensitive ATP currents in HEK293WT cells stimulated by a hypo-osmotic challenge or by ZPT exposure (30µM). These ATP currents were not recorded in LRRC8A-KD cells. In conclusion, these results suggest that LRRC8 mediate ATP release, but further experiments are needed to confirm a direct ATP permeation through the channel pore.

P20- MONO AND BIALLELIC VARIANTS IN HCN2 CAUSE A NEURODEVELOPMENTAL DISORDER

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Channelopathies are frequently involved in neurodevelopmental disorder often associated with epilepsy, likely due to an impairment of membrane excitability regulation. The HCN2 channel subtype, a member of the Hyperpolarization-activated cyclic nucleotide-gated channels family, could be involved. HCN2 has a central role in the electrical properties of neurons, including pace-making activity and excitability regulation, thus malfunction of HCN2 caused by mutations can be responsible of neuronal dysfunction. Here, we report four different missense HCN2 variants individuals who exhibited developmental delay associated with an epileptic encephalopathy or a syndromic intellectual disability without epilepsy. Three variants were monoallelic, two occurring de novo (p.Ala363Val, and p.Met374Leu) and one being inherited from a paucisymptomatic father (p.Arg324His). The last variant was biallelic (p.Leu377His). Interestingly, all these mutations affected highly conserved amino acids in HCN1-4 sequences. These mutations were introduced into the wild-type HCN2 sequence for expression in *Xenopus laevis* oocytes and subsequent electrophysiological characterization. The biallelic HCN2 variant, namely p.(Leu377His) produced silent channels in response to hyperpolarized pulses. Both p.(Ala363Val) and p.(Met374Leu) variants in homomeric or heteromeric channels namely (wt/p.(Ala363Val) and wt/p.(Met374Leu) respectively, resulted in lower current densities, slower currents and a stronger negative shift of activation compared to those of wild-type-HCN2 channel. The inherited dominant heterozygous p.(Arg324His) variant displayed only a negative shift of the voltage-dependent activation. Finally, 3D models of HCN2 carrying the p.(Ala363Val), p.(Met374Leu) and p.(Leu377His) mutations showed that they concern crucial amino acid positions involved in HCN2 gating. Our data indicate that the biallelic variant p.(Leu377His) induced a loss-of-function of HCN2 channel likely due to altered trafficking, that should be further confirmed by other experimental approach, while p.(Arg324His), p.(Ala363Val), as well as p.(Met374Leu) induce a dominant negative effect on HCN2

channel. In conclusion, we described mono and biallelic variants of HCN2 inducing loss-of-function as a novel cause of neurodevelopment disorder with or without epilepsy.

P21- ROLE OF CALCIUM ENTRIES IN THE PHYSIOPATHOLOGY OF GLIOBLASTOMA STEM CELLS

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Deregulations of the calcium toolkit have been involved in cancer physiopathology. Transcriptome analysis in glioblastoma stem cells (GSC), the cells that are responsible for tumor initiation, growth and recurrence, showed that GSC are enriched in calcium signaling genes. As variations in calcium concentration can regulate various cellular processes, we analyzed the expression of calcium channels in human GSC and determined their involvement in the regulation of GSC activity. We focused SOC (store operated calcium channels) and ROC (receptor operated calcium channels) that are a widespread type of calcium entry in non-excitable cells.

Our data show that SOC and ROC are functional in GSC derived from patients with glioblastoma. Pharmacological inhibition of SOC decreases GSC proliferation and self-renewal, and reduces expression of the stem cell marker SOX2. GSC migration has been examined using tridimensional environments to mimic the different substrates that GSC will find in the brain. Biocompatible polyacrylonitrile-derived nanofibrous scaffolds with two nanofibers organizations (preferentially aligned and planar isotropic orientation) and two stiffnesses (3 kPa and 166 kPa) were developed in « Institut Européen des Membranes » and were used to analyze the impact of stiffness and organization on calcium influx as well as on migration of GSC. This study on the role of calcium in GSC has been completed with the analysis of ROCE, especially TRPC6 and TRPC3, in GSC.

Our study indicates that calcium signaling plays a crucial role in GSC physiopathology and could be an interesting therapeutic target to regulate GSC.

P22- MECHANISMS OF PH PERMEABILITY AND SELECTIVITY OF TWIK1

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The interest for the family of two-pore-domain potassium channels (K2P) has grown dramatically in recent years. Because of the essential role of this 15-member family in the membrane potential homeostasis, the literature suggests that targeting and altering their activity will contribute to treating conditions as diverse and severe as neuropathic pain, affecting 7-8% of the population, depression, memory disorders, and cardiovascular disease, the leading cause of death globally, and disorders of the immune system and epilepsy. The recent excitement of the pharmaceutical industry

for the development of K2P modulators reflects the scientific community's recognition of the importance of these channels in neuronal, cardiac, and immune physiology.
While all members are selective for potassium, TWIK1 stands out in this family because its selectivity depends on the extracellular pH. Selective for K+ at physiological pH, it becomes permeable to Na+ when exposed to slightly acidic pH (pH ~ 6.0 to 6.5). This change is reversible.
It was shown by our lab that this variability of the selectivity is partly related to the presence of a pH-sensor residue, His122. However, a unique pH-sensor does not suffice to explain the selectivity changes of TWIK1, because they occur in two different phases at different pH values. From pH7.4 to pH6.5, the activity of TWIK1 increases upon titration of H122. The loss of selectivity per se occurs at lower pH values, when H122 is already protonated, which clearly indicates that other "acid-sensing" residues are involved.
I will present how, using molecular dynamics simulations (MD), candidate residues were identified, which were assumed, according to the calculations, to be involved in the pH induced selectivity changes. Mutagenesis and electrophysiological experiments confirmed the predictions, opening new hypotheses to explore. Detailed analyses of the MD trajectories combined with elementary machine learning approaches offer a glimpse in the possible mechanism behind the pH-induced permeability changes.

P23- LEBECETIN, A VIPER VENOM PROTEIN, ENHANCES MYELINATION IN THE DEMYELINATING EXPERIMENTAL MODEL OF MULTIPLE SCLEROSIS

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With approximately 2.8 million people afflicted worldwide and no definitive cure in sight, multiple sclerosis (MS) poses a great socio-economic burden around the globe. This autoimmune neurodegenerative disease, characterized by neuroinflammation and axonal demyelination, progressively renders the patient not able to function properly in society and to cope with everyday life. Available treatments are lacking efficiency and fraught with side effect. In this vein, we assessed the effects of lebecetin, a C-type lectin protein isolated from the venom of *Macrovipera lebetina* (M. lebetina) viper, on experimental models of multiple sclerosis. Our results showed that Lebecetin (LCT) showed neurotrophic and anti-inflammatory effects in neuron-like cellular model. Indeed, it promoted neurite outgrowth in cuprizone-treated N2a cell line and modulated the production of pro-inflammatory cytokines TNFα and IL-6 and chemokine CXCL10 as well as anti-inflammatory cytokine IL-10 in LPS-stimulated C8D1A astrocytic cell line. LCT was also shown to increase the expression of the basic protein of myelination (MBP) in stressed 158N oligodendrocyte cell line. Anti-inflammatory of LCT is confirmed by in vivo studies, on the cuprizone model, which mimics the demyelinating aspect of the disease. Indeed, treating cuprizone-intoxicated mice with LCT addressed motor dysfunction, as proven by key behavioral studies. Furthermore, LCT was shown to promote myelination through tracking the increased expression MBP in the corpus callosum of treated mice. Based on these

findings, LCT could be a tool to design a potential alternative or complementary therapeutic agent in the treatment of MS.

P24- IDENTIFICATION OF SIGNALING PATHWAYS OF OCTOPAMINE RECEPTORS IN APIS MELLIFERA AND VARROA DESTRUCTOR

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Octopamine receptors (OctoR) are G protein-coupled receptors specific to invertebrate, which are expressed in many neurons in *Apis mellifera*. They control or regulate neuromuscular transmission, learning, memory or responses to odorant by modulating responses of odorant receptors, interneurons or motoneurons. In honeybee, 5 types of OctoR have been identified, with functional homology to a- or b-adrenoreceptors, able to activate different intracellular signaling pathways (Gs, Gi/o, Gq), although no precise characterization has been done yet. Amitraz, a synthetic OctoR agonist is used extensively in the hive worldwide to fight against the Varroa destructor, a parasite mite partly responsible for the increasing number of colonies collapse disorders recorded last years. The specificity of amitraz for the honeybee or varroa OctoR is however is not really known as well as the precise pharmacology and signaling profiles of the mite OctoRs.
We have cloned the 5 honeybee and 2 varroa OctoR and expressed them in oocytes to (1) analyze their pharmacological profile (octopamine, tyramine, dopamine and amitraz) and (2) identified the signaling pathway they activate.
Our data show that these OctoR can activate multiple G-protein dependent pathways (Gi/o, Gs, Gq) but can also activate directly (G-protein independent) a cation channel that is tentatively identified as TRPC in *Xenopus* oocyte. The presence of these different types of responses is dependent upon the type of receptors expressed and the agonist used.
We now verify the existence of these OctoR-specific signaling pathways in bee neurons from the antennal lobes and mushroom bodies.

P25- NEW INSIGHTS IN THE UNDERSTANDING OF TREK-1 CHANNEL ACTIVATION BY FATTY ACIDS

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Among the two-pore domain K+ channels (K2P), TREK-1 channel is one of the most studied K2P channel members due to its involvement in neuroprotection and pain perception but suffers from a poor specific pharmacological profile. TREK-1 channel is polymodulated by voltage, pH changes, membrane stretch and polyunsaturated

fatty acids (PUFAs) and generates an outwardly rectifying current. The PUFAs-mediated activation is supposedly correlated to the length of the carbon chain suggesting a link with FAs membrane insertion and a stretch effect through an increase of membrane tension.
Using the patch-clamp technique in ruptured whole-cell configuration, we compared the effects of FAs, from stearic acid (C18:0, 18 carbons; 0 double bound) to docosahexaenoic acid (C22:6, 22 carbons; 6 double bounds) on ITREK-1 in a HEK293 cell line overexpressing TREK-1 channel. C18:0 and C18:3 failed to activate ITREK-1. Surprisingly, the shortest PUFA, linoleic acid (LA C18:2) produced the largest activated ITREK-1, while Alpha-linolenic acid (ALA C18:3) failed to activate TREK-1. At 0 mV, ITREK-1 was increased by 23.8±3.9 folds for LA (C18:2); DHA(C22:6): 29.7±4.1; AA(C20:4): 15.7±2.1. The activation kinetic by FAs was fast (in the range of 1 min) and the current reversibility was immediate when FA solution was washed. The FAS-related kinetics were comparable with the kinetics of ML402, a direct and specific activator of TREK-1. Thus, we supposed that FAs interact directly with TREK-1 rather than FAs membrane insertion changing membrane tension. In inside-out configuration, TREK-1 is also activated by FAs (and ML402) excluding an activation through a transmembrane partner. At last, whatever the application side of FAs, they activate the channel while the supposed FAs-induced curvatures of the membrane are opposite. Taken together, all these data suggest that the activation of TREK-1 by PUFAs is not a consequence of change in membrane tension but more complex than previously assumed. As ML402, the suggested activation site of TREK-1/FAs interaction should be accessible from the inside and the outside of the cell.

P26- CRYO-ELECTRON MICROSCOPY UNVEILS UNIQUE STRUCTURAL FEATURES OF THE HUMAN KIR2.1 CHANNEL

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Kir2.1 channels are strongly inward rectifying K+ channels that play a key role in maintaining resting membrane potential. Their gating is modulated by phosphatidylinositol-4,5-bisphosphate (PIP2). Genetically-inherited defects in Kir2.1 channels are responsible for several rare human diseases, including Andersen's syndrome. We present here the first structure of the human Kir2.1 channel containing both transmembrane (TMD) and cytoplasmic (CTD) domains solved at 3.7-4.3 Å resolution. The structural analysis (cryo-EM), surface plasmon resonance, and electrophysiological experiments revealed a well-connected network of interactions between the PIP2-binding site and the G-loop through residues R312 and H221. Normal mode analysis showed the intrinsic tendency of the CTD to tether to the TMD leading to a compaction of the Kir2.1 channel structure even without PIP2. Moreover,

normal mode analysis also described a swing motion that results in a lateral opening of the area located at the interface between CTD and TMD in adjacent chains which leads to an increase in the accessibility of the PIP2-binding site. Remarkably, these structural conformations predicted by the normal mode analysis were clearly identified in the experimental cryo-EM data set. Thus, Kir2.1 can present large amplitude motions that can lead to more favorable conformations for PIP2 binding. It is the first time that these motions are identified in the Kir channels. Finally, molecular dynamics simulations showed a movement of the secondary anionic binding site to the membrane. Our results revealed structural features unique to human Kir2.1 and provided insights into the connection between G-loop and gating and the pathological mechanisms associated with this channel.

P27- DECREASED PAIN RESPONSE IN A VALPROATE-INDUCED MOUSE MODEL OF AUTISM; TRPV1 CHANNEL AS A POTENTIAL CULPRIT?

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Autism Spectrum Disorders (ASD) are neurodevelopmental disorders in which abnormalities in sensory information processing have been described. Among sensory functions, nociception and its potential result, pain, are of a critical importance due to their crucial role for survival and the high prevalence of potentially painful comorbidities in ASD. However, there is no consensus among studies in humans and hypo-, hyper- as well as normal sensitivity to pain have been described. We decided to use an animal model of ASD to try to answer this question, allowing us to do standardize measures of pain sensitivity as well as study of the underlying cellular and molecular mechanisms.
Using an environmental model of ASD taking advantage of teratogenic drug sodium valproate (VPA) in CD1 mice, we explored behavioral response to nociceptive and painful situations and tried to elucidate underlying molecular and cellular mechanisms using electrophysiology and calcium imaging.
Studying pain related behaviors, our model showed a reduced sensitivity to capsaicin, a direct agonist of transient receptor potential vanilloide 1 (TRPV1) channels, only present in males and at a post-puberty age. Using calcium imaging we show that dorsal root ganglia neurons response to capsaicin is reduced and could potentially explained the behavioral phenotype.
These data altogether point toward a potential modification of TRPV1 channels expression or function.

P28- MECHANOSENSITIVE CHANNEL MRNA EXPRESSION IN CARDIAC DISEASES: NOVEL TARGETS AND FOCUS ON TREK-1 AS A SWITCH IN FIBROBLASTS PHENOCONVERSION

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Cardiac cell mechanical environment changes on a beat-by-beat basis and in the course of various cardiac diseases. Cells sense and adapt to such mechanical cues because of specialized mechanosensors mediating adaptive signaling cascades. Here, we report TREK-1 mRNA expression and activity in human atrial fibroblasts and reveal a cross talk between TREK-1 and fibroblast phenoconversion. With the aim to reveal additional candidates underlying mechanotransduction relevant to cardiac diseases, we investigated mechanosensitive ion channels (MSC) mRNA expression in diseased and non-diseased human hearts.
Our results showed higher TREK-1 expression and activity in fibroblasts compared to myofibroblasts and we found that TREK-1 down-regulation leads to a more myofibroblastic phenotype suggesting a role for this mechanosensor in phenoconversion. In addition, TREK-1 is preferentially expressed in the left atrium compared to the right one and its expression is not significantly changed when fibroblasts from patients in sinus rhythm vs. sustained atrial fibrillation are compared. At the whole-heart level, numerous MSC were differentially expressed between atrial and ventricular or between non-diseased and diseased tissue samples, revealing novel candidates to investigate.
Thus, we identify atrial fibroblast-specific TREK-1 expression and activity and highlight a role of TREK-1 in atrial fibroblast phenoconversion. We also provide a comprehensive overview of cardiac MSC mRNA expression in atrial and ventricular tissue from diseased and non-diseased patients, identifying potential novel candidates underlying mechanotransduction in cardiac diseases.

P29- REGULATORY PARTNERS INVOLVEMENT IN SODIUM CHANNEL TRAFFICKING IN CARDIOMYOCYTES

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Kir2.x potassium channels and Nav1.5 sodium channel are key players in cardiomyocytes excitability. In inherited arrhythmias, mutations in genes encoding ion channels affect either their biophysical properties or their functional expression. Moreover, in acquired arrhythmias, ion channel dysfunctions are often the consequence of structural remodeling of cardiomyocytes. These observations point out the major role of both cardiomyocytes architecture's integrity and dynamic regulation of ion channels expression, from intracellular trafficking to membrane anchoring.

This work asks if ion channel partners may participate in cardiomyocytes organization by their ability to regulate current properties and by their predisposition to localize into specific membrane domains.

To complete this study, real-time trafficking and targeting in cardiomyocytes microdomains of Nav1.5 and its partners would be investigated with Retention Using Selective Hooks (RUSH) system. RUSH is a two-state assay based on the reversible interaction of a hook protein fused to core streptavidin, with a fluorescent reporter protein of interest fused to streptavidin-binding peptide. The addition of biotin causes a synchronous release of the reporter from the hook.
After several sub-cloning for RUSH tools preparation, our preliminary data showed the RUSH system permits retention and release of Nav1.5 and two transmembrane partners, Kir2.1 and Connexin-43 successfully, in Human Embryonic Kidney (HEK) cells. HEK cells have been transfected with two RUSH plasmids coding for Nav1.5 and Kir2.1 or Connexin-43, tagged with eGFP and mCherry respectively. This

method enables the trapping of these proteins into the Endoplasmic Reticulum with Streptavidin, the subsequent synchronous release after addition of biotin and their co-trafficking followed by fluorescence. We show that without the addition of biotin, the proteins are localized throughout the ER, whereas the addition of biotin at the time of transfection redistributes them to the cell periphery, of which a fraction is localized at the membrane, at 48 hours post-transfection.

We will use our RUSH constructs into adult polarized cardiomyocytes of Rat to test the hypothesis of a sorting hub at early stages of trafficking where interactions between NaV1.5 channels and its regulatory partners orientate the final targeting of channels into membrane microdomains.

P30- C-JUN N-TERMINAL KINASE (JNK) POST-TRANSLATIONAL REGULATION OF PAIN-RELATED ASIC1B AND ASIC3 CHANNELS

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Neuronal proton-gated Acid-Sensing Ion Channels (ASICs) participate in the detection of tissue acidosis, a hallmark of several painful diseases. Such conditions often involve in parallel the activation of various signaling pathways such as the Mitogen Activated Protein Kinases (MAPKs) that ultimately leads to phenotype modifications of sensory neurons. Here, we identify one member of the MAPKs, c-Jun N-terminal Kinase (JNK), as a new post-translational positive regulator of ASIC channels in rodent sensory neurons. Recombinant H+-induced ASIC currents in HEK293 cells are potently inhibited within minutes by the JNK inhibitor SP600125 in a subunit dependent manner, targeting both rodent and human ASIC1b and ASIC3 subunits (except mouse ASIC3). The regulation by JNK of recombinant ASIC1b- and ASIC3-containing channels (homomers and heteromers) is lost upon mutation of a putative phosphorylation site within the intracellular N- and the C-terminal domain of the ASIC1b and ASIC3 subunit, respectively. Moreover, short-term JNK activation regulates the activity of native ASIC1b- and ASIC3-containing channels in rodent sensory neurons and is involved in the rapid potentiation of ASIC activity by the proinflammatory cytokine TNF-alpha. Local JNK activation in vivo in mice induces a short-term potentiation of the acid-induced cutaneous pain in inflammatory conditions that is partially blocked by the ASIC1-specific inhibitor mambalgin-1. Collectively, our data identify pain-related channels as novel physiological JNK substrates in nociceptive neurons, and propose JNK-dependent phosphorylation as a fast post-translational mechanism of regulation of sensory neuron-expressed ASIC1b- and ASIC3-containing channels that may contribute to peripheral sensitization and pain hypersensitivity.

P31- ROLE OF CA2+ ACTIVATED K+ CHANNELS (SKCA) IN OVARIAN CANCER CHEMORESISTANCE

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In epithelial ovarian cancer, 75% of patients are diagnosed at an advanced stage. Carboplatin and Taxol® constitute the first-line treatment. The survival rate of patients is 20-30%, and chemoresistance is observed in 70% of cases.
Our laboratory has documented the role of Ca2+-activated K+ channels (SKCa), as a key player for cancer cell migration (in breast, prostate and colon cancer cells) and the development of metastases in vivo (Chantôme et al., 2013; Guéguinou et al., 2016). While those channels are not well described in ovarian cancer, the aim of our study is to characterize their roles in the tumor behaviors (survival, migration, adhesion) and their involvement in chemoresistance.
We choose a high grade ovarian epithelial cancer cell line, COV504, and we generated a Taxol®-resistant high-grade serous ovarian cancer cell line (COV504 TX) by repeated application of Taxol® (fixed dose of 5nM). The cell survival and cell proliferation (for 7 days, MTT), cell adhesion (on fibronectin, 2h) and cell migration (inserts, 24 hours) assays are all performed on sensitive and resistant cell lines. Plasma membrane Ca2+ fluxes are evaluated by spectrofluorescence (ratiometric probe Fura2-AM) and ion channels are invalidated by siRNA (20 nM, 72h) and expression is analyzed by qRT-PCR.
The Taxol® resistance phenotype, in COV504 TX subline, is associated with a decrease of cell adhesion and a significant increase of cell migration in vitro compared to COV504 sensitive cell line. SK1, SK2 and SK3 mRNA and protein levels are significantly increased in COV504 TX subline. Invalidation of SK2 and SK3 by siRNA in COV504 TX restores cell adhesion, decreases cell migration without affecting cell proliferation. SK1 does not seems to be involved in cell adhesion, migration and proliferation of both COV504 and COV504 TX cell lines. Pharmacological inhibition of SKCa by apamin and invalidation of SK2 reduce chemoresistance of COV504 TX cell line and increase the constitutive calcium entry.
Thus SK2 channel expression in COV504 ovarian cancer cells seems contribute to the emergence of a pro-migratory phenotype and to the chemoresistance to Taxol®.

P32- ASSESSMENT OF CARDIAC TOXICITY OF MANGANESE CHLORIDE FOR CARDIOVASCULAR MAGNETIC RESONANCE

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MRI is widely used in cardiology to visualize the structure and function of the heart. Currently gadolinium is the contrast agents use to improve sensitivity and specificity of diagnostic images, enabling the assessment of scar and fibrosis. Manganese, a calcium analogue, allows detection of viable cells within altered tissue. However, the cardiac safety of manganese is not fully assessed. In this study, we investigated the effect of MnCl2 on cardiac function and electrical parameters. Hemodynamic function was determined ex vivo using the isolated working rat heart preparation. HL-1 cell line was used to investigate cell viability (loading with calcein AM) and calcium cycling (loading with CAL-520 AM). Rat ventricular cardiomyocytes were dissociated by enzymatic digestion. Action potentials and calcium currents were recorded using the patch clamp technique. The effect of ascending concentrations of MnCl2. (1, 10, 25, 50 and 100 µM) were evaluated. MRI experiments (1.5T and 9.4T) were performed on formalin-fixed rat hearts, previously perfused with 25 or 100 µM of MnCl2. Left ventricular hemodynamic parameters from isolated working hearts show

that heart rate is not modified by µM [MnCl2]. Contractility and relaxation index were not altered up to 50 µM MnCl2. At the cellular level, µM [MnCl2] did not impact HL-1 cell viability. A reduction of calcium transient was highly marked at 50 and 100µM MnCl2 (12.2±0.9% and 17.1±1.0%, respectively). In isolated ventricular myocytes, action potential duration at 20%, 50% and 90% of repolarization were not modified up to 10 µM of MnCl2. A small but significant decrease of action potential duration was observed at 25, 50 and 100 µM (APD90 decrease by 23.35±6.321% vs control). Peak L-type calcium current amplitude was significantly decreased by 50 and 100 µM of MnCl2. MRI on heart perfused with 25 µM of MnCl2 (no impact hemodynamic function) and 100 µM of MnCl2 showed a dose dependent decrease in the T1 relaxation time, a parameter for altered and disease state. In conclusion, our results show that µM [MnCl2] (up to 25 µM) can be used as a contrast agent in MRI, without effects on cardiac hemodynamic parameters.

P33- CONTROL OF THE FIGHT-OR-FLIGHT RESPONSE OF HEART RATE BY L-TYPE CAV1.3 CHANNELS

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The mechanisms underlying the increase in the frequency of heartbeat induced by catecholamines, so called fight-or-flight response (FFR) of heart rate, are incompletely understood and controversial. Indeed, while it is known that catecholaminergic activation positively modulates the activity of ion channels, pumps and exchangers of the primary heart pacemaker -the sinus node (SAN) - all attempts to block the FFR response of heart rate by targeting membrane ion channels or cardiac ryanodine receptors (RyR2) have so far failed. Mice in which the predominant hyperpolarization-activated “funny” (f)-channels isoform Hcn4 has been inactivated or modified do not show diminished SAN FFR. Second, impairment of RyR2-mediated Ca2+ intracellular release by ryanodine only reduces beta-adrenergic regulation of pacemaker activity. Consistently, mouse models with altered function of CaMKII, RyR2 or NCX have only partially reduced rather than abolished SAN FFR. Thus, we still do not really know which membrane ion channels constitute the key downstream effectors of the SAN FFR. We show that concurrent genetic or pharmacologic targeting of L-type Cav1.3 and f- channels eliminates the beta-adrenergic regulation of heart rate and SAN FFR response. Mutant mice carrying genetic ablation of Cav1.3 channels and expressing a dominant-negative human HCN4-channel subunit lacking cAMP dependent regulation in a time-controlled and heart specific way (Cav1.3-/-/HCN4-CNBD) showed no significant heart rate increase following administration of isoproterenol or during spontaneous physical activity. Mutant mice in which L-type Cav1.2 channels have been rendered insensitive to dihydropyridines (Cav1.2DHP-/-) showed that concomitant selective inhibition of Cav1.3 and funny channels prevented the positive chronotropic response to isoproterenol of beating hearts and intact SAN ex vivo, as well as of pacemaker activity in isolated SAN pacemaker cells. Finally, we could obtain loss of the beta-adrenergic regulation of

heart rate via selective concurrent pharmacologic inhibition of Cav1.3 conductance and of cAMP –dependent regulation of f-channels using di-cyclic nucleotides (di-cGMP). Our study identifies synergic interplay between Cav1.3 and f-channels as the key effector of beta-adrenergic regulation of SAN pacemaker activity and explains, for the first time, the mechanism underlying the FFR response of the heartbeat.

P34- VOLTAGE-GATED SODIUM CHANNEL NAV1.5 CONTROLS NHE1-DEPENDENT INVASIVE PROPERTIES IN COLORECTAL CANCER CELLS

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Colorectal cancer is a major cause of morbidity and mortality throughout the world and affects men and women equally. Increasing evidence has shown that voltage-gated sodium channels (Nav) are key contributors to the aggressive behavior of cancer cells. In this study, we investigated the expression of the SCN5A gene (encoding for Nav1.5) in normal, tumor and metastatic tissue samples and identified an increase in expression correlated with colon cancer progression. In addition, elevated levels of the SCN5A mRNA were associated with poor prognosis in malignancies of the gastrointestinal tract. In four colorectal cancer cell lines (HCT116, SW480, SW620 and HT29), we identified that SCN5A was the most abundantly expressed pore-forming isoform and TTX-resistant Nav1.5-mediated currents were recorded at the plasma membrane of cancer cells. Voltage-gated sodium currents recorded in cancer cells showed similarities in voltage dependence, activation and inactivation kinetics and recovery from inactivation. In addition, we identified TTX-resistant voltage-dependent sodium currents in primary cultures of colon cancer-derived cells. Silencing the expression of SCN5A, or inhibiting the activity of Nav1.5 by using tetrodotoxin, or new small-molecule inhibitors for Nav1.5 reduced colon cancer cells invasiveness, measured in 2-dimensions through collagen I or Matrigel. We also identified that Nav1.5 contributed to the invasive potential of colon cancer cells through the activation of the Na+-H+ exchanger type 1 (NHE1), which was also overexpressed in colorectal cancer patients biopsies compared to non-cancer tissues, and correlated efflux of protons. Our findings suggest that both Nav1.5 and NHE1 proteins could serve as molecular targets for anti-cancer therapy using drug repurposing strategies or new molecules based on rational design

P35- INFLAMMATION INDUCES MITOCHONDRIAL ION CHANNELS OVEREXPRESSION ON LIVER CELL LINE

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Some pathological conditions concerning liver like hepatic steatosis, hepatic cancer and cancer cachexia, can affect hepatocyte metabolism inducing hypermetabolism in vivo. These conditions have shown to affect mitochondrial bioenergetics and ATP production by hepatocytes, showing an increased energy wasting by mitochondria; the mechanism linked in this mitochondrial metabolism effect is only partially explained. Ion channel expression in mitochondria, even in physiological condition, is not totally clear. Some ion channels, mainly calcium and potassium are known to be expressed in mitochondrial membranes. Mitochondrial ion channels are known to be able affect energetic mitochondrial metabolism and could partially explain the mitochondrial energy wasting observed in these pathological conditions. We studied the expression of some calcium and potassium mitochondrial ion channels in a hepatocyte cell line in physiological conditions. We then observed the expression of these ion channels in hepatocytes cultured with pro-inflammatory cytokine, observing an effect on the expression of these. More data are needed, but this could participate to mitochondrial energy wasting observed.
This study has been partially founded by Ligue Contre le Cancer 85.

P36- SODIUM - CALCIUM SIGNALLING NETWORK UNDERLYING METASTATIC POTENTIAL OF PROSTATE CANCER CELLS

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Genomic instability is a primary cause and fundamental feature of human cancer. Among the genes affected, those encoding ion channels are present. Previous study from our laboratory have described that ion channels control some of the "hallmarks of cancer" such as tumor angiogenesis, migration, and metastasis, thereby paving the way to a new chapter in oncology. Recently we have performed bioinformatic analysis of prostate tumors, revealing several mutations of NALCN a sodium channel which we previously described as actor of invasion during prostate cancer (PCa). During my thesis, I am investigating the role(s) of these mutations that are totally unknown and when do they appear during PCa progression.

P37- STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A HUMAN POTASSIUM CHANNEL, KIR2.1

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Inwardly rectifying potassium (Kir) channels selectively control the permeation of K+ ions in and out of the cell. The inward rectification allows them to be associated with the control of many vital physiological functions, including the heartbeat. These channels open and close in response to modulators like phosphatidylinositol 4,5-bisphosphate (PIP2) – a lipid required for function in eukaryotic channels. Dysfunctions in Kir channels often lead to diseases called channelopathies. In our case, we are interested in Andersen's syndrome, where mutations of the human Kir2.1 protein are directly involved.
My Ph.D. work aims to identify the differences between the wild-type and two mutant channels in order to find links between the structure and the function of human Kir2.1 using a combination of biochemical, structural, and functional approaches. In this study, we recombinantly expressed the full-length His-tagged human Kir2.1 channel in yeast and purified it in detergent. We characterized the interaction between Kir2.1 (WT and loss-of-function mutant) and the essential activator PIP2 and determined the kinetic parameters by surface plasmon resonance. The findings of our study will provide a structural and functional base to better understand the mechanisms involved in Kir channels and the effects of their mutations.

P38- IN VIVO SPATIOTEMPORAL CONTROL OF VOLTAGE-GATED ION CHANNELS BY ENGINEERED PHOTOACTIVATABLE PEPTIDIC TOXINS

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Photoactivatable drugs targeting ligand-gated ion channels opened up new opportunities for light-guided therapeutic interventions. Photoactivable toxins targeting ion channels have the potential to control excitable cell activities with low invasiveness and high spatiotemporal precision. We developed caged HwTxIV-Nvoc, a UV light-cleavable and photoactivatable peptide that targets voltage-gated sodium (NaV) channels. We first validated physico-chemical parameters of photolysis and using high-throughput patch-clamp system (SyncroPatch364, Nanion), we validated activity and photosensitivity of caged HwTxIV-Nvoc in vitro in HEK293 cells. We further pursued our investigations in ex vivo brain slices and in vivo on mice neuromuscular junctions and zebrafish models. We found that caged HwTxIV-Nvoc enables precise spatiotemporal control of neuronal NaV channel function under all conditions tested. We developed multiple photoactivatable toxins and therefore

demonstrated the broad applicability of the toxin-photoactivation technology as a tool for experiments but also as a relevant clinical approach in disease management.

P39- EVALUATION OF CROTALPHINE EFFECTS, A RATTLESNAKE POTENT ANALGESIC TOXIN, ON THE TETRODOTOXIN-SENSITIVE NA CURRENT OF ADULT MOUSE DORSAL ROOT GANGLION NEURONS

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Crotalphine is an analgesic peptide identified in the venom of the South American rattlesnake Crotalus durissus terrificus. Although its antinociceptive effects are well documented, its direct mechanisms of action are still under investigation. The aim of the present work was to study the action of the crotalid peptide on the sodium channel subtype NaV1.7, a genetically validated pain target. To this purpose, the effects of crotalphine were evaluated on the tetrodotoxin-sensitive Na current of dorsal root ganglion neurons of adult mice, using the whole-cell patch-clamp configuration, and on the cell viability, using the propidium iodide fluorescence and trypan blue assays. The results show that 18.70 µM of peptide were necessary to inhibit 50% of the Na current, the blocking effects occurring without any marked change in the current activation and inactivation kinetics and voltage-dependencies. In addition, a crotalphine-induced increase in leakage current amplitude of approximately 150% and a maximal decrease of 28% of cell viability were detected in the presence of 50 µM of peptide. Taken together, these results point out, for the first time, the effectiveness of crotalphine to act on the NaV1.7 channel subtype, directly and/or indirectly by affecting cell membrane integrity. This mechanism of action could contribute to the peptide analgesic properties.

P40- FROM IDENTIFICATION TO MULTI-SCALE PHARMACOLOGICAL EVALUATION OF VENOM PEPTIDES AGAINST PAIN

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Pain care is a global public health priority and current pain relievers, although effective in many cases, are too often associated with side effects. A promising approach to manage pain and avoid central side effects is to target the dorsal root ganglia (DRG) neurons, located in the periphery. These neurons are well-known to express various proteins involved in pain transmission, including the 1.7 subtype of voltage-gated sodium channels (NaV1.7), a major and genetically validated target for chronic pain treatment. Thanks to their high affinity and selectivity, venom peptides of animal origin have been identified as an original source of antinociceptive molecules.
The objective of my PhD is to identify and characterize, by multi-scale studies, a large number of novel antinociceptive venom peptides possessing high selectivity and potency for NaV1.7. For this purpose, an electrophysiological screening on cells overexpressing the human NaV1.7 allowed to identify novel hits from a unique and large venom collection of Smartox Biotechnology. The different studies, currently undertaken on some selected peptides, aim (1) to chemically synthesize these peptides, (2) to characterize their selectivity profile and safety issue, notably on cell lines overexpressing NaV subtypes (from 1.1 to 1.8) and on native systems such as the mouse cardiomyocytes and neuromuscular system, (3) to specify their efficiency and mechanism of action on mouse DRG neurons, (4) to evaluate in vivo their analgesic properties in vigilant mice, (5) to determine their pharmacophore and optimize their functional properties, and (6) to provide evidence for the clinical potential of the best candidates in correcting de novo identified NaV channelopathies.

P41- ASIC1A CHANNELS PARTICIPATE TO PAIN PROCESSING BY DORSAL SPINAL CORD NEURONS

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The dorsal horn of the spinal cord is a key point of the pain neuraxis, where sensory-nociceptive informations coming from the periphery, enter the central nervous system to be integrated, processed, and sent to the brain. Spinal inputs come from peripheral Aβ, Aδ and C fibers and, importantly, these inputs can be subject to different facilitation/sensitization processes, leading to pain hypersensitivity and allodynia. Among these processes, windup is a “short term” facilitation of C-fiber inputs following peripheral low-frequency repetitive stimulations, resulting in a progressive increase in the number of action potentials (APs) evoked by wide dynamic range (WDR) neurons. Windup is therefore an interesting way to study the processing of nociceptive information by spinal cord neurons. Moreover, although windup was initially described several decades ago, its exact mechanism is still not fully understood.

Here, we combine both in vivo and ex vivo electrophysiological recordings to demonstrate a role for ASIC1a channels in the windup process. Pharmacological inhibition of spinal ASIC1a channels by different venom peptides (PcTX1 and Mambalgin-1) lead to significant decreases of the windup process, and we characterize a native ASIC current in WDR neurons. The kinetics and pharmacological sensitivities of this native current suggest the functional presence in WDR neurons of ASIC1a channels, either in homomeric or heteromeric (i.e., ASIC1a/ASIC2a, ASIC1a/ASIC2b) forms. We next characterized the properties of

these different ASIC1a channel subtypes in a heterologous expression system, and we show that the native ASIC current matches the properties of ASIC1a homomeric channels.

This work indicates that ASIC1a homomeric channels in WDR neurons of the dorsal spinal cord positively participate to the pain facilitation process of windup.

P42- THE ROLE OF THIK2 POTASSIUM CHANNEL IN NOCICEPTION

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The widespread expression of K+ channels in the nervous system, their ability to control resting membrane potential and to modulate action potentials make them a valuable target for the search for neuronal modulators. Among the K+ channels, the members of the THIK subfamily, THIK1 and THIK2, are expressed in the central and peripheral nervous system but their neuronal functions have not yet been studied. THIK1 and THIK2 do not only exist as homomers but can also form functional heteromeric channels, which implies that they should be considered together when studying their functions. In addition to their cellular distribution, THIK channels have a unique subcellular localization as they are expressed in both the plasma membrane and the endoplasmic reticulum. Transcriptomic data showed that THIK1 and THIK2 transcripts are particularly present in non-peptidergic nociceptive C-fiber neurons of dorsal root ganglia. By using the RNAscope technique, we were able to demonstrate the presence of THIK1 and THIK2 together in sensory fibers composed of small neurons (<650µm^2) and expressing the purinergic receptor P2RX3. We are now investigating whether they are present as homomers or heteromers, whether they are expressed at the membrane or in intracellular compartments, and their role in transmission of sensory messages. A THIK2 KO mouse was generated in which the deleted gene was replaced by the LacZ gene encoding β-galactosidase. X-gal labeling in DRG neurons shows activation of the THIK2 promoter in nociceptive C-fibers and preliminary studies have shown differential sensitivity of KO mice to thermal stimulation. We are pursuing our investigations on the role of THIK2 in sensory perception and we are attempting to differentiate the functions attributed to homomeric and heteromeric THIK channels. This distinction is essential for the further development of specific pharmacology and targeted therapy.

P43- IS NAVβ3 A NEW ACTOR OF MECHANOTRANSDUCTION IN ENDOTHELIAL CELLS?

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The endothelial cell (EC) is the central actor of vascular tone and homeostasis and as such endothelial dysfunction promote cardiovascular diseases including atherosclerosis. This endothelial function is mainly controlled by shear stress generated by blood flow. This physical force is detected by many mechanosensors and mechanotransductors in EC, which trigger intracellular signalling pathways

influencing endothelial behaviour. Among the mechanoproteins, several ion channels have been identified.
Despite the fact that the voltage-gated Na+ (NaV) channels have been shown to be mechanosensitive in cardiac and intestinal cells, these ion channels also expressed in vascular cells, have not been extensively studied in EC. Here, the aim of this study is to explore their contribution in the endothelium mechanotransduction.
Human Umbilical Vein EC (HUVEC) and Human Aorta EC (TeloHAEC) have been exposed either to a physiological laminar shear stress (LSS) at 20 dynes/cm² or to a pathological oscillatory shear stress (OSS) at 20 dynes/cm² with 1 Hz frequency using the Ibidi pump system. Only LSS induced cell orientation in the direction of flow (quantified by local gradient orientation method) and activated the atheroprotective signalling pathway (KLF2/KLF4, eNOS). Interestingly, this physiological LSS lead to an important modification of the expression of the NaV channel subunit transcripts. Notably, SCN3B expression, encoding NaVβ3 protein, belonging to Ig-CAM superfamily, was increased with a 2.1-fold at 24 hours, 3.9 at 4 days and 12.6 at 7 days in HUVEC and 2.9 at 4 days in TeloHAEC, whereas OSS did not lead to a significant increase of SCN3B expression. Moreover, the atheroprotective KLF2 transcription factor might be involved in SCN3B regulation since trans-resveratrol-induced KLF2 expression and KLF2 plasmid transfection led to increase SCN3B expression in EC. Furthermore, extinction of SCN3B by siRNA seems to impaire EC alignment in response to LSS. Finally, immunoprecipitation of NaVβ3 and mass spectrometry analysis revealed that integrin α5 could be a novel protein partner of NaVβ3.
Taken together, our results suggest that the Ig-CAM NaVβ3 could be a novel actor of the endothelial mechanotransduction involved in cell alignment in response to atheroprotective flow through the KLF2 pathway activation.

P44- THE PROTECTIVE ROLE OF TRPM8 IN PROSTATE CANCER PROGRESSION

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Prostate cancer (PCa) is the second most lethal tumor among men and its mortality is mainly due to metastasis. Thus, it is critical to understand the mechanisms by which tumors grow and how metastases can diffuse throughout the body. Several Transient Receptor Potential (TRP) channels are deregulated in cancer cells and have been suggested as valuable markers in predicting cancer progression as well as potential targets for pharmaceutical therapy.
In this context, a protective role of TRPM8 in PCa progression consistent with its strong down-regulation during late metastatic stages has been suggested. By using a prostate orthotopic xenograft mouse model, we confirmed in vivo the protective role of TRPM8 in PCa showing that its overexpression in PC3 cells not only reduces tumor growth through cell cycle arrest in G0/G1 and reduced clone forming capacity

but also inhibits metastatic dissemination by impairing the motility of these cancer cells. In particular, investigating the molecular mechanism underlying this biological effect, we found that, as previously described for endothelial cells, TRPM8 inhibits PCa cell migration and adhesion independently from its channel function. More specifically, TRPM8 intracellularly traps the small GTPase Rap1A in its inactive form, thus avoiding its translocation to the plasma membrane and the subsequent activation of adhesion signaling pathways. Moreover, we identified and validated the residues involved in the interaction between TRPM8 and Rap1A which are residues E207 and Y240 in the sequence of TRPM8 and Y32 in that of Rap1A.
Overall, these data deepen our knowledge of the role of TRPM8 in PCa progression, providing new insight into its possible use as new therapeutic target in PCa treatment.

P45- CROSS-TALK BETWEEN THE CALCIUM CHANNEL TRPV4 AND REACTIVE OXYGEN SPECIES INTERLOCKS ADHESIVE AND DEGRADATIVE FUNCTIONS OF INVADOSOMES.

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Invadosomes support cell invasion by coupling both acto-adhesive and extracellular matrix degradative functions, which are apparently antagonistic. Beta1 integrin dynamics regulates this coupling but the actual sensing mechanism and effectors involved have not yet been elucidated. Usinggenetic and reverse genetic combined with biochemical and imaging techniques, we now show that the calcium channel TRPV4, colocalizes with Beta 1 integrins at the invadosome periphery, regulates its activation and the coupling of acto-adhesive and degradative functions. TRPV4-mediated regulation of podosome function depends on its ability to sense reactive oxygen species in invadosome's microenvironment, and involves activation of the ROS/calcium-sensitive kinase Ask1 and binding of the motor Myo1C. Furthermore, disease-associated TRPV4 gain-of-function mutations that modulate ECM degradation are also implicated in the ROS response, which provides new perspectives in our understanding of the pathophysiology of TRPV4 channelopathies.

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USEFUL INFORMATION

The attendees are expected on Sunday, between 4PM and 7PM. During this period, they would have the time to proceed to their check-in and meet each other before a welcome drink and the diner. The congress will begin on sunday evening with the plenary lecture and finish on Wednesday after lunch.

The conference will take place at Centre de vacances du Lazaret, a leisure center at Sète on the Mediterranean coast of France, close to the city of Montpellier.

A forum has been setup if you want to share a taxi or a car (personal or rental one).

Directions to "Le Lazaret"

Le Lazaret
La Corniche
223 Rue Pasteur Benoît
34200 Sète

Tel: +33 (0)4 67 53 22 47
Fax: +33 (0)4 67 53 36 13

Web: www.lazaret-sete.com

Mail: le-lazaret@capfrance.com

GPS coordinates:
43°23'40.01 N
003°40'26.60 E

Arriving

By Air:

Montpellier Méditerranée Airport is the closest airport. At you arrival you will find taxi. We strongly encourage you to share a taxi using the forum.

If you want to book a taxi in advance you can use

Taxi Valenti, +33 611 57 18 05, taxi.valenti@sfr.fr

By train:

In Sète, there is a SNCF railway station, which is covered by TGV. At your arrival you can find taxis and public transportation. For city bus you can take a bus of the n°23 line operating to the Centre Malraux and stop at the halt "Plan de la Corniche". Alternatively, you can take a bus of the n°9 line operating to Marseillan Plage and stop at the halt "Le Lazaret". You can check for timetables, directions and prices at the following web address: http://www.thau-agglo.fr

By Car :

Free parking are available at the center of "le Lazaret".

Le Lazaret;223 Rue Pasteur Benoît, 34200 Sète
Sète
Pointe Longue
Port de Sète
Quai d'Alger
Avenue Victor Hugo
Quai de Bosc
Gare de Sète
Avenue Gilbert Martelli
Route de Carnon
Rue de Bruxelles
Rue de Copenhague
Rue de Madrid
Avenue des Eaux Blanches
Michelot Herve
Rue Paul Bousquet
Rue Previt d'Auglier
Rue Jean Moulin
Rue de la Révolution
Rue de la Caraussane
Rue de Belfort
Eglise Saint Joseph
OPEN SPACE Galerie
Centre Régional d'Art Contemporain
Grande Rue Haute
Le Môle Saint Louis
Musée Paul Valéry
Musée De La Mer
Prom. Maréchal Leclerc
Rue Jean Vilar
Chemin de Saint-Clair
Crique de l'Anau (la Conque)
Chemin des Hirondelles
Rue Robespierre
Boulevard Chevalier de Clerville
Chemin du Glacis
Chemin des Pierres Blanches
Combe d'Aubagnac
Chemin du Grenet
Rue Roland Dorgelès
Rue du Football
Rue des Cormorans
Rue des Cigales
Avenue de la Source
Rue des Rotheles
Rue des Lotois
Auchan Sète
Espace Georges Brassens
Chemin de la Pipe
Rue d'Artois
Montée des Pierres Blanches
Rue d'Aquitaine
Boulevard Joliot Curie
Corniche de Neuburg
Plage du Lazaret
Casino de Sète
Plage de la Corniche
Boulevard Jean Mathieu Grangent
Chemin des Quilles
Cimetière de Py
Route des Quatre Chansons
Avenue du Pointu
Rue de Palavas
Plage des Quilles
Le Dôme
Canal des Quilles
Chemin Château de Vialongue
Plan
Satellite
Google
Données cartographiques ©2017 Google
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Conditions d'utilisation
Signaler une erreur cartographique

Gare SNCF
Noël Guignon
Passage Le Dauphin
Épi d'Or
Jean Moulin
Mathieu Grangent
Centre Malraux
Le Vallon
Notre-Dame Souveraine du Monde

MISCELLANEOUS

Lazaret Holiday Village
www.lazaretsete.com
Rue du Pasteur Lucien Benoît, 34200 Sète Téléphone : 04 67 53 22 47

Tourist office
www.tourisme-sete.com/
60, Grande Rue Mario Roustan, 34200 Sète, 04 99 04 71 71

Public bus
http://mobilite.thau-agglo.fr/eng, 04 67 53 01 01
The direct bus line between the Sète SNCF railway station and the Lazaret are the line 3 and 9

Espace Georges Brassens
http://www.espace-brassens.fr/
67 Boulevard Camille Blanc, 34200 Sète, France 04 99 04 76 26

Musée Paul Valéry
http://www.museepaulvalery-sete.fr/
Rue François Desnoyer, 34200 Sète, France 04 99 04 76 16

Others activities
https://www.tripadvisor.fr/Attractions-g660465-Activities-Sete_Herault_Occitanie.html#ATTRACTION_LIST