CANAUX IONIQUES

30th Ion Channel Meeting
Association "Canaux Ioniques"

VILLAGE VACANCES - SETE - LE LAZARET -

8-11 September 2019, Sète, France

PROGRAM & ABSTRACTS

www.canaux-ioniques.fr

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ORGANIZING COMMITTEE

SCIENTIFIC COMMITTEE CANAUX IONIQUES

Bruno CONSTANTIN (Chair)
Laboratoire STIM, CNRS ERL 7368, Université de Poitiers
bruno.constantin@univ-poitiers.fr

Sylvie DIOCHOT (Co-Chair)
IPMC, CNRS UMR 7275, Université de Nice-Sophia Antipolis
Sylvie.diochot@ipmc.cnrs.fr

Dimitra GKIKA
Laboratoire PhyCell, INSERM U1003, Université de Lille
dimitra.gkika@univ-lille.fr

Raphël RAPETTI-MAUSS
IBV, CNRS UMR 7277, INSERM U1091, Université de Nice Sophia Antipolis
rrapettima@unice.fr

Matteo MANGONI
IGF-CNRS, CNRS UMR 5203, INSERM U1191, Université de Montpellier
Matteo.mangoni@igf.cnrs.fr

Jean louis BOSSU
CNRS - INCI Université de Strasbourg
jlbossu@inci-cnrs.unistra.fr

Laurent FAGNI
CNRS, Université de Montpellier
laurent.fagni@orange.fr

Aubin PENNA
Laboratoire STIM, CNRS ERL 7368, Université de Poitiers
aubin.penna@univ-poitiers.fr

Lise RODAT-DESPOIX
Laboratoire de Physiologie Cellulaire et Moléculaire, Université de Picardie
lise.despoix@u-picardie.fr

Pietro MESIRCA
IGF-CNRS, CNRS UMR 5203, INSERM U1191, Université de Montpellier
pietro.mesirca@igf.cnrs.fr

Arnaud MONTEIL (President)
Institut de Génomique Fonctionnelle, CNRS UMR 5203 - INSERM U1191
Université de Montpellier,
arnaud.monteil@igf.cnrs.fr

Caroline STRUBE (vice President)
Centre de Recherche en Neurobiologie et Neurophysiologie de Marseille, CNRS UMR 7286, Université Aix Marseille,
caroline.strube@univ-amu.fr

Bruno CONSTANTIN (Treasurer)
Laboratoire STIM, CNRS ERL 7368, Université de Poitiers
bruno.constantin@univ-poitiers.fr

Sylvie DIOCHOT (Secretary)
IPMC, CNRS UMR 7275, Université de Nice-Sophia Antipolis
Sylvie.diochot@ipmc.cnrs.fr

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FOREWORD

The ionic channel is a very potent mechanism of exchange between intra and extracellular compartments, between the extracellular medium and some cell organelles, and also from a cell to another coupled cell. Transiently open it is a signaling mechanism. Open in basal conditions, it defines the resting potential of a cell. Obviously ionic channels are a mechanism common to all cell types. For instance they are involved in cell motility, reproduction, hormone secretion and brain activity. They are also present in plants were they regulate roots turgidity and nutrient exchange. Thus the diversity of the ion channels effects has stimulated an ever increasing interest .

Facing thirty years ago a wealth of incoming informations, researchers issued from all domains of physiology, animal and plant physiology as well, wished to discuss of their progress in their work on ionic channels. A modest INSERM funded club at its origin in 1989, the Ionic channels Association has gathered young and established researchers of the field in an unformal way. on basis of a single four days event in september at the beginning of the universitary year, The composition of the each year renewed organizing committee spontaneously followed the development of one or the other branch of the ionic channel related field.

This year again, our meeting will cover a broad spectrum of researches. It will bring together specialists of epithelia and specialists of the synapse who will introduce the most recent developments of their research including technical and pharmacological tools. Cation channels, mainly potassium channels will be discussed for their role not only in tumor development but also in the drug resistance tumor cells may develop. Prediction of the effect of a channel blocking drug is uneasy, particularly in the CNS where each single neuron generate a distinct response due the specific topology of an often similar population of channels, What is the expected effect of A-typical channels will be discussed in different neuronal cell types. This meeting will be again for us a source of new projects and new collaborations !

Anne Feltz - IBENS - Paris

AVANT-PROPOS

Importée d'Allemagne en 1983, la technique de patch clamp fut implantée à Paris puis à Strasbourg sous la bienveillance du Professeur Erwin Neher. Elle se dissémina rapidement dans de nombreux laboratoires de l'hexagone. La communauté française de chercheurs passionnés par cette technique nouvelle qui permet d'enregistrer les canaux ioniques unitaires se réunit à de nombreuses reprises pour discuter des problèmes variés qui concernaient le filtrage, l'analyse et l'interprétation de ses ouvertures et fermetures complexes. En 1989, le nombre de « patcheurs » avait grandi de façon exponentielle, ainsi que les types cellulaires étudiés. Anne Feltz et François Couraud entourés de plusieurs confrères eurent l'idée qu'il serait utile de permettre à cette communauté de « canalophiles » de se rencontrer lors d'un colloque qu'ils nommèrent Colloque des Canaux Ioniques. Il se déroula pour la première fois au bord de la méditerranée à Carry le Rouet. La philosophie de cette réunion partagée par tous, était qu'il fallait favoriser la participation de jeunes étudiants et que tout se passe dans la plus grande convivialité à l'écart de la compétition scientifique. Le choix du Français s'imposa pour permettre aux étudiants d'être plus à l'aise, et fut remplacé une dizaine d'années plus tard par l'Anglais ce qui permettait d'accueillir des collègues venus des quatre coins du monde. Ces trente années ont permis à plus de 3500 étudiants et chercheurs de tous horizons (du monde animal au monde végétal) de se rencontrer dans la bonne humeur dans des lieux pleins de charme comme Lalonde Les Maures, La presqu'ile de Giens, l'ile d'Oléron et Sète. Discussions scientifiques passionnées jusqu'au petit matin, côtoyèrent pas de danse endiablé, éclats de rire, le tout arrosé par de subtiles boissons. De nombreuses collaborations et amitiés naquirent de ces rencontres. Voilà, trente années ont passé et je pense que l'on fêtera encore longtemps, tous les dix ans, l'anniversaire du colloque des canaux ioniques et qu'il gardera cette réputation de convivialité qui fait son succès et sa réputation.

Jean louis BOSSU

CNRS - INCI Université de Strasbourg

PROGRAM

Sunday, September 8th 2019

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

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

21:00 **Plenary lecture**

Anne Feltz (Paris, France): 1989- 2019: ***30 years of ion channel history***

Monday, September 9th 2019

08:15 Opening session

08:30 Symposium 1: "Ionic channels and autism"

Organized by **Jean-Louis Bossu** *(Strasbourg, France)*

Diane Papazian (Los Angeles, USA): *Altered gating in mutant Kv4.2 channel associated with autism and epilepsy*

Igor Medina (Marseille, France): *Causal links between impaired developmental neuronal chloride homeostasis and formation of Autistic Spectrum Disorders*

Enrico Cherubini (Roma, Italy): *Impairment of GABAergic signalling in Autism Spectrum Disorders*

Selected speaker: Eric Hosy (Bordeaux, France): *Nanoscale co-organization and co-activation of AMPAR, NMDAR and mGluR at the synapse*

10:15 *Coffee break*

10:30 Oral communication session 1: « Ion Channel Pharmacology »

Organized by **Sylvie Diochot** (Nice-Sophia Antipolis, France)

Ines ELBini Dhouib (Tunis, Tunisia): *Potential neuroprotective effect of bee venom on aluminum chloride-induced Alzheimer's disease on rats*

Zhong Peng (Lausanne, Switzerland): Acid-sensing ion channels the hypothalamus are regulated by hydrogen sulfide

Léa Réthoré (Angers, France): *Brevenal prevents ciguatoxin and brevetoxin activation of Nav channels*

Osbaldo Lopez-Charcas (Tours, France): *Biophysical characterization of new small-molecule blockers of nNaV1.5 channels expressed in breast cancer cells*

11:30 1-minute oral presentation for posters

12:15 *Lunch*

14:00 Poster session 1 - Odd numbers

15:30 Poster session 2 - Even numbers

17:00 Symposium 2: "Ion channels and skin functions"

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

Hongzhen HU (St. Louis, USA): *Piezo/TRP channels in dermal pain and Itch*

V'yacheslav LEHEN'KYI (Lille, France): *TRP channels and Skin homeostasis*

Elena OANCEA (Providence, USA): *Ion channels in skin pigmentation and response to UV light*

Selected speaker: Adélaïde Doray (Tours, France): *The role of the auxiliary NaVβ4 subunit in maintaining epithelial phenotype*

18:30 Annual meeting of the association « Canaux ioniques »

19:30 *Apéritif Sétois*

20:30 *Dinner*

Tuesday, September 10th 2019

08:30 Symposium 3: Glutamate ligand-gated channels

Organized by **Laurent Fagni** *(Montpellier, France)*

Pierre Paoletti (Paris, France): *New types of NMDA receptors*

Christophe Mulle (Bordeaux, France): *Presynaptic plasticity in hippocampal circuits: role of kainate receptors*

Ludovic Tricoire (Paris, France): *The delta family of glutamate receptors: role in excitatory synaptic transmission*

Selected speaker: Perrine Inquimbert (Strasbourg, France): *Plasticity of inhibitory synaptic transmission under the control of NMDA receptors in the dorsal horn of the spinal cord*

10:15 *Coffee break*

***10:30* Symposium 4: Ion channels in cancer therapy resistance**

Organized by **Lise Rodat-Despoix** *(Amiens, France)*

Federica Wolf (Roma, Italy): *Magnesium-Specific Ion Channels in Chemoresistance: Is There a Role for Magnesium?*

Geert Bultynck (Leuven, Belgium): *Ca^{2+}-signaling modulation by antiapoptotic Bcl-2 proteins: from molecular mechanisms towards translational avenues*

Annarosa Arcangeli (Florence, Italy): *Targeting potassium channels to overcome chemoresistance in gastrointestinal cancers*
Selected speaker: Charlotte Dubois (Lille, France)**:** *Apoptosis mediated by Mitochondrial Ca^{2+} Overload Requires Inhibition of Autophagy Revealing a Widespread Vulnerability of Cancer Cells*

12:15 Lunch

14:00 Special social events for 30th anniversary of association « Canaux ioniques »: Boat excursion with Sète Croisières : One hour guided tour of

the fishing port, commercial port and marina, followed by a sea excursion along the rocky 'corniche' with underwater viewing. (http://www.sete-croisieres.com/)

19:00 Poster Prize session

19:30 ***Apéritif and special dinner***

22:00 Evening Party

Wednesday, September 12th 2019

09:30 ***Oral communication session 2: « Ion Channel Structural Aspects»***

Organized by **Dimitra Gkika** (Lille, France)

Olivier Bignucolo *(Lausanne, Switzerland:) Biomolecular modelling of acid-sensing ion channels*

Côme Camus *(Bordeaux, France): Phosphorylation of PSD95 as potential link between synaptic and structural plasticities*

Mathilde Folacci *(Grenoble, France): Design of light-modulated inward rectifying potassium channels*

Kerstin K. Viet *(Mainz, Germany): Structure and Ca^{2+} interaction of the Extracytosolic / Lumenal Domain (ELD) of the human TRPML2 ion channel*

10:30 ***Coffee break***

10:45 Symposium 5: A-typical' Ion channels/currents

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

Rajan Sah (Iowa City, USA): *SWELL1 is a glucose sensor regulating β-cell excitability and systemic glycaemia*

Arnaud Monteil (Montpellier, France): *The sodium leak channel: NALCN*
Anna Moroni (Milan, Italy)**:** *Blue-light–induced K+ channel 1 (BLINK1) channel: a promising tool in optogenetics*

Selected speaker: Mallory Ducrozet (Lyon, France): *Role of Translocon in calcium exchanges in cell compartments and in cell death, during myocardial infarct*

12:30 Meeting closure

13:15 ***Lunch***

14:00 Airport shuttle departure

SYMPOSIA AND ORAL COMMUNICATIONS ABSTRACTS

Sunday, September 8th 2019

21:00 **Plenary lecture**

1989- 2019 : 30 YEARS OF ION CHANNEL HISTORY

Anne Feltz IBENS, Paris, France

Around 1970 the mechanisms of communication between cells were universally accepted. Hodgkin & Huxley(1952) had provided the basis for understanding the nerve action potential. And chemical transmission at most synapses was experimentally established (Katz, 1966), In their seminal papers, Hodgkin and Huxley introduced the concept of gates to describe the observed conductance changes, and by a mathematical modelling of the proposed "K+ channel " they assumed that a certain number of charged particles had to move in a voltage-dependent manner, to allow K+ to pass. The voltage-dependent ionic channel was conceptually born. In 1972, Katz & Miledi and Anderson and Stevens described the acetylcholine current as the sum of unitary currents generated by ligand activated ion channels. Many of the properties of electrically and chemically excitable cells could be explained by the properties of transmembrane channels. Only the underlying molecular mechanisms were to be identified. With the development in parallel of electrophysiology, biochemistry and soon after of molecular biology, these membrane proteins were to be identified one by one.
From 1989 when these Canaux ioniques meetings started to now, not less than five Nobel prizes were awarded which each points to a breakthrough in our understanding of the ion channels functioning. The channel became a reality that could be described when Erwin Neher and Bert Sakmann developed the patch clamp technique and succeeded in measuring miniscule ~pA amounts of transmembrane current. Our understanding of Ca2+ movements in the cell arises mainly from the work of Roger Tsien who devised a number of fluorescent Ca2+-sensitive small molecules before taking advantage of the GFP (green fluorescent protein) properties. Optogenetics in a way is in line with his dream of seeing cells at work. But the ion translocation process itself remained a pending question until Rod MacKinnon produced X-ray crystallography pictures allowing to understand the chemical principles of ion selectivity in K+ channels. With the development of cryoEM (cryoelectromicroscopy ; J. Dubochet, J. Frank and R. Henderson) focus is now not so much on the ions but on the subtile structural changes occurring in a membrane protein when transiently going from rest to activated or inactivated states. The so numerous channel-connected molecules will start to take life. These successive major steps will be illustrated and ongoing developments in the field will be outlined. By the way, did you notice that I only referred to four Nobel prizes. We will discuss at the meeting the message brought forward by the fifth one, the 2015 prize attributed to Campbell and Omura.

Monday, September 9th 2019

08:30 Symposium 1: "Ionic channels and autism"

Organized by **Jean-Louis Bossu** *(Strasbourg, France)*

ALTERED GATING IN MUTANT KV4.2 CHANNEL ASSOCIATED WITH AUTISM AND EPILEPSY

Diane PAPAZIAN;
UCLA LOS ANGELES

Kv4 subunits form the pore in inactivating, somatodendritic K+ channels. Mutations in Kv4.2 are associated with seizures and behavioral phenotypes in human patients. The V404M mutation was identified in twin boys with infant-onset epilepsy and autism, whereas the nearby V402L mutation was identified in a boy with epilepsy onset at 4 years of age and behavioral dyscontrol, but without a diagnosis of autism. Both mutations have dominant effects on closed state inactivation (CSI), a mechanism in which inactivation is coupled to voltage sensor movement rather than pore opening. Due to CSI, neuronal Kv4 channels inactivate without opening in response to excitatory synaptic input. CSI modulates neuronal excitability and the back propagation of action potentials, thereby regulating Ca2+ influx into dendrites and changes in synaptic strength. V404M enhances the inactivation of channels that have not opened, but dramatically impairs inactivation after opening. V404M gives rise to these opposing effects by increasing the stability of the inactivated state and in parallel, profoundly slowing the closure of open channels, which is required for CSI. The larger volume of methionine compared to the original valine is a major factor underlying altered CSI gating. V402L also impairs the inactivation of Kv4.2 channels after opening, but the change in kinetics is not as large as in V404M. The effects of V404M and V402L on CSI are expected to disturb the regulation of neuronal excitability and the induction of spike timing-dependent plasticity. Our results strongly support the idea that altered CSI gating is a major contributor to the clinical phenotypes of human patients, with a more severe clinical presentation associated with more dramatic changes in inactivation.

CAUSAL LINKS BETWEEN IMPAIRED DEVELOPMENTAL NEURONAL CHLORIDE HOMEOSTASIS AND FORMATION OF AUTISTIC SPECTRUM DISORDERS

Igor MEDINA;

Aix- Marseille University UMR 1249, INMED, INSERM, Marseille, France

Emerging number of studies indicate that modified neuronal Cl- homeostasis is critically involved in etiology of autism spectrum disorders (ASD). Treatments directed to compensate Cl- changes alleviate symptoms both in patients and animal models of ASD, but yet the Cl- related mechanisms contributing to ASD are little understood. The malfunction of KCC2, a vital neuronal K+/Cl- co-transporter, was suggested as an important factor contributing to etiology of ASD. By studying the properties of heterozygous mice with phospho-mimetic mutations T906E and T1007E (KCC2E/+) to prevent the normal developmental dephosphorylation of KCC2, we have found that

the mice exhibited typical ASD-like phenotype including altered GABAergic inhibition, modified neuronal network excitability and impaired neurobehavior. In the talk I will present an overview of recent findings in the field as well as provide experimental data illustrating the causal link between KCC2 dysfunction and formation of ASD-like symptoms.

IMPAIRMENT OF GABAERGIC SIGNALING IN AUTISM SPECTRUM DISORDER

Enrico CHERUBINI;

European Brain Research Institute, Rome, Italy

Autism Spectrum Disorders (ASDs) comprise a heterogeneous group of neuro-developmental abnormalities with a strong genetic component, characterized by deficits in verbal and non-verbal communication, impaired social interactions and stereotyped behaviors. In a small percentage of cases, ASDs are associated with alterations of genes involved in synaptic function. Although rare, these point to synapses as possible sites of ASDs origin. One class of non-syndromic forms of ASDs has been found to be associated with mutations/deletions of genes encoding for neuroligins (NLGs). These are postsynaptic adhesion molecules that, interacting with their presynaptic partners neurexins, ensure the cross-talk between pre- and postsynaptic specializations and synaptic stabilization, necessary for maintaining a proper excitatory/inhibitory balance within local neuronal circuits.
Here, transgenic mice carrying the human R451C mutation of the Nlgn3 gene, found in some families with autistic children, were used to study GABAergic signaling in the hippocampus at early stages of postnatal development. We assumed that activity-dependent alterations in synaptic plasticity represent a convergent mechanism underlying neuro-developmental disorders including ASDs. In particular, we tested the hypothesis that a disruption of GABAergic signaling affects spike time dependent plasticity (STDP), a particular Hebbian type of learning crucial for information processing.
We found that, unlike littermate controls, in the hippocampus of NL3R451C knock-in pups, positive pairing consistently induces a loss of LTP at mossy fibers-CA3 synapses, known to exhibit, at early developmental stages, a GABAergic phenotype. A similar effect was observed in NL3 knock-out mice, indicating a loss of function. These results were associated with a dysfunction of BDNF/TrkB signaling and could be rescued by exogenous application of BDNF. These data clearly show that a dysfunction GABAergic signaling early in postnatal life leads to alterations in the functional refinement of developing circuits and synaptic plasticity processes possibly underlying cognitive deficits in autistic children. The beneficial effects induced by exogenous application of BDNF opens new therapeutic perspectives for the treatment of these devastating disorders.

NANOSCALE CO-ORGANIZATION AND CO-ACTIVATION OF AMPAR, NMDAR AND MGLUR AT THE SYNAPSE

Julia GONCALVES; Daniel CHOQUET; Eric HOSY;

IINS, CNRS Bordeaux

The nanoscale organization of neurotransmitter receptors relative to pre-synaptic release sites is a fundamental determinant of the amplitude and the reliability of synaptic transmission. Thanks to dual color super-resolution imaging techniques, the alignment between AMPARs and the pre-synaptic release site has been demonstrated, and in parallel, the nanoscale clustered organization of NMDARs has been shown. However, the co-organization between the two main ionotropic glutamate receptors is still undefined. Here we used dual color super-resolution to characterize the AMPAR-NMDAR co-organization with a precision of 10 nm. In parallel we demonstrated that mGluR5 presents a non-clustered distribution at synapses, and an even lower density at the PSD localization. Based on this precise organization, and combined with electrophysiology, we built a model which mimics the dynamic organization of the three glutamate receptor families, and succeeded in predicting their activation.

10:30 Oral communication session 1: « Ion Channel Pharmacology »

Organized by **Sylvie Diochot** (Nice-Sophia Antipolis, France)

POTENTIAL NEUROPROTECTIVE EFFECT OF BEE VENOM ON ALUMINUM CHLORIDE-INDUCED ALZHEIMER'S DISEASE ON RATS

Ines ELBINI DHOUIB1; Chedly ELLEJIMI1; Raoudha DOGHRI2; Asma GATI3; Najet SRAIRI ABID1;
1: Laboratory of Venoms and Therapeutic Biomolecules, LR11IPT08, Pasteur Institute of Tunis, University of Tunis El Manar, Tunis, Tunisia
2: Laboratory of Anatomo-Pathology, Salah AzaiezInstitute, Tunis, Tunisia
3: Laboratory of Genetics, Immunology and Human Pathology,Department of Biology, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive depositions of amyloid protein plaques among nerve cells, and neurofibrillary tangles on the neuron. The essential components of plaques are Aβ peptides which are cytotoxic and may play a role in the pathogenesis of AD. In addition, Aβ–membrane interaction event may be followed by the insertion of Aβ into the membrane in a structural configuration which forms an ion channel. Remarkably, the blockage of Aβ ion channels can prevent the activation of cytotoxicity and inflammation processes at different intracellular levels, thereby preserving the life of the nerve cells. Bee venom (BV), also known as apitoxin, is widely used in traditional oriental medicine to treat inflammation-related diseases. We investigated the neuroprotective effect of BV, against neurobehavioral and neuropathological alterations induced by chronic administration of aluminium chloride (Al). The results revealed that treatment with bee venom significantly prevented Al- rat from impairment in the performance of neurobehavioral tests in comparaison with control ones. In addition, bee venom protects rats against Al-induced brain toxicity such as increase in AChE activity, decrease on cells viability, apoptosis, oxidative stress, and inflammation. The protective effects of BV was further confirmed by histopathological study. Taken together, our results demonstrate, for the first time, that BV provided protective effects against neurotoxicity induced by aluminium chloride on male rats and could be beneficial for the treatment of Alzheimer’s disease.

ACID-SENSING ION CHANNELS OF THE HYPOTHALAMUS ARE REGULATED BY HYDROGEN SULPHIDE

Zhong PENG; Stephan KELLENBERGER;
Department of Pharmacology and Toxicology, University of Lausanne, Rue du Bugnon 27, 1011 Lausanne, Switzerland

Hydrogen sulfide (H2S), known as 'gas of rotten eggs', is a toxic and colorless gas. In mammals, the expression of the enzymes of H2S biosynthesis is predominantly found in the brain. Recently, H2S has emerged as a new gasotransmitter, and was shown to exert cellular effects by interacting with ion channels. The aim of this study was to investigate whether acid-sensing ion channels (ASICs) are regulated by H2S. In a mammalian cell line transfected with ASICs, we found that the H2S donor NaHS time- and concentration-dependently increased the acid-induced ASIC1a peak currents. The enhancing effect of NaHS on ASIC1a peak currents was detectable at a concentration of 100 µM, exposed during 40 seconds. This concentration can be commonly attained under several physiological and pathological conditions. Similarly to its effect on ASIC1a, NaHS also time-dependently increased the acid-induced peak currents of ASIC1b, ASIC2a and ASIC3, as well as the sustained ASIC3 current. Notably, the enhancing effect of NaHS on the peak ASIC1a currents was not due to a change of the acid sensitivity of ASIC1a. In cultured hypothalamus neurons, H2S donors enhanced the acid-induced endogenous ASIC peak currents in a time- and concentration-dependent manner. Our study indicates that H2S regulates time- and concentration-dependently recombinantly expressed ASICs as well as the endogenous ASICs of the hypothalamus.

BREVENAL PREVENTS CIGUATOXIN AND BREVETOXIN ACTIVATION OF NAV CHANNELS

Christian LEGROS1; Claire LEGENDRE1; Léa RETHORE 2; Lucille CRESPIN2; Mireille CHINAIN3; Andrea BOURDELAIS4; César MATTEI2;
1: Institut MITOVASC, UMR CNRS 6015 - INSERM U1083, SFR ICAT, 3 Rue Roger Amsler 49100 Angers, France - Université d'Angers - IUT Angers-Cholet, 4 boulevard Lavoisier 49000 Angers, France
2: Institut MITOVASC, UMR CNRS 6015 - INSERM U1083, SFR ICAT, 3 Rue Roger Amsler 49100 Angers, France
3: Institut Louis Malardé (ILM), UMR 241-EIO, Laboratoire de recherche sur les Microalgues Toxiques BP 30, 98713 Papeete, Tahiti, French Polynesia
4: University of North Carolina, Center of Marine Science, Wilmington, NC, 28409, USA

The voltage-gated sodium channel (Nav channel) is the target of numerous neurotoxins. Among them, phycotoxins like ciguatoxins (CTXs) or brevetoxins (PbTXs) exert deleterious effects on Nav channels, frequently inducing human food intoxication in Pacific, Caribbean and Indian areas termed ciguatera or neurotoxic shellfish poisoning respectively, for which there is no specific treatment.
Brevenal, a metabolite produced by the dinoflagellate Karenia brevis, has been shown to compete with PbTXs over Nav channels on an unknown molecular site. We hypothesized that brevenal may counteract the effects of PbTXs and CTXs on Nav channels.
We showed that brevenal did not modify the biophysical properties of the rat brain Nav1.2 channel expressed in Xenopus oocytes, using the two-electrode voltage clamp technique. Moreover, brevenal antagonized the effet of PbTx-2 on Na+ currents mediated by Nav1.2 channel.
To further characterize the pharmacological properties of brevenal, we measured its effects on the calcium response induced by PbTx-2 and P-CTX-1B in the rat pituitary GH3b6 cell line, which expresses several Nav channels. Our results show that PbTX-2- and P-CTX-1B- evoked Nav channel activation induces TTX-sensitive transient

increase of intracellular Ca2+,. Brevenal (1 and 10 µM) antagonizes PbTX-2- and P-CTX-1B- evoked calcium response in GH3b6 cells.
Altogether, our findings suggest that brevenal binds silently to Nav channels and thereby antagonized CTX and PbTX effects. This in vitro proof of concept allows us to consider the therapeutic development of natural molecules such as brevenal for the management of toxin-induced channlopathies linked to Nav channels.

BIOPHYSICAL CHARACTERIZATION OF NEW SMALL-MOLECULE BLOCKERS OF NNAV1.5 CHANNELS EXPRESSED IN BREAST CANCER CELLS

Osbaldo LOPEZ-CHARCAS1; Shilpa DUTTA2; Samuel TANNER2; Katri SELANDER3; Wayne BROUILLETTE2; Sébastien ROGER1; Sadanandan VELU2;
1: EA4245 Transplantation, Immunologie et Inflammation (T2I), Université de Tours, Faculté de Médecine, 10 Boulevard Tonnellé, 37032 Tours, Cedex, France
2: Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294-1240, USA
3: Division of Hematology and Oncology, Department of Medicine, University of Alabama at Birmingham, 1808 7th Ave S, Birmingham, AL 35294-0012, USA

Voltage-gated sodium channels (NaV) are well-established drug targets for anti-epileptic, anti-arrhythmic and pain medications due to their crucial role in cellular excitability. A non-canonical NaV expression has been recognized in non-excitable cancer cells and their over-expression has been associated with cell invasion properties in a variety of human cancers. The neonatal isoform of the NaV1.5 subtype (nNaV1.5) is over-expressed in human breast cancer and its activity has been associated with metastasis and poor patient prognosis. Currently, there is not treatment available to specifically prevent or treat breast cancer metastasis. Recently, we synthesized new small-molecule drugs acting as nNaV1.5 blockers predicted by 3D-QSAR modeling. The aim of the present study was to investigate the potency and blocking mechanism of these compounds on nNaV1.5 currents expressed in MDA-MB-231 cells, a highly metastatic human breast cancer cell line. By performing whole-cell patch-clamp recordings in MDA-MB-231 cells, we analyzed the acute effect of 7 compounds and found that all of these blocked the nNaV1.5 channels in a dose-dependent manner, with the most potent compound exhibiting an IC50 value of 4.8 +/- 1.3 µM. We also observed a slow-down of the activation and inactivation kinetics of nNaV1.5 currents in presence of each drug. In addition, these compounds caused a leftward shift in steady-state inactivation-voltage relationships with a moderate effect on the conductance-voltage relationships. Two of the compounds tested exhibited a state-dependent inhibition of NaV1.5. Finally, these compounds also decreased the invasive capacity of MDA-MB-231 cells without affecting cell viability. These small-molecule compounds studied have the potential to be developed as new inhibitors of breast cancer metastases.

17:00 Symposium 2: “Ion channels and skin functions”

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

CUTANEOUS ION CHANNEL MECHANISMS OF ITCH SIGNALING

Hongzhen HU;
Department of Anesthesiology The Center for the Study of Itch Washington University School of Medicine in St. Louis

Skin is the largest sensory organ of the human body. Emerging evidence suggests that ion channels serve as molecular sensors of thermal, chemical, and physical stimuli in the skin. Activation of these ion channels by tissue damage and inflammation initiates complex signaling pathways in both primary sensory nerve endings and numerous tissue resident cells in the skin and subsequently produces pain and itch sensations. Although several G-protein coupled receptors have been shown to play an itch-specific role in the primary sensory neurons and spinal cord, the molecular and cellular mechanisms underlying itch initiation in the skin are less understood. We reported that TRPV4 channels expressed by the keratinocytes and dermal macrophages contribute to persistent itch in mouse models of dry skin and allergic contact dermatitis, respectively. Moreover, both TRPV1 and TRPA1 channels expressed by primary sensory nerve endings mediate persistent itch but TRPV1 protects skin inflammation in a mouse model of allergic contact dermatitis. Surprisingly, the mechanosensitive Piezo2 channels expressed by the mechanosensory Merkel cells modulate mechanical itch through promoting the activity of the slowly-adapting type I (SAI) afferent. This modulatory effect is lost in the setting of aging and dry skin, resulting in exaggerated mechanical itch (also known as alloknesis). Collectively, these studies demonstrated that ion channels have versatile functions in the regulation of chronic itch. Identification of cutaneous cells and ion channels that are critically involved in the pathogenesis of chronic itch will provide novel insights into the molecular and cellular basis of chronic itch, which is essential to the development of effective anti-pruritic drugs.

TRP CHANNELS AND SKIN HOMEOSTASIS

V'yacheslav LEHEN'KYI;
Laboratoire de Physiologie Cellulaire INSERM U1003 Laboratoire d'Excellence Ion Channels Science and Therapeutics Département de Biologie Faculté des Sciences et Technologies Université de Lille

Transient Receptor Potential (TRP) channels are a superfamily of non-selective cationic channels defined firstly as mechanoreceptors. Being ion channels they mediate ion signalling in the skin, such as skin permeability and barrier, wound healing, angiogenesis, immune function, and cellular senescence.
Calcium is one of the most important second messengers involved in the skin homeostasis. Within this superfamily of TRP channels, the role of TRPV6 highly calcium channel in the process of differentiation of human keratinocytes has been shown as well as some other channels as TRPC1 and TRPC4 whose role was also suggested in basal cell carcinoma. The other key players of calcium homeostasis such as STIM1 and Orai1 were also considered. The focus of the recent studies was done on the Orai1 channel which is a pore subunit of a store-operated Ca2+ channel (SOC) which is a major molecular counterpart for Ca2+ influx in nonexcitable cells. To elucidate the physiological significance of Orai1 in skin, its role and function were studied in epidermis of mice with targeted disruption of the Orai1 gene, human skin sections and primary keratinocytes. Orai1 protein was mainly confined to the basal layer of epidermis where it plays a critical role to control keratinocyte proliferation and polarized motility.
Another channel, TRPM8 has been recently reported as a cold receptor in kératinocytes playing a role in cold-dependent balance between keratinocyte proliferation and differentiation. Altogether, TRP channels are important regulators of skin homeostasis and deserve particular attention in both health and disease.

INTRACELLULAR ION CHANNELS REGULATING SKIN MELANOCYTE PIGMENTATION

Elena OANCEA;
Department of Molecular Pharmacology, Physiology, and Biotechnology, Brown University, 171 Meeting St., Box G–E304, Providence, Rhode Island 02912, USA.

Skin melanocytes are pigment cells that produce melanin, the major pigment in animals. Melanin is synthesized and stored in unique lysosome-related organelles named melanosomes. Ion transport across melanosomal membrane and its contribution to pigmentation remain poorly understood, although many genes encoding putative melanosomal transporters have been identified as key regulators of melanin synthesis. We recently identified two ion channels that regulate pigmentation of skin and eye melanosomes: an anion channel encoded by the protein mutated in oculocutaneous albinism II (OCA2) and a cation channel encoded by the two-pore channel 2 (TPC2) that is a negative regulator of pigmentation. We found that OCA2 functions as an anion channel that affects melanin synthesis by regulating melanosomal pH. We also identified two-pore channel 2 (TPC2), previously implicated in human pigmentation and melanoma, as the first reported melanosomal cation channel. We demonstrate that the vesicular signaling lipid phosphatidylinositol bisphosphate PI(3,5)P2 modulates TPC2 activity in melanosomes. TPC2 serves as a negative regulator of pigmentation by increasing the melanosome's membrane potential and acidity, resulting in decreased melanin content. More recently, we identified a Cl-/H+ antiporter that functions in melanosomes as a negative regulator of pigmentation, in conjunction with OCA2 to regulate melanosomal pH and luminal Cl- concentration. In addition, we found that the TRPV2 ion channel is localized to a subset of mature melanosomes and functions as a positive regulator of pigmentation. Our studies suggest that melanosomal pH is a key regulator of melanogenesis and it is controlled by a complex mechanism that might be deficient in different forms of oculocutaneous albinism.

THE ROLE OF THE AUXILIARY NAVβ4 SUBUNIT IN MAINTAINING EPITHELIAL PHENOTYPE

Adélaïde DORAY1; Lucie BRISSON2; Stéphanie CHADET1; Lucile POISSON1; Osbaldo LOPEZ CHARCAS1; Caroline GOUPILLE2; Lobna OULDAMER2; Christophe BARON1; Pierre BESSON2; Sébastien ROGER1;
1: University of Tours, EA4245, Tours, France
2: University of Tours, Inserm UMR1069, Tours, France

Cancer metastasis is the main cause for the high mortality attributed to carcinomas. As such, understanding the cellular mechanisms leading to metastases development is crucial. Our team has described the SCN4B gene, and its expression product, the NaVβ4 auxiliary subunit of voltage-gated sodium channels (NaV), to be implicated in the invasive progression of breast cancer, independently of NaV subunit (Bon et al., Nat Commun 2016).

SCN4B is highly expressed in epithelial cells of normal breast, but is reduced in breast cancer tissues and cells. SCN4B is reduced in grades II and III compared to grade I, and is lower in HER2 and triple-negative compared to luminal A and B breast tumours. In vitro, the loss of SCN4B expression is responsible for the acquisition of a very aggressive mesenchymal-amoeboid hybrid phenotype and promotes breast cancer cell invasiveness. Correlatively, the overexpression of NaVβ4 reduces breast cancer cell invasiveness and mammary tumour progression in animal models.

Recently, we have generated several clones of MCF-10A non-cancer mammary cells knocked-down the expression of SCN4B (CRISPR-Cas9) and have identified important morphological changes, with the loss of their epithelial phenotype. Our objectives are to understand the mechanisms leading to these phenotypical changes in epithelial cells when SCN4B expression is reduced, and to study consequences on the epithelial function and carcinogenesis. Our preliminary results showed that the loss of NaVβ4 in normal breast cells decreased both total and plasma membrane expression of the cell adhesion protein β-catenin, as observed in western blotting and immunocytochemistry experiments, but not at the transcriptional level (RT-qPCR). The use of MG132 (10 µM) partially restored β-catenin levels in SCN4B-knocked-out MCF-10A cells, suggesting that NaVβ4 could prevent β-catenin degradation by the proteasome. A similar effect was observed in MCF-10A cells transiently knocked-down for the expression of NaVβ4 (siRNA). In MDA-MB-231 breast cancer cells stably knocked-down for the expression of NaVβ4 (CRISPR-Cas9), the expression of β-catenin is slightly reduced at both protein (western blot) and mRNA (RT-qPCR) levels, as compared to control MDA-MB-231 cells (wild-type cells). The functional link between NaVβ4 and β-catenin remains unclear and is currently studied.

Tuesday, September 10th 2019

08:30 Symposium 3: Glutamate ligand-gated channels

Organized by **Laurent Fagni** *(Montpellier, France)*

NEW TYPES OF NMDA RECEPTORS

Pierre PAOLETTI;
pierre.paoletti@ens.fr

Since their discovery over three decades ago, NMDA receptors (NMDARs) have kept drawing attention of neuroscientists because of their central roles in CNS development and function. These glutamate-gated ion channels are essential mediators of brain plasticity converting specific patterns of neuronal activity into long-term changes in synapse structure and function that are thought to underlie higher cognitive functions. The well characterized conventional NMDARs are heterotetramers composed of two GluN1 and two GluN2 subunits, and require two agonists, glutamate and glycine (or D-serine) for activation. They are highly Ca2+ permeable, exhibit strong voltage-dependency due to Mg2+ pore block, and cluster at excitatory synapses where they control synaptic strength by acting as coincident detectors.
Far less is known regarding NMDARs incorporating the glycine-binding GluN3 subunits (GluN3A-B), which decrease Ca2+ permeability and confer resistance to Mg2+ block. Remarkably, in heterologous expression systems, GluN1 and GluN3 subunits can co-assemble to form 'unconventional' NMDARs that lack glutamate-binding sites and are gated by glycine only, thus forming GluN1/GluN3 excitatory glycine receptors. The physiological relevance of such excitatory glycine NMDARs however, has sparked intense controversy since their activity was undetectable in native neuronal tissues, pointing to potential artifacts of expression systems. Moreover, in recombinant systems, glycine currents produced from GluN1/GluN3 receptors are conspicuously small, unstable and difficult to quantify. Based on the discovery of a pharmacological agent (CGP-78608) that uniquely and massively potentiates GluN1/GluN3 receptors (by preventing their desensitization), we now provide ample evidence that GluN1/GluN3A receptors are functionally expressed at high levels in specific regions of the mouse brain, where they constitute a new type of neuronal receptors. We also uncover that these receptors are highly plastic in their gating, capable of switching modes between low and high agonist affinity.
Overall, this work provides important novel information about the biophysics, pharmacology and physiology of 'unconventional' glycine-activated GluN1/GluN3 receptors. By demonstrating that glycine excitatory NMDARs are bona fide brain receptors and not just artifacts of heterologous expression systems, it also reshapes our current understanding of these underappreciated receptors, and open new perspectives on their characterization and on their role in brain development and function.

PRESYNAPTIC PLASTICITY IN HIPPOCAMPAL CIRCUITS: ROLE OF KAINATE RECEPTORS.

Christophe MULLE;
CNRS, University of Bordeaux

Neuronal circuits can be optimized to allow for transmission, storage, and recall of information. It is widely accepted that these processes depend on multiple forms of activity-dependent synaptic and intrinsic plasticity with distinct temporal dynamics. Within the hippocampus, most of the attention has focused on long-term changes in synaptic efficacy which occurs at the postsynaptic level. Neurons often encode information not as spikes in isolation but as bursts of spikes at high frequency, which give rise to a wide dynamic range of presynaptic plasticity, depending on the synaptic contacts and on the history of synapse activity. In this lecture, I will discuss the cell-biological mechanisms of various forms presynaptic plasticity within the hippocampal CA3 region. I will show the implication of presynaptic ionotropic receptors of the kainate type in presynaptic plasticity, and provide experimental evidence indicating how these processes are likely to determine the dynamics of neural processing and to underlie mechanisms of memory encoding.

ROLE OF THE DELTA FAMILY OF GLUTAMATE RECEPTOR IN SYNAPTIC TRANSMISSION.

Ludovic TRICOIRE;
Neuroscience Paris Seine, Sorbonne université, paris, France

Despite the strong sequence homology with other ionotropic glutamate receptors (iGlu), the delta family of iGlu, comprising GluD1 and GluD2, is unable to bind glutamate. Until recently, these subunits were considered as orphan since no endogenous ligand was found to open their ion pore. Nonetheless, over the past decade, several genetic studies have linked mutations in GluD1/2 encoding genes with psychiatric disorders such as schizophrenia and autism. Furthermore, many advances have been achieved in the elucidation of the role of GluD in synaptic transmission such as the identification of Cbln1 and D-serine as ligand regulating synapse formation and synaptic long term plasticity. Recently, our lab showed a tight coupling between group I glutamate metabotropic receptors (mGlu1/5) and GluD1/2 subunits. We found that pharmacological or synaptic activation of mGlu1/5 induced opening of the GluD1/2 channel in heterologous systems, in cerebellar Purkinje cells and in dopaminergic neurons. In this latter case, we discovered the crucial role of GluD1 ion pore in the in vivo firing activity of these neurons. During my talk, I will review the main features of GluD1/2 subunits regarding synapse formation, long term plasticity and ion channel. I will also provide new unpublished data regarding the role of GluD1 in glutamatergic transmission. We are currently characterizing several missense mutations in GluD1 encoding gene observed in human patient affected with intellectual disabilities. We observed that one of these mutations located at the interface between Cbln1 and D-serine binding sites caused defects in synapse maturation and alteration in mGlu1/5 intracellular signaling as well as D-serine sensitivity. We are generating a transgenic mouse line carrying this human mutation associated with intellectual disabilities using the CRIPR/Cas9 technology. Aside to this project, we are developing a new pharmacology of GluD1/2 subunits based on light molecular switch attached to these subunit allowing reversible blockade of the ion pore in a subunit-, cell type-, and time-dependent manner.

PLASTICITY OF INHIBITORY SYNAPTIC TRANSMISSION UNDER THE CONTROL OF NMDA RECEPTORS IN THE DORSAL HORN OF THE SPINAL CORD.

Perrine INQUIMBERT1; Benjamin LEONARDON2; Louise VIAL2; Rémy SCHLICHTER1;
1: INCI UPR32 / Université de Strasbourg
2: INCI UPR 3212

The dorsal horn (DH) of the spinal cord is an important structure involved in the integration of nociceptive messages. Plastic changes in the properties of neuronal networks in the DH underlie the development of analgesia as well as, of hyperalgesia and allodynia in acute and chronic pain states. Two key mechanisms are involved in these chronic pain states: increased electrical activities and glutamate release leading to the recruitment of NMDAr and plastic changes in the synaptic inhibition. Although 1) the balance between excitation and inhibition is known to play a critical role in the spinal network and 2) plastic changes in spinal excitation and inhibition have been studied separately, the relationship between these two mechanisms has not been investigated in detail.
Our work aims at exploring mechanisms responsible for activity-dependent plasticity of synaptic inhibition engaging NMDAr in the DH.
Our results show that, in a subset of neurons recorded in lamia II, NMDAr activation facilitates GABAergic synaptic transmission with a target specificity on GABAergic interneurons. Using a pharmacological approach, we investigated the composition of NMDAr involved in this facilitation. We found that the NMDA-induced facilitation was mediated by the activation of NMDA receptors containing GluN2C/D subunits. Finally, we found that spinal glial cells were involved in the NMDAr-induced facilitation of GABAergic synapse.
Altogether, our results bring new insights on the activity-dependent plasticity of inhibitory synaptic transmission in the spinal cord network that could underlie the development and maintenance of neuropathic pain.

10:30 Symposium 4: Ion channels in cancer therapy resistance

Organized by **Lise Rodat-Despoix** *(Amiens, France)*

MAGNESIUM-SPECIFIC ION CHANNELS IN CHEMORESISTANCE: IS THERE A ROLE FOR MAGNESIUM?

Federica i. WOLF; Valentina TRAPANI;
Istituto di Patologia Generale e Centro di Ricerche Oncologiche Giovanni XXIII, Università Cattolica del Sacro Cuore, Rome, Italy.

Magnesium in an essential cation for life, it affects hundreds of enzyme, stabilizes membranes and takes part in cell ionic balance. In particular, Mg2+- ATP complex is the active form triggering phosphorylation reactions, such as in signal transduction, DNA duplication and repair, cell cycle regulation, cytoskeletal activity. Thus, it is not surprising that Mg2+ availability affects carcinogenesis, tumor development and progression. Currently we know that the biological effects of magnesium are due not only to its availability but also to the expression and regulation of its specific transporters. Indeed, TRPM7 expression, the most studied magnesium transporter in oncology, correlates with growth rate, aggressiveness and tumor spreading of different epithelial tumors. In this context, we investigated whether magnesium could influence sensitivity to doxorubicin, one of the most used anti-tumor chemotherapeutic agent.

We used two strains of LoVo colon cancer cells sensitive and resistant to doxorubicin and investigated the relationship between TRPM7 and MagT1 expression with cell magnesium homeostasis, growth rate and doxorubicin sensitivity. We also assessed the relationship of Mg2+ with MDR-glycoprotein expression and doxorubicin uptake. We will discuss these data trying to elucidate the role of magnesium and its transporters in this crucial though complex phenomenon.,

CALCIUM SIGNALING MODULATION BY ANTI-APOPTOTIC BCL-2 IN HEALTH & DISEASE

Geert BULTYNCK;
Laboratory of Molecular & Cellular Signaling, KU Leuven, Belgium

It has become increasingly clear that anti-apoptotic Bcl-2-family members, including Bcl-2 and Bcl-XL, execute part of their cell biological function through Ca2+-signaling modulation. These proteins reside in intracellular membranes, including at contact sites between the endoplasmic reticulum (ER) and the mitochondria. At these sites, also IP3 receptors (IP3Rs), ryanodine receptors (RyRs) and voltage-dependent anion channels (VDACs), Ca2+-flux system of the ER and mitochondrial outer membrane, respectively, reside. Their Ca2+-flux properties critically control several cell fate processes, whose dysregulation has been implicated in oncogenesis and several cancer hallmarks but also underlie disease burden like in acute pancreatitis. We and others have found that several Bcl-2-family members can directly targets these channels and impact their function. Our team has been working on (i) characterizing the effects of Bcl-2-family members on IP3Rs, RyRs and VDACs and the consequences for the cell, (ii) identifying the underlying molecular determinants responsible for complex formation and channel regulation by these proteins and (iii) exploiting these insights to develop novel therapeutic strategies to fight diseases. This revealed a unique role for the “BH4-domain biology” of the Bcl-2 family. Bcl-2 targets several regions in IP3R channels, ensuring dynamic modulation of Ca2+ signaling outputs. Besides a Bcl-2-binding site in the modulatory region of the IP3R, we identified the ligand-binding domain of IP3R as a novel target for Bcl-2 via its BH4 domain. Excitingly, binding of Bcl-2 to this site was antagonized by IP3, the physiological agonist of IP3Rs. Vice versa, Bcl-2’s BH4 domain could prevent IP3 binding to IP3Rs. This allows a dynamic regulation of IP3R inhibition by Bcl-2 dependent on IP3 signaling strengths. Indeed, IP3R inhibition by Bcl-2 or its BH4 domain occurred at moderate level of IP3/agonist signaling but not at high level of IP3/agonist signaling. Targeting the Bcl-2 interaction network with IP3Rs and RyRs also held therapeutic potential. On the one hand, antagonizing Bcl-2 using IP3R-derived peptides representing the Bcl-2-binding site proved to be successful to kill B-cell cancer cell models by triggering Ca2+ overload. The cancer-specific actions of such peptides appear to rely on chronic IP3 signaling and high IP3R2 expression representing a deadly cocktail rendering cancer cells addicted to Bcl-2 at the ER. Ongoing work points towards a key role for mitochondrial Ca2+ overload. On the other hand, antagonizing IP3Rs/RyRs using Bcl-2-derived peptide representing the BH4 domain proved to be successful to suppress excessive Ca2+ signaling and necrosis in primary pancreatic acinar cells exposed to bile acids, key features in disease burden associated with acute pancreatitis. BH4-domain peptides prevent mitochondrial Ca2+ overload and might be combined with other therapeutic avenues.

TARGETING POTASSIUM CHANNELS TO OVERCOME CHEMORESISTANCE IN GASTROINTESTINAL CANCERS

Annarosa ARCANGELI;
Department of Experimental and Clinical Medicine University of Florence Italy

Ion channels have been intensively studied for their pivotal role in the control of cell excitability and ion transport equilibria. More recently, Ion channels have also been shown to be implicated in cancer establishment and progression, since they regulate different hallmarks of cancer (Prevarskaya N. et al., 2018). Overall, ion channels can now be considered novel cancer biomarkers (Lastraioli et al., 2015). Moreover, ion channels may be good targets for anti neoplastic therapy, once their side effects are appropriately controlled (Arcangeli A and Becchetti A., 2017). We provided several evidences that a voltage dependent K+ channel, Kv 11.1 (also known as hERG1), is aberrantly expressed in cancer cells, where it regulates different aspects of neoplastic cell behaviour (from cell proliferation and apoptosis regulation, to cell migration and invasiveness, hence impacting on tumour angiogenesis and metastases) and can hence be considered a relevant cancer biomarker. Kv 11.1 regulates so different cancer hallmarks, since it forms a molecular complex with the Beta1 subunit of integrin receptors and, by this way, can regulate several intracellular signalling pathways (Becchetti A. et al., 2017, Becchetti A et al., 2019). We also provided evidence that Kv 11.1 can modulate chemoresistance in gastrointestinal, in particular colorectal, cancers, since it (1) regulates cisplatin uptake, in conjunction with Ca2+ activated K+ channels (Pillozzi & D'Amico et al., 2018); (2) modulates the response to 5-fluorouracil (5FU), through a process of activation/inhibition of autophagy, which occurs since Kv 11.1 forms a signaling complex with the p85 subunit of PI3K; (3) represents a positive biomarker of response to anti-VEGF therapy (Bevacizumab) in metastatic colorectal cancer patients. Overall, the targeting of K+ channels, either Kv or KCa, can contribute to overcome chemoresistance in colorectal cancer.

APOPTOSIS MEDIATED BY MITOCHONDRIAL CA2+ OVERLOAD REQUIRES INHIBITION OF AUTOPHAGY REVEALING A WIDESPREAD VULNERABILITY OF CANCER CELLS.

Charlotte DUBOIS; Morad ROUDBARAKI; Natalia PREVARSKAYA; Fabien VANDEN ABEELE ;
Inserm U1003, Equipe labellisée par la Ligue Nationale Contre le Cancer, SIRIC ONCOLille, Université Lille1, Villeneuve d'Ascq, France. Laboratory of Excellence, Ion Channels Science and Therapeutics;

Mitochondria play key roles in regulating cell fate and mitochondrial Ca2+ overload is one of the ways to induce apoptosis. Here we demonstrate against all odds that sustained mitochondrial Ca2+ overload by itself is poorly effective in inducing cell death. In fact, this well-accepted apoptotic stimulus requires concomitant inhibition of autophagy to counteract its prosurvival action. Such conditions are a prerequisite for mitochondrial membrane depolarization, by a DRP1-dependent mitochondrial fission. Such conditions are a prerequisite for release of cyto-chrome c thus promoting cell death. Using xenograft mouse models, cell line-based functional studies, and ex-vivo tumor models from clinical specimens, we evaluated the translational relevance of this dependency with drugs in clinical evaluation. Here, we confirmed that a dual-targeting strategy based on mitochondrial Ca2+ overload and autophagy inhibition synergically sensitizes multiple cancer cell types to chemotherapies independently of their mechanisms of action. In conclusion, these findings challenge a crucial paradigm in cell death revealing novel combinatorial therapeutic strategies to improve clinical outcomes.

Wednesday, September 11th 2019

09:30 ***Oral communication session 2: « Ion Channel Structural Aspects»***

Organized by **Dimitra Gkika** (Lille, France)

BIOMOLECULAR MODELLING OF ACID-SENSING ION CHANNELS
Olivier BIGNUCOLO; Sabrina VULLO; Ivan GAUTSCHI; Stephan KELLENBERGER;
University of Lausanne, Switzerland

Acid-sensing ion channels (ASICs) are pH-sensing, Na+-selective ion channels in the nervous system, where they exert important physiological and pathological roles. ASICs respond to an extracellular acidification with a transient inward current, which is due to a rapid transition from the closed to the open and subsequently a slower transition to the non-conducting desensitized state. We are interested in understanding in molecular details the mechanisms by which protons and associated ligands control the behaviour of the ASIC channel.
To this end, in close collaboration with the experimental lab, we use a variety of computational tools in order to predict and explain in atomic details the behaviour of ASIC channels. Central to our work, molecular dynamics simulations (MD) solve the classical laws of motion to compute the time evolution of coordinates and momenta of atoms within a system representing the membrane embedded channel, water and ions. Time-dependant variables, e.g. atomic distances, hydrogen bonds, salt bridges formations/breakages, are extracted from the resulting trajectory. Crystal structures of chicken ASIC1a, in the closed, toxin-opened and desensitized state have been published. Since chicken ASIC1a shares 90% homology with human ASIC1a, we rely on homology modelling to construct human ASIC1a models. The pKas of all ASIC titratable residues in the closed, open and desensitized states were calculated by solving the Poisson-Boltzmann (PB) equation. Since applications of PB represent the solvent as a continuous medium, we wrote a wrapper to the Charmm PB module, which takes the ion dynamics into account when using structures extracted from a MD trajectory. Comparison of the individual values allowed us to identify pH-sensor candidates, which are currently under experimental investigations. Using classical MD, we study the conformational changes induced by specific mutations, which cause pH50 activation shifts. Constant-pH MD simulations constitute a recent further development of MD, in which protonation/deprotonation events can be sampled in addition to the usual conformational sampling. We currently conduct several constant-pH MD trajectories in order to describe at atomic level conformational changes associated with acidification. Thus, a variety of biomolecular modelling approaches are used to link functional and structural information.

PHOSPHORYLATION OF PSD95 AS POTENTIAL LINK BETWEEN SYNAPTIC AND STRUCTURAL PLASTICITIES

Côme CAMUS; Eric HOSY; Daniel CHOQUET;
Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, Bordeaux (FRANCE)

The synaptic strength, which corresponds to the efficacy of synaptic transmission, evolves all along life time and experiences. The molecular mechanisms underlying such phenomenon define the synaptic plasticity. A particular form of synaptic plasticity, called Long Term Depression (LTD), leads to a decrease of the synaptic strength. This change has been associated with a rapid and persistent removal from the synapse of a major post-synaptic scaffold protein, PSD95, several minutes after LTD induction. Moreover, few hours after induction, LTD triggers a decrease of the spines density due to the suppression of some synapses via a process called structural plasticity. Nonetheless, a clear comprehension of the relation between LTD and synapse selection is still missing.
Interestingly, it has been reported that the mutation of PSD95 at the site of GSK-3β phosphorylation on threonine-19 impairs the expression of LTD. However, data are lacking to clearly conclude about the mechanisms implicated in such impairment. Here, using whole-cell patch-clamp recordings, super-resolution microscopy and confocal imaging on cultured hippocampal neurons, we investigated the implication of the phosphomutant PSD-95-T19A in this LTD blockade, and its possible impact on synaptic pruning.

DESIGN OF LIGHT-MODULATED INWARD RECTIFYING POTASSIUM CHANNELS

Mathilde FOLACCI 1; Jean REVILLOUD1; Ana sofia ERIA OLIVEIRA1; Zlatomir TODOROV1; Gina catalina REYES-MEIJA1; Guillaume SANDOZ2; Michel VIVAUDOU1;
1: Institut de Biologie Structurale, Grenoble, France
2: Institute of Biology Valrose, Nice, France

We are designing light-sensitive Kir channels as tools for controlling cell excitability and deciphering the physiological roles of these channels in various tissues. We focus on the Kir3.1 and Kir3.4 subunits of the G-protein-gated inward rectifying K+ (GIRK) and the Kir6.2 subunit of the ATP-sensitive potassium (KATP) channel. These channels have important functions in heart (rhythm control), pancreas (insulin secretion), as well as in the nervous system.
To confer light sensitivity to Kir channels, we use the photoswitched tethered ligand (PTL) approach, based on the grafting of a light-sensitive blocker, here Maleimide Azobenzene Quaternary ammonium (MAQ), to a cysteine introduced in the channel, at a position identified as suitable by molecular model analysis. The MAQ molecule, like other azobenzene compounds, adopts a long, trans conformation under 500-nm light and a short, cis conformation under 380-nm light. It relaxes to trans in the dark. Functional tests were carried out by expressing channels in Xenopus laevis oocytes and analyzing their response to light using patch clamp and Two-Electrode Voltage Clamp (TEVC) techniques.
Recently our team succeeded in achieving complete and reversible light-inhibition of Kir6.2, as well as Kir3.4. However, channels are inhibited by the trans conformation of MAQ, which is the conformation adopted in 500-nm light and in the dark and therefore not suitable for native cell expression. We present here a new Kir3.4 construct, which is functional in the dark and can be blocked reversibly by UV illumination. We are now in the process of designing UV-inhibited Kir3.1 channels as well as Kir6.2 channels.
After full characterization in Xenopus oocytes, we plan to test these channels in more relevant models, from mammalian cell lines to native cells.

STRUCTURE AND CA2+ INTERACTION OF THE EXTRACYTOSOLIC/LUMENAL DOMAIN (ELD) OF THE HUMAN TRPML2 ION CHANNEL

Kerstin k. VIET1; Annika WAGNER1; Kevin SCHWICKERT2; Tanja SCHIRMEISTER2; Hermann SCHINDELIN3; Ute a. HELLMICH1;
1: Institute for Pharmacy and Biochemistry, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany & Center for Biomolecular Magnetic Resonance (BMRZ), Goethe-Universität, 60438 Frankfurt am Main, Germany
2: Institute for Pharmacy and Biochemistry, Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany
3: Rudolf-Virchow-Centre for Experimental Biomedicine, Universität Würzburg, 97080 Würzburg, Germany

Intracellular ion channels experience vastly different physiological conditions (e.g. pH, ion concentration) depending on their respective localisation. Importantly, the structural and functional (?) consequences of these conditions are often poorly understood. The three members of the human transient receptor potential mucolipin (TRPML) family are unselective cation channels located in the endolysosmal system (and to a lesser extent in the plasma membrane). They are involved in e.g. membrane trafficking, ion homeostasis and autophagy. The hallmark of the TRPML family is a large loop (~25 kDa) between the first two transmembrane helices, which forms the Extracytosolic/Lumenal Domain (ELD) comprising one third of the channel. This domain faces either the endolysosomal lumen or the extracellular space and therefore experiences drastic changes in pH and Ca2+ concentrations. With our crystal structures of the human TRPML2 ELD at pH 6.5 (2.0 Å) and 4.5 (2.95 Å), corresponding to the pH values in recycling endosomes and lysosomes1, we present the first structural information available for TRPML2. The ELD forms tetramers even in the absence of the rest of the channel, not only in crystals, but also in solution as assessed by size exclusion chromatography (SEC) and small angle X-ray scattering (SAXS). One important feature of the ELD is the highly acidic pre-pore loop, which is located in the ELD center. At neutral pH, Ca2+ ions bind to this loop, as verified by isothermal titration calorimetry (ITC), possibly forming a “cation block”. In contrast, at lower pH values, similar to what the channel would experience in the lysosome, Ca2+ binding is abrogated. We therefore suggest a pH- and Ca2+-dependent regulation mechanism for TRPML2 similar to what was proposed for TRPML12. Furthermore, our crystal structures of the human TRPML2 ELD complete the high-resolution set of human TRPML channel ELDs, allowing the first structural comparisons of this fascinating domain at different pH values.

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10:45 Symposium 5: A-typical' Ion channels/currents

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

SWELL1/LRRC8A MEDIATED NUTRIENT SENSING

Rajan SAH;
Washington University School of Medicine, USA

Our research program is broadly centered around ion channel modulation of cellular metabolism, with the aim of identifying innovative biological targets to open new, untested therapeutic avenues for cardio-metabolic disease. By taking a "wide-angle" view of ion channel signaling, my laboratory has established several independent research directions that emanate from our findings over the past 6 years, all exploring a singular, fundamental question in cell biology: Can ion channels sense shifts in cellular metabolism via cell swelling to regulate growth and energy homeostasis – a ubiquitous form of cell-autonomous "swell-signaling"? Our work on SWELL1 (LRRC8a) in adipocytes and pancreatic β-cells has led us to uncover a pathway linking cell expansion (due to either lipid content or glucose metabolism) to both insulin-sensitivity (adipocyte) and insulin secretion (pancreatic β-cell). These findings suggest that SWELL1 represents a critical control point connecting swell-mediated nutrient sensing with rheostatic regulation of systemic metabolism.

THE SODIUM LEAK CHANNEL NALCN IN PHYSIOLOGY AND DISEASE

Malik BOUASSE; Hathaichanok IMPHENG; Zoé SERVANT; Nathalie GUERINEAU; Philippe LORY; Arnaud MONTEIL;
Institut de Genomique Fonctionnelle CNRS UMR5203 - INSERM U1191 - UM 141, rue de la Cardonille 34094 Montpellier Cedex 5, FRANCE

The leak sodium channel NALCN, an atypical member of the four-domain ion channel family, plays a crucial role in the maintenance of the resting membrane potential of several cell types including neurons. Although this ion channel was first identified in 1999, the first functional data were only reported in 2007. Since then, several studies reported a role in pacemaking activity of different types of excitable cells including neurons, myometrial smooth muscle cells and interstitial cells of Cajal. Studies of animal models indicated a large panel of NALCN-related phenotypes including an altered locomotor behaviour and anaesthetic sensitivity, a disruption of respiratory and circadian rythms as well as a neonatal lethality when nalcn is knocked out in mouse. NALCN mutations lead to complex neurodevelopmental syndromes, including infantile hypotonia with psychomotor retardation and characteristic facies (IHPRF) and congenital contractures of limbs and face, hypotonia and developmental delay (CLIFAHDD), which are recessively and dominantly inherited, respectively. In order to decipher the mechanisms involved in the IHPRF and CLIFAHDD syndromes, we have developped cellular and zebrafish models to examine functional and physiological impacts of mutations of NALCN. We will present our data showing that IHPRF and CLIFAHDD pathogenic variants are loss- and gain-of function ones respectively when expressed in recombinant system. We also found that zebrafish models in which expression level of NALCN is changed resulted in altered locomotor behaviour, viability, and body weight. Our cellular and animal models provide powerful tools to develop strategies to rescue NALCN-induced deficiency.

ENGINEERING SYNTHETIC TOOLS FOR THE INHIBITION OF CELL EXCITABILITY

Andrea SAPONARO; Michal LASKOWSKI ; Anna MORONI;
Department of Biosciences, University of Milan, Italy

Chronic pain is an important public health problem. Many patients remain refractory to pharmacotherapy and there is a substantial need for novel treatments. In peripheral neuropathic pain, somatosensory neurons show hyper-excitability leading to abnormal sensations (dysesthesia and allodynia). Since neuronal excitability can be efficiently prevented by optogenetics, we tested whether our light-activated K+ channel BLINK2 could be used in the treatment of chronic pain in vivo. BLINK2 was transfected in the DRG of a rat model of chemotherapy-induced peripheral neuropathy. A day later the rats were tested for light-induced reversion of neuropathic pain. Short (1 min) exposure to blue light significantly reduced the pain sensation in the illuminated paw, but not in the control (dark) paw. BLINK2 expression in DRG and in cutaneous nerve terminals was confirmed by immunohistochemistry. Our data indicate that BLINK2 is a viable strategy for developing treatments for reversing neuropathic pain.
Another synthetic tool that I will present is a cell-penetrating peptide (TAT-nanoTRIP) that prevents HCN modulation by cAMP. HCN channels are crucial in the development of neuropathic pain and they are a pharmacological target. We are engineering an optogenetic tool, LOV-nanoTRIP, that releases the active peptide upon stimulation with blue light.

ROLE OF TRANSLOCON IN CALCIUM EXCHANGES IN CELL COMPARTMENTS AND IN CELL DEATH, DURING MYOCARDIAL INFARCT

Mallory DUCROZET1; Nolwenn TESSIER2; Ducreux SYLVIE3; Ovize MICHEL4; Van coppenolle FABIEN5;
1: mallory.ducrozet@univ-lyon1.fr
2: nolwenn.tessier@univ-lyon1.fr
3: sylvie.ducreux@univ-lyon1.fr
4: Michel.Ovize@chu-lyon.fr
5: fabien.van-coppenolle@univ-lyon1.fr

Introduction: Cardiovascular diseases are one of the main causes of death in the world. Myocardial infarct (MI) is characterized by an occlusion of an artery, causing emergence of an ischemic area. Even if reperfusion is necessary, it still causes myocardial injuries. At the cellular level, cardiomyocytes sustain an alteration of calcium (Ca2+) homeostasis between reticulum (ER) and mitochondria, which contributes to cell death. Translocon (TLC), a major component of the translation machinery, is one of the main reticular Ca2+ leak channel and is involved in Ca2+ homeostasis.
Objectives: The aim of our work is to better understand the role of TLC. Firstly, we want to know where this channel is precisely located. Then, we assess whether the pharmacological modulation of TLC could have an impact on ER-mitochondria Ca2+ exchanges. Finally, the goal is to know the impact of TLC modulations on cell death after hypoxia-reoxygenation (HR) sequence.
Methods: Rat cardiomyoblast H9C2 cell line was used in all of our experiments. Ca2+ and ATP levels in cell compartments were assessed in imaging experiments, using Ca2+ and ATP sensors. Puromycin (Puro) and anisomycin (Aniso) were used to open and close TLC, respectively. The location of this channel was obtained with immunoblot and the level of cell death was obtained by flow cytometry.
Results: Our data show that TLC is a functional Ca2+ leak channel: the application of 40µM of Puro leads to a Ca2+ leak from ER, and Aniso pretreatment prevents this effect. These treatments impact Ca2+ exchanges between ER and mitochondria,

especially by increasing IP3-R response to ATP stimulation in Ca2+ Hot spots. Puro pretreatment increases the mitochondrial ATP content by 39%. Moreover, TLC modulations during HR sequence show a decrease of cell death.
Conclusion: Pharmacological modulations of TLC could be an effective cardioprotection strategy, by regulating Ca2+ exchanges between ER and mitochondria, to reduce cell death after a MI.

POSTER ABSTRACTS

P1- FUNCTIONAL ROLE OF ION CHANNELS IN THE NEOPLASTIC PROGRESSION STEPS OF CANCER CELLS, ELUCIDATED BY SCORPION PEPTIDES: EMERGING NEW SIGNALING PATHWAY

Najet SRAIRI-ABID; Dorra AISSAOUI; Houcemeddine OTHMAN; Rym BENAISSA; Saoussen MLAYAH-BELLALOUNA;

Laboratoire des Venins et Biomolécules Thérapeutiques, Institut Pasteur de Tunis, Université Tunis El Manar

Scorpion toxins have been the subject of many studies exploring their pharmacological potential. The high affinity and the overall selectivity to various types of ionic channels endowed scorpion toxins with a potential therapeutic effect against many channelopathies. Cancer is considered as a channelopathy since ion channels are shown to be over expressed and play a pivotal role in the progression of various cancer cell types. Ionic channels are thus considered as new targets for designing anti-cancer therapy. However, the fact that many ion channels are expressed in different cell lines makes it difficult to ascribe a functional role for a given ion channel on a specific aspect of the tumorogenesis. We focused on the anti-cancer activity of some scorpion toxins and their effect on the multiple hallmarks of cancer. We also shed light on effectors and receptors involved in signaling pathways in response to scorpion toxins effect. We showed that although some ion channels are expressed in different cancer cell lines, these respond differently to a given scorpion venom peptide. Until now, the anticancer mechanisms described for scorpion peptides consist on targeting ion channels to (i) inhibit cell proliferation and metastasis; and (ii) induce cell cycle arrest and/or apoptosis through membrane depolarization leading to hemostasis deregulation and caspase activation. Putative targets such as metalloproteinases, integrins and/or growth factor receptors, beside ion channels, have been unveiled to be affected by scorpion peptides. Our results showed that besides they can elucidate the implication of ion channels in molecular mechanisms of neoplastic progression, scorpion peptides may be used as therapeutic tools against different cancers.

P2- CALCIUM SIGNALS TRIGGERED BY THE MICROENVIRONMENT REGULATE NEURAL STEM CELL PROLIFERATION AND SELF-RENEWAL.

Florence DOMENICHINI[1]; Elodie TERRIE[1]; Cyrine BEN DHAOU[2]; Patricia ARNAULT[1]; Thomas HARNOIS[1]; Christophe MAGAUD[3]; Patrick BOIS[3]; Emmanuel MOYSE[2]; Bruno CONSTANTIN[1]; Valérie CORONAS[1];

1: Laboratoire STIM, Université de Poitiers-CNRS ERL7003- 86022 Poitiers Cedex- France
2: UMR85 PRC INRA, Nouzill
3: Laboratoire STIM, Université de Poitiers- 86022 Poitiers Cedex- France.

In the brain of adult mammals, neural stem cells (NSC) persist in specific brain germinative niches where they proliferate, self-renew and produce neurons, astrocytes and oligodendrocytes. NSC are regulated by signals of the microenvironment that

adjust stem cells response to the needs of the organism. Evidence accumulated during the last decade points to a possible regulatory role of ion channels over postnatal neurogenesis. A recent analysis of adult NSC transcriptome highlighted that proteins belonging to the calcium-signalling pathway are the first set of transcripts enriched in NSC. Among calcium channels, the store-operated calcium channels (SOC) have the ability to transduce extracellular signals in calcium signals. Aberrant functioning of SOC has been linked to a growing number of diseases, including brain disorders. Accordingly, we addressed the possible roles of SOC in NSC.
For these studies, we used NSC derived from two adult mice brain areas: the subventricular zone that is the major stem cell reservoir and the area postrema. We found that NSC derived from both areas express TRPC1 and Orai1, known to form SOC, as well as their activator STIM1. Calcium imaging indicated that NSC display store-operated calcium entries (SOCE). Pharmacological blockade of SOCE with SKF-96365 or YM-58483 resulted in decreased proliferation and self-renewal of NSC from both areas. As area postrema is a site of action of hormones controlling food intake, we assessed whether leptin, a hormone that controls satiety, affects NSC from this region. Interestingly, leptin decreased SOCE and reduced self-renewal of NSC derived from area postrema.
Overall, our data show that SOC are major actors in the control of NSC activities and that SOC alteration in NSC may lead to brain pathologies.

P3- THE ROLE OF P2X7 RECEPTOR IN CANCER CELL INVASIVENESS, MAMMARY TUMOUR GROWTH AND METASTATIC PROGRESSION

Lucie BRISSON[1]; Stéphanie CHADET[2]; Stéphanie LERONDEL[3]; Alain LE PAPE[3]; Isabelle COUILLIN[4]; Aurélie GOMBAULT[4]; Stéphan CHEVALIER[1]; Lin-hua JIANG[5]; Pierre BESSON[1]; Sébastien ROGER[2];

1: University of Tours, Inserm UMR1069, Tours, France
2: University of Tours, EA4245, Tours, France
3: CNRS UPS44 TAAM, Centre d'Imagerie du Petit animal, Orléans, France
4: University of Orléans, CNRS UMR7355, Orléans, France
5: University of Leeds, Leeds, United-Kingdom

ATP is the main source of free energy for cellular functions and is found at high concentrations in intracellular compartments. In physiological conditions, the extracellular concentration of ATP is very low, but is importantly increased in tumours. Extracellular ATP acts as a signalling molecule and activates P2 purinergic receptors such as the ionotropic P2X7 receptor, which is highly overexpressed in numerous tumours from epithelial origin, as in breast cancer. P2X7 (encoded by the P2RX7 gene) is highly expressed in aggressive human MDA-MB-435s and murine Balbc-derived 4T1 mammary cancer cell lines.
In these cells, we showed that P2X7 was fully functional, giving rise, under agonist (ATP or BzATP) stimulation, to inward ion currents, cytosolic increases of Ca2+, and YO-PRO1/ ethidium uptake, all of which were prevented by the use of specific pharmacological antagonists. We demonstrated that P2X7 activity induced cell morphology changes, with the formation of cellular extensions and fast F-actin reorganization, and promoted cancer cell invasiveness through extracellular matrices in vitro. Cancer cells grown on Matrigel-composed matrices formed particular structures called invadopodia, which are specific of aggressive cancer cells, enriched in F-actin, penetrating into the extracellular matrix and responsible for its proteolytic degradation. P2X7 appeared to be expressed in invadopodia, and its functioning increased invadopodial activity, with no change in the number of these structures.

In order to test the potential role of P2X7 in tumour progression, and the potential benefit of using pharmacological antagonists of P2X7 for the treatment of tumours, we used two strains of syngeneic, immunocompetent Balbc mice: wildtype (WT) p2rx7+/+ and p2rx7-/-, for the orthotopic graft of 4T1 cells. Our results showed that tumour growth was similar when WT 4T1 cells were implanted into p2rx7+/+ or p2rx7-/- mice, suggesting that expression of P2X7 by host environment do not interfere with mammary tumour progression. However, the down-regulation of P2X7 expression in 4T1 cancer cells strongly reduced mammary tumour growth and metastases development in WT mice. Furthermore, the pharmacological inhibition of P2X7 reduced primary tumour growth in WT mice, suggesting that systemic P2X7 targeting could offer new strategies to reduce tumour progression.

P4- EXPRESSION OF PORE-FORMING AND AUXILIARY SUBUNITS OF VOLTAGE-GATED SODIUM CHANNELS (NAV) IN COLORECTAL CANCER, ROLES IN PH REGULATION AND INVASIVE PROPERTIES

Lucile POISSON[1]; Osbaldo LOPEZ CHARCAS[1]; Widad LAHLOU[2]; Adrien PETEREAU[1]; Emiliane TAILLEBOIS[3]; Adélaïde DORAY[1]; Mehdi OUAISSI[4]; Sébastien ROGER[1]; Pierre BESSON[3]; Driffa MOUSSATA[2];

1: Université de Tours, EA4245 Transplantation, Immunologie et Inflammation, 10 Boulevard Tonnellé, 37032 Tours, France
2: Service d'hépato-gastroentérologie et de cancérologie digestive, hôpital Trousseau, CHRU de Tours, 37000 Tours, France
3: Inserm UMR1069, Nutrition, Croissance et Cancer, Faculté de Médecine de Tours, 10 Boulevard Tonnellé, 37032 Tours, France
4: Service de Chirurgie viscérale et oncologique, hôpital Trousseau, CHRU de Tours, 37000 Tours, France

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world. Increasing evidence suggests that ion channels, and more specifically voltage-gated sodium channels (NaV) are key contributors to the oncogenic behaviour of cancer cells. In breast cancer, the isoform NaV1.5 is abnormally expressed de novo in tumours and cancer cells. NaV1.5 contributes to the invasive potential of breast cancer cells by acidifying the extracellular pH through the allosteric activation of the Na+-H+ exchanger type 1 (NHE1) and the subsequent activation of extracellular matrix-remodelling proteases.
In this study, we assessed the expression levels of pore-forming α- and auxiliary β-subunits of NaV in different human colon cancer cell lines and colon or rectal cancer patients biopsies. In all cell lines tested, transcripts of NaV1.5 and NaV1.6 were the more abundantly expressed pore-forming isoforms. The mRNA encoding for the β1 subunit was the most expressed among all regulatory β subunits. Concerning biopsies, a trend to an overexpression of the isoforms NaV1.6 was observed in the rectal cancerous samples. A study from a retrospective cohort with clinical follow-up involving more than 100 patients is currently underway. Nav1.6 seemed to be overexpressed in left and right colon tumors, compared to matched normal tissues. The expression of β1 appeared higher in left colon and NHE1 expression seemed overexpressed in right colon tumors. NaV functionality was confirmed in SW620 and SW480 cancer cell lines by the recording, using the patch clamp technique, of TTX-inhibited fast sodium currents. Sodium currents have been also recorded on primary cultures of colonic tumors. Similarly to breast cancer, NaV channels and NHE appeared to be functionally coupled for the regulation of the intracellular pH in SW620 cells. In SW620 cancer

cells, the expression and activity of NaV1.5 seem to promote cellular invasiveness, measured in 2D through an insert covered with collagen I, or matrigel and in 3D with the realization of spheroids in matrigel. Other studies are necessary to explain the involvement and the link between NaV/NHE in the invasiveness of colonic cancer cells.

P5- A204E MUTATION IN DIS3 OF NAV1.4 EXERTS GAIN- AND LOSS-OF-FUNCTION EFFECTS THAT LEAD TO PERIODIC PARALYSIS COMBINING HYPER- WITH HYPO-KALAEMIC SIGNS

Yosuke KOKUNAI[1]; Carine DALLE[1]; Savine VICART[2]; Damien STERNBERG[2]; Valérie POULIOT[3]; Said BENDAHHOU[4]; Emmanuel FOURNIER[2]; Mohamed CHAHINE[3]; Bertrand FONTAINE [2]; Sophie NICOLE[5];

1: Inserm U1127 ; CNRS UMR 7225 ; Sorbonne Université; ICM, Paris, France
2: AP-HP, Hôpital de la Pitié-Salpétrière, Paris, France
3: 3 Centre de recherche CERVO, Institut Universitaire en Santé Mentale de Québec; Department of Medicine, Université Laval, Quebec City, QC, Canada
4: CNRS UMR7370, LP2M, Labex ICST, University Nice Sophia-Antipolis, Nice, France
5: Inserm U1127 ; CNRS UMR 7225 ; Sorbonne Université; ICM, Paris, France. Novel affiliation : Institut de Génomique Fonctionnelle, Univ Montpellier, CNRS, INSERM, LabEx ICST, Montpellier, France

Periodic paralyses (PP) are characterized by episodic muscle weakness classified into the hypokalaemic (hypoPP) and hyperkalaemic (hyperPP) forms. Dominant hyperPP is caused by overactivity of Nav1.4 — the skeletal muscle voltage-gated sodium channel — whereas hypoPP results from a gating pore current induced by mutations in Nav1.4 or Cav1.1 — the skeletal muscle voltage-gated calcium channel. Determining whether PP is hypo- or hyper-kalaemic is clinically important. We recently reported two individuals who suffered from an unusual phenotype of PP combining hyperkalaemic and hypokalaemic episodes of muscle paralysis due to one Nav1.4 mutation (p.Arg1451Leu, DIVS4). Here, we report a third individual with hyperPP and hypokalaemic episodes of muscle paralysis who was heterozygous for p.Ala204Glu (DIS3) in Nav1.4. A204E induced a significant decrease of current density, increased the window current, enhanced fast and slow inactivation, and did not cause gating pore current in functional analyses done in h cells heterologous cells. Interestingly, the negative impact of A204E on activation was strengthened in low extracellular K+. Our data prove the existence of a phenotype combining hyperPP and hypoPP due to Nav1.4 mutations. We propose that the hyperPP component results from gain-of-function effects and the hypoPP one from loss-of-function effects strengthened by low K+ of A204E.

P6- PHENOTYPIC CHARACTERIZATION OF A ZEBRAFISH MODEL FOR THE IHPRF1 SYNDROME, A RARE GENETIC DISEASE LINKED TO LOSS-OF-FUNCTION MUTATIONS IN NALCN CHANNEL

Zoé SERVANT; Andie GODO; Adèle FAUCHERRE; Chris JOPLING; Philippe LORY; Arnaud MONTEIL;

IGF Montpellier

Infantile Hypotonia with Psychomotor retardation and characteristic facies of type 1 (IHPRF1, OMIM #615419) is a complex neurodevelopmental disorder characterized by a large panel of symptoms including hypotonia, developmental delay, large cognitive and locomotor deficits, disturbances of vital functions, deformities, brain atrophy, cerebellar degeneration, epileptic seizures and premature death. IHPRF1 is linked to recessive loss-of-function mutations of the sodium-leak channel NALCN. NALCN is expressed in several cell types including neurons, endocrine/neuroendocrine cells, and intestitial cells of Cajal where it contributes to setting the resting membrane potential and thereby regulates cellular excitability. Our goal is to establish a zebrafish model of the IHPRF1 syndrome in order to precisely investigate the physiological defects in this disease. First, in situ hybridization experiments demonstrate that the expression pattern (mRNA) of NALCN and its ancillary subunits is reminiscent in zebrafish to what has been described in humans and rodents. Then, in order to mimic the Q642x mutation described in 2 IHPRF siblings, we used the CRISPR-Cas9 approach to introduce a nonsense mutation in the intracellular loop between the transmembrane domains II and III of zf-NALCN. Our results show that homozygous mutant animals at the larval stage exhibit a reduced viability, an altered basal and evoked locomotor activity, as well as a propensity to develop dysmorphic morphologies. We also found that adult heterozygous animals become dysmorphic and dramatically loose weight starting at 6 months post-fertilisation. Taken together, our data validate our zebrafish mutants as a relevant model to study NALCN channel and to investigate the pathogenic mechanisms involved in IHPRF1. This zebrafish IHPRF1 model should be valuable to evaluate drug-based therapeutic approaches to correct IHPRF1 syndrome.

P7- THE ROLE OF THE P2X4 RECEPTOR IN BREAST CANCER

Stéphanie CHADET[1]; Lucie BRISSON[2]; Stéphanie LERONDEL[3]; Roseline GUIBON[2]; Gaëlle FROMONT[2]; Alain LE PAPE[3]; Sébastien ROGER[1]; Ruth MURRELL-LAGNADO[4];

1: EA4245 Université de Tours France
2: INSERM UMR U1069 Université de Tours France
3: CNRS UPS44 Orléans France
4: University of Sussex Brighton UK

Purinergic signalling is associated with cancer cell invasiveness and metastasis formation with P2X receptors involved on the side of the host immune cells as well as the tumour. The unusually high levels of extracellular ATP within the tumour microenvironment activate the low affinity P2X7 receptor to promote both the growth and metastatic potential of breast cancers. P2X4 is commonly co-expressed with P2X7 and their synergistic activity is often questioned. However, unlike P2X7, P2X4 is targeted to endolysosomes. In this study we assessed the role of P2X4 in mammary tumour growth and metastases, and its functional association with P2X7. At the transcript level, P2X4 is over-expressed in human breast cancers compared to normal breast tissue. We showed by immunohistochemistry, elevated P2X4 expression in >50% of human breast cancer samples compared to normal breast tissue. A combination of in vitro and in vivo approaches was carried out, utilizing highly invasive human MDA-MB-435s and murine 4T1 mammary cancer cell lines, both endogenously expressing P2X4 and P2X7. Western blot and immunocytochemistry analyses showed expression of P2X4 and its targeting to lysosomes. Knocking down the expression of P2X4 using the CRISPR/Cas9 system reduced basal invasive

capacities of cells through Matrigel-coated inserts by 50%. It also inhibited the 2-fold enhancement of invasion produced by BzATP and the secretion of cathepsin D, suggesting a role for P2X4 in lysosome exocytosis triggered by P2X7 stimulation. A comparison was made of tumour growth and metastases in BALB/c mice following implantation of either P2X4-CRISPR or CTL-CRISPR cells into mammary fat pads. For two clones of the P2X4-CRISPR cells, tumour growth was inhibited by 85.5% and 91.5% compared to control cells, and metastases development was prevented. In conclusion, our results show a prominent role of P2X4 in cancer cell invasiveness, tumour growth and metastases, which could be potentiated by P2X7 stimulation.

P8- STRETCH-ACTIVATED PIEZO1 CHANNEL IN ENDOTHELIAL CELLS RELAXES MOUSE INTRAPULMONARY ARTERIES

A LHOMME[1]; G GILBERT[1]; J DEWEIRDT[1]; D HENRION[2]; C GUIBERT[1]; I BAUDRIMONT[1]; T DUCRET[1]; Jf QUIGNARD[1];

1: CRCTB INSERM U1045 PTIB Hopital xavier Arnosan 33600 talence 2: MITOVASC Institut, UMR CNRS 6015, INSERM U1083, Université d'Angers, Angers

In intrapulmonary arteries (IPA), endothelial cells (EC) respond to mechanical stimuli by releasing vasoactive factors to set the vascular tone. Piezo1, a stretch-activated, calcium-permeable channel, is a sensor of mechanical stress in EC. The present study was undertaken to investigate the implication of Piezo1 in the endothelium-dependent regulation of IPA tone and potential involvement of Piezo1 in pulmonary hypertension, the main disease of this circulation. IPA tone was quantified by means of a myograph in control Piezo1+/+ mice and in mice lacking endothelial Piezo1 (EC-Piezo1-/-). Endothelial intracellular calcium concentration ([Ca2+]i) and nitric oxide (NO) production were measured, in mouse or human EC, with Fluo-4 or DAF-FM probe, respectively. Immunofluorescent labeling and patch-clamp experiments revealed the presence of Piezo1 channels in EC. Yoda1, a Piezo1 agonist, induced an endothelium-dependent relaxation that was significantly reduced in pulmonary arteries in EC-Piezo1-/- compared with Piezo1+/+ mice. Yoda1 as well as mechanical stimulation (by osmotic stress) increased [Ca2+]i in mouse or human EC. Consequently, both stimuli increased the production of NO. NO and [Ca2+]i increases were reduced in EC from Piezo1-/- mice or in the presence of Piezo1 inhibitors. Furthermore, deletion of Piezo1 increased α-adrenergic agonist-mediated contraction. Finally, in chronically hypoxic mice, a model of pulmonary hypertension, Piezo1 still mediated arterial relaxation, and deletion of this channel did not impair the development of the disease. The present study thus demonstrates that endothelial Piezo1 contributes to intrapulmonary vascular relaxation by controlling endothelial [Ca2+]i and NO production and that this effect is still present in pulmonary hypertension.

P9- ROLE OF TRPV6 CHANNEL IN MIGRATION AND INVASION OF CANCER CELL LINES

Aline GHESQUIER[1]; Aurélien HAUSTRATE[1]; Corentin SPRIET[2]; V'yacheslav LEHEN'KYI[1]; Natalia PREVARSKAYA[1];

1: Bâtiment SN3, 2ème étage, Cité Scientifique
2: Bâtiment C9, 1er étage, Cité Scientifique

TRPV6 channel belongs to the superfamily of transient receptor potential (TRP) channels, subfamily vanilloid, member 6. Among all TRP channels TRPV6 is highly Ca2+ selective, and such high Ca2+ selectivity is unique within the TRP superfamily and makes this channel quite distinguishable, especially in Ca2+-related intracellular pathways.
A correlation between TRPV6 channel expression and Gleason score of PCa has been previously demonstrated. By modulating calcium homeostasis, this highly selective calcium channel plays a role in tumorigenesis process. Despite the discovery of its crucial role in cancer cell proliferation and survival in vitro, nothing is known yet as to its role in prostate cancer cell migration and invasion. The first data demonstrate its direct action in the formation of osteoblastic lesions. Nevertheless, the inherent mechanisms of this channel remain to be revealed, and more precisely its role in the invasion and the migration of the prostate cancer cells.
To address this issue we established for our experiments, PC-3Mtrpv6-/- and PC-3Mtrpv6+/+ cell lines (prostate cancer cells) and HAP1trpv6-/- and HAP1trpv6+/+ (leukemia cell line). On the other hand, stable clones HAP1trpv6-/- containing either wild-type TRPV6 (pTRPV6wt (mCherry)), or the mutated pore channel (pTRPV6D541A (mCherry)), or the mCherry vector alone (pmCherry) were generated. The migration and invasion capacities of these differents cell lines and clones were studied using wound healing assay, Boyden chamber assay and by video microscopy. The calcium-dependent mechanisms involved were studied by Western-Blot. In video microscopy, PC-3Mtrpv6-/- cells migrated significantly slower than PC-3Mtrpv6+/+ cells. Wound healing assays and Boyden chamber assays show that functional TRPV6 channel expression promotes migration and invasion.
This TRPV6 involvement in the migration and the cellular invasion could be explained by the establishment of an epithelial–mesenchymal transition (EMT) and Matrix MetalloProteinase 9 (MMP9) production.
Thus, these data show that TRPV6 plays a canonical role in the migration and invasion capacities of prostatic cancer cells.

P10- ENHANCED CALCIUM CONSTITUTIVE ENTRIES THROUGH OPTOGENETICALLY DRIVED MEMBRANE HYPERPOLARIZATION : IMPACT ON C2C12 MYOBLAST BEHAVIOR.

Guénaëlle LIZOT*[1]; Charles-albert CHAPOTTE-BALDACCI*[1]; Cyrielle JAJKIEWICZ[1]; Christophe MAGAUD[1]; Vincent THOREAU[2]; Aubin PENNA[1]; Patrick BOIS[1]; Stephane SEBILLE[1]; Aurélien CHATELIER[1];

1: STIM laboratory, Université de Poitiers, Pôle Biologie Santé, 1 Rue Georges Bonnet, TSA 51106 86073 POITIERS Cedex 9 France
2: EA3808 NEUVACOD, Université de Poitiers, Pôle Biologie Santé, 1 Rue Georges Bonnet, TSA 51106 86073 POITIERS Cedex 9 France
*Equivalent contributors

Adult skeletal muscle growth and regeneration are guaranteed by satellite cells, which constitute a pool of muscle stem cells located between sarcolemma and basal lamina. Upon activation, these cells proliferate as myoblasts that migrate and fuse together or with preexisting muscle fibers, resulting in muscle regeneration. Membrane potential and calcium (Ca2+) homeostasis have been shown to regulate many cellular processes, including proliferation, migration and fusion. For example, myoblast fusion is a Ca2+-dependent mechanism, which requires the hyperpolarization of the cell membrane. In human myoblasts, this hyperpolarization results from the expression of potassium channels and mediates calcium entry through voltage-dependent Ca2+ channels. In addition, calcium entries mediated by TRP channels are also supposed to play an important role in myoblast proliferation, migration and differentiation processes.
In this context, we took advantage of optogenetics to mediate hyperpolarization upon light stimulation of C2C12 myoblasts expressing the light-sensitive chloride pump, halorhodopsin (eNpHR). The objective was to assess whether these membrane potential variations impact calcium constitutive entries, calcium homeostasis and cellular properties such as proliferation, migration and fusion.
Patch-clamp experiments demonstrated that light stimulation is sufficient to mediate significant hyperpolarization of eNpHR expressing C2C12 myoblasts. Furthermore, Ca2+ imaging showed that light stimulation of these cells was also accompanied by an increase in intracellular Ca2+, dependent on the presence of external Ca2+ and blocked by Tranilast, a TRPV2 inhibitor. Therefore, the aim of this study is to understand the consequences of membrane potential and Ca2+ homeostasis modulation on proliferation, migration and fusion of myoblasts.

P11- ACCELERATED CURRENT DECAY KINETICS OF A RARE HUMAN ACID-SENSING ION CHANNEL 1A VARIANT THAT IS USED IN MANY STUDIES AS WILD TYPE

Anand VAITHIA; Sabrina VULLO; Zhong PENG; Omar ALIJEVIC; Stephan KELLNEBERGER;

Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland

Acid-sensing ion channels (ASICs) are sodium-selective and voltage-insensitive ion channels which are localized both in the peripheral and the central nervous system. As the name implies, ASICs are activated by extracellular acidification. They are involved in learning and memory formation, pain sensation, fear conditioning, mechanosensation and neurodegeneration after ischemic injury. Our laboratory has been investigating the structure-function relationship of human ASIC1a for several years. The hASIC1a clone used by us and by many other laboratories had been cloned decades ago. We found recently that this clone contains a mutation of the highly conserved Gly residue at position 212 to Asp. This mutation is extremely rare. Therefore, our aim was to verify whether previous findings obtained with the hASIC1a mutant (D212) remain valid. The functional analyses showed that the pH dependence of activation and desensitization, as well as the concentration dependence of well-known ASICs modulators and inhibitors such as GMQ and amiloride, the toxin peptides Psalmotoxin 1 and Mambalgin-1, were very similar between the two channel types. In contrast, we observed a higher cell surface channel expression and current amplitudes, and slower current decay kinetics in hASIC1a WT (G212) in comparison to hASIC1a mutant (D212). We also performed here, in the hASIC1a (G212) background, key experiments of previous structure-function studies of our laboratory

that had been carried out in the background of hASIC1a (D212). These control experiments demonstrated very similar effects of mutations in hASIC1a WT (G212). In conclusion, our experimental findings show that the hASIC1a WT (G212) and mutant hASIC1a D212 are similar in their pharmacological and biophysical properties, except for the cell surface channel expression and current decay kinetics. We further validate structure-function experiments that were carried out in the hASIC1a (D212) background. In spite of the similarity between the two clones, it is important that researchers using hASIC1a switch now to the real hASIC1a wild type.

P12- HONEYBEE CAV4 CHANNEL : A NEW TYPE OF CA2+ CHANNEL

Anaïs BERTAUD[1]; Olivier THERIAULT[2]; Claudine MENARD[1]; Thierry CENS[1]; Matthieu ROUSSET[1]; Mohamed CHAHINE[2]; Pierre CHARNET[1];

1: IBMM, UMR 5247, Montpellier France
2: CERVO, Univ Laval, Québec Canada

Neurotoxic products used in plant protection or veterinary pharmacy are often derived from a limited number of molecules of biological origin (nicotine, pyrethroids, toxins from spider venoms…), and selected for their ability to control pests while being safe for mammals. These molecules mainly target ion channels involved in the propagation of axonal signal in synaptic transmission. Pyrethroids which affect voltage–gated Na (NaV) channels are one of the most widely used insecticides. In honeybee, 2 genes with homology with the Drosophila NaV channel para have been isolated: Am-para (NaV1) and NaV2.
We have cloned and characterized the honeybee NaV2 channel and demonstrated a strong Ca2+ permeation. NaV2 appears to be a member of a new class of CaV channel and may rather be named CaV4. Sequence analysis suggests that the structural parts involved in inactivation of this new CaV channel are similar to those involved in NaV channel inactivation : they should be located within the loop between domains III and IV.
In this work, we present a detailed analysis of CaV4 channel permeation and inactivation in the presence of Ca2+ , Ba2+ and Na+. We demonstrate that CaV4 is permeable to Ca2+ and Ba2+ but not Na+, and displays an unusual Ca2+-dependent inactivation. The role of the connecting loop between domains III and IV and of the C-terminal region in channel inactivation has been analyzed by site-directed mutagenesis. The role of calmodulin has been tested by coexpression of a mutant calmodulin. Altogether our data suggest that CaV4 somehow fills the gap between NaV and CaV channels, displaying the selectivity of a CaV channel but the inactivation mechanism of a NaV channel.

P13- ACTIVATION OF NAV CHANNELS WITH THE NEUROTOXIN, VERATRIDINE INDUCES VASORELAXATION MEDIATED BY NO-PATHWAY OF MURINE MESENTERIC ARTERIES.

Joohee PARK; Claire LEGENDRE; Coralyne PROUX; Daniel HENRION; Christian LEGROS;

MitoVasc Laboratory, Angers, France

Blood vessel cells express voltage-gated Na+ channels (Nav channels) and their activation induces a Ca2+ response mediated by Na+-Ca2+ exchangers (NCX) in Ca2+ entry mode (Boccara et al., 1999; Figueroa et al., 2007; Andrikopoulos et al., 2011; Ho et al., 2013). Nevertheless, the role of Nav channels in vascular function (VF) still needs to be clarified. The aim of our study was to identify the Nav channel subtypes in resistance artery from mice and to define their implication in VF by physiological and pharmacological approaches. To this end, we used mesenteric arteries (MA), as a suitable model of resistance artery from 5-month-old male and female mice. Our RT-qPCR results showed the expression of three transcripts encoding Nav1.2 (scn2a), Nav1.3 (scn3a) and Nav1.5 (scn5a) in MA from male and female mice. The presence of Nav channels in these arteries were confirmed by histoimmunostaining. We showed by wire myography that the activation of tetrodotoxin-sensitive Nav channel by veratridine (VTD) induced a vasorelaxation in both genders. This VTD-induced vasorelaxation was totally abolished by L-NNA, a NO synthase inhibitor, indicating that the NO pathway is involved in this response. We also investigated the implication of NCX in VTD-induced vasorexalation response. We established the gene expression profile of NCX in murine MA by RT-qPCR, revealing the detection of slc8a1 and slc8a2, encoding NCX1 and NCX2. In presence of the NCX inhibitor, KB-R7943, the relaxation induced by VTD is almost abolished. Altogether, our data highlight for the first time the role of Nav channels in vasorelaxation response in murine MA. The activation of Nav channels induces Na+ entry and subsequent membrane depolarization, which trigger Ca2+ entry through NCX. This possible Nav channels-NCX cross-talk might be an important feature of the link between Na+ and Ca2+ homeostasis in vascular cells. Further experiments will be necessary to define the role of each Nav channel subtype in VTD-induced vasorelaxation and to characterize the cellular pathway of this response evoked by Na+ entry.

P14- REGULATION OF TRANSMEMBRANE WATER TRANSPORT BY THE CFTR CHLORIDE CHANNEL.

Jodie LLINARES; Anne CANTEREAU; Frederic BECQ;

Laboratoire STIM, Université de Poitiers, France

The CFTR chloride channel is a key regulator of fluid secretion in airway epithelia. Impairment of CFTR activity can lead to dehydration and production of viscous mucus in Cystic Fibrosis patients. Thus, the physiological regulation of water and ion transport in the respiratory tract is crucial. Water flow across cell membranes following osmotic gradient is facilitated by water-selective channels called aquaporins (AQP). Previous studies reported a functional interaction between the AQP3 isoform and CFTR however this regulation remains poorly understood.
We used the Quantitative Phase Imaging (QPI), a technique based on the light wave shift when passing through living cells, to record transmembrane water flow. Stimulation of the cAMP-pathway induced an Optical Path Difference (OPD) increase

in CHO cells overexpressing wt-CFTR, corresponding to a water efflux. This cAMP-dependent response was also observed in mutated CHO G551D although the OPD increase was significantly smaller than for CHO wt-CFTR. Potentiation of the channel activity by VX770 did not further stimulate the water efflux. In CHO F508del cells, the cAMP-dependent water efflux was not significantly different from the response observed in non-expressing CHO cells (CHO K1). However, reinsertion of the F508del CFTR channels at the plasma membrane with the corrector VX809 significantly increased the cAMP-dependent water efflux. But VX770 did not enhance the OPD response of VX809-corrected F508del cells. The selective inhibitors CFTR-inh172 and GlyH101 did not inhibit the OPD response suggesting that CFTR does not directly conduct water. On the other hand, the broad-spectrum aquaporins inhibitor, HgCl2, abolished the cAMP-dependent OPD response in all cell types tested including CHO-K1. Whole-cell patch clamp data confirmed no inhibition of CFTR currents by HgCl2. Taken together, our results show that CFTR at the plasma membrane is necessary to potentiate a cAMP-dependent water efflux mediated by endogenous aquaporins but independently of the CFTR activity. In CF cells cAMP-dependent water efflux is abnormal but can be restored by re-addressing F508del-CFTR at the plasma membrane. This suggests that the corrected cAMP-dependent water efflux might improve the hydration of airways of CF patients.
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P15- TRP EXPRESSION SIGNATURE IN TUMOR-DERIVED ENDOTHELIAL CELLS: FUNCTIONAL ROLES IN PROSTATE CANCER ANGIOGENESIS

Michela BERNARDINI[1]; Giorgia CHINIGò[2]; Alessia BROSSA[3]; Guillaume GROLEZ[4]; Virginie MATTOT[5]; Gaelle FROMONT-HANKARD[6]; Luca MUNARON[7]; Natalia PREVARSKAYA[8]; Alessandra FIORIO PLA[2]; Dimitra GKIKA[8];

1: Department of Life Science and Systems Biology, University of Torino, Turin, Italy
2: Department of Life Science and Systems Biology, University of Torino, Turin, Italy
3: Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Centre, University of Torino, Turin, Italy
4: Inserm U1003, Université Lille 1,Villeneuve d'Ascq, France
5: Institute de Biologiede Lille, Lille, France
6: Inserm UMR 1069, Université de Tours, Tours, France.
7: Department of Life Science and Systems Biology, University of Torino, Turin, Italy
8: Inserm U1003, Université Lille 1,Villeneuve d'Ascq, France

Introduction: TRP channels play a key role in cancer progression, modulating cell proliferation and survival, cancer invasion of surrounding tissues and angiogenesis. TRP expression could therefore characterize the prostate cancer (PCa) cell phenotype. Another well-established concept is that TRPs deeply modulate endothelial cell (EC) biology and tumor angiogenesis. However, a specific TRP expression signature of PCa angiogenesis is still lacking. Our aim was therefore to define a TRP expression signature during PCa angiogenesis providing novel therapeutic targets.

Methods: By means of a qPCR screening and Western blotting, we fully profiled the expression of all TRPs in normal ECs and tumor endothelial cells (TECs) derived from PCa, as well as from breast and renal tumors. TRP channel function on TEC was analyzed by Ca2+ imaging and compared with healthy EC. Moreover, we characterized the role of the 'prostate specific' TRPs in the modulation of EC biological

processes such as cell proliferation, motility and ability to form tubules in vitro, as well as in vivo angiogenesis.

Results: We identified three 'prostate-associated' genes whose expression is upregulated in prostate TECs: TRPV2 as a positive modulator of TEC proliferation, TRPC3 as an endothelial PCa cell attraction factor and TRPA1 as a critical TEC angiogenic factor in vitro and in vivo.

Conclusions: We provide here the full TRP signature of PCa vascularization among which three play a profound effect on EC biology. These results contribute to explain the aggressive phenotype previously observed in PTEC and provide new putative therapeutic targets.

P16- GENETIC ABLATION OF G PROTEIN-GATED INWARDLY RECTIFYING K+ (GIRK)4 CHANNELS PREVENTS HEART RATE REDUCTION INDUCED BY INTENSIVE EXERCISE TRAINING

Isabelle BIDAUD[1]; Alicia D'SOUZA[2]; Denis GREUET[1]; Antony CHUNG YOU CHONG[1]; Angelo TORRENTE[1]; Julien ROUSSEL[1]; Mark BOYETT[2]; Matteo MANGONI[1]; Pietro MESIRCA[1];

1: Département de Physiologie, Institut de Genomique Fonctionnelle, UMR-5203 - INSERM U661 - CNRSUniversité de Montpellier, 34094 Montpellier, France 2: Institute of Cardiovascular Sciences, University of Manchester, Manchester M13 9NT, United Kingdom

Background: Athletes are considered the healthiest members of society and, paradoxically, the incidence of arrhythmias, ranging from the benign to the pathological, is known to be higher in athletes. In rodent models of exercise training, it has been demonstrated that training-induced bradycardia results from downregulation of hyperpolarization-activated cyclic nucleotide-gated 4 (f-HCN4) channels leading to a reduction of the "funny" (If) current in the heart sino-atrial node (SAN).

Objectives: To test if genetic ablation of G-protein-gated inwardly rectifying potassium 4 (Girk4) channel prevents sinus bradycardia induced by intensive exercise training in mice.

Methods: Control (WT) and Girk4 knock-out (Girk4KO) mice were separated in two different groups: trained and sedentary. Mice in trained group swam for 28days, 1 h twice a day, 7days per week. In sedentary group, mice went in the water as frequent as trained mice but only for 5min. We performed telemetric electrocardiogram recordings in both groups during 12h-night time before and during the entire training period. At the end of training protocol, mice were euthanized and SAN tissues were isolated. Half were dissociated into single cells by enzymatic digestion for patch clamp experiments and the rest was frozen for transcriptomic test.

Results: Heart rate (HR) reduction in trained WT mice became significant at day 17 of swimming (550±7bpm vs 522±4bpm*, day0 and day17, respectively). We did not record differences in HR all along the training period in sedentary WT or in Girk4KO mice (both sedentary and trained). In line with heart rate reduction, current clamp recordings in isolated SAN cells from trained WT mice showed lower rates of spontaneous action potentials in comparison to SAN cells from animals of the other groups (146±7bpm WT-trained vs 218±12bpm WT-sedentary**, 235±33bpm Girk4KO-

sedentary* and 240±14bpm Girk4KO-trained****). Unlike in SAN myocytes collected from WT-trained, GirkKO-sedentary and -trained animals If current density was statistically reduced in WT-trained SAN cells. Quantitative PCR confirmed that HCN4 mRNA expression was lower in WT-trained SAN tissues than in SAN collected from others animals.

Conclusion Genetic ablation of Girk4 channels prevents sinus bradycardia induced by downregulation of f-HCN4 channels in trained WT mice.

P17- TRPV1 INHIBITION SUPPORTS CELL SURVIVAL DURING HYPOXIA/REOXYGENATION IN THE RAT CARDIOMYOBLAST H9C2 CELL LINE

Nolwenn TESSIER[1]; Mallory DUCROZET[2]; Sally BADAWI[3]; Christophe CHOUABE[4]; Claire CROLA DA SILVA[5]; Gabriel BIDAUX[6]; Michel OVIZE[7]; Fabien VAN COPPENOLLE[8]; Sylvie DUCREUX[9];

1: nolwenn.tessier@univ-lyon1.fr
2: mallory.ducrozet@univ-lyon1.fr
3: sally.badawi@univ-lyon1.fr
4: christophe.chouabe@univ-lyon1.fr
5: claire.crola-da-silva@univ-lyon1.fr
6: gabriel.bidaux@univ-lyon1.fr
7: michel.ovize@chu-lyon.fr
8: fabien.van-coppenolle@univ-lyon1.fr
9: sylvie.ducreux@univ-lyon1.fr

Introduction: Transient receptor potential vanilloid 1 (TRPV1) is a nonselective ion channel that gated calcium (Ca2+). Over the last decade, several TRPV1-targeted strategies based on remote, pre- and post-conditioning in order to decrease infarct size have been successful. In fact, during myocardial infarction, Ca2+ dysregulation occurs leading to cell death and causing irreversible myocardial injury. TRPV1 modulation would counteract these deadly effects. Objective: The purpose of this study is to analyze the TRPV1-dependent mecanisms in cell survival during hypoxia-reoxygenation (H/R). Here, we used H9C2 cell line as an alternative model to adult cardiomyocytes in order to perform live imaging with genetic probes. Method and results: First, using RT-PCR, Western blot and confocal microscopy, we demonstrated that TRPV1 is expressed in H9C2 and seems to be localized at endoplasmic reticular (ER) membrane. Secondly, thanks to cytosolic and reticular Ca2+ imaging (respectively with Fura-2 and ErGAP1), we showed that TRPV1 is a functional ER Ca2+ leak channel. Since ATP synthesis and cell fate are dependent on Ca2+ exchanges between ER and mitochondria, we then analyzed the role of TRPV1 on the mitochondrial [Ca2+] using R-GECO probe. We observed that pharmacological TRPV1 modulation increases both cytosolic and mitochondrial Ca2+ contents by at least 20%. Finally, cell death was evaluated by flow cytometry after H/R sequences. In particular, we showed that TRPV1 inhibition improves cell survival (at least by 22%). Conclusion: Many physiological processes (including cell death/survival balance) rely on precise and spatiotemporal changes in the intracellular Ca2+ concentration. In this study, we show: (1) that TRPV1 could precisely regulates Ca2+ signals in a localization-specific way, (i.e. between ER and mitochondria) and (2) that H9C2 is a valuable model to evaluate the role of TRPV1 in Ca2+ fluxes during H/R.

P18- CONTRIBUTION OF A SODIUM-LEAK CONDUCTANCE TO THE ELECTRICAL ACTIVITY OF MOUSE CHROMAFFIN CELLS: NALCN OR NOT NALCN?

Alexandre MILMAN[1]; Stéphanie VENTEO[2]; Arnaud MONTEIL[1]; Philippe LORY[1]; Nathalie c. GUERINEAU[1];

1: Institute of Functional Genomics, Montpellier; LabEx "Ion Channel Science and Therapeutics", Montpellier
2: Institute of Neurosciences, Montpellier

Regulation of cell excitability is a crucial process whereby cells ensure their physiological function. Adrenal chromaffin cells are excitable neuroendocrine cells responsible for the secretion of catecholamines (epinephrine and norepinephrine), which are the first hormones released into the blood in response to stress. Catecholamine secretion is allowed by a tightly coordinated sequence of cellular and tissular mechanisms ("stimulus-secretion coupling"), in which chromaffin cell excitability plays a crucial role. Elucidating the mechanisms regulating the firing discharge is therefore of interest. While the ion channels involved in action potential generation are well-described, the conductances operating near the resting membrane potentials are less known. The recent discovery of the sodium leak channel (NALCN) and its contribution to set the resting membrane potential in neuronal cells prompted us to investigate whether NALCN could participate to chromaffin cell excitability. We describe here, in mouse adrenal acute slices, a background sodium-permeable conductance active around the resting potential. Indeed, reducing external [Na+] from 125 to 15 mM leads to a robust membrane hyperpolarization, abrogating thus action potentials. Neither TTX application nor intracellular Cs+ impairs the hyperpolarization. Depolarizing voltage ramps show that lowering external [Na+] blocks a current in a linear manner between -130 and -50 mV. Calculated reversal potential argues for a non-selective conductance and permeability challenges unveil a PNa/PK ratio of 2.9. Collectively, these results contribute to the first evidence for a background conductance, mainly permeable to [Na+], in modulating chromaffin cell excitability in the mouse adrenal medulla. Interestingly, our data match with attributes to NALCN. In addition, the selective expression of NALCN mRNA detected by in situ hybridization in mouse chromaffin cells consistently argues for a possible involvement of this channel in regulating chromaffin cell excitability. Even though our data are consonant with features of NALCN current, the precise contribution of NALCN channels to chromaffin cell excitability remains to be ascertained. Because of the lack of selective inhibitors for NALCN channels, it will be necessary to manipulate NALCN gene expression, in the adrenal medulla in vivo, to decipher the role of NALCN in chromaffin cells.

P19- CHARACTERISATION OF A NEW MOUSE LINE TO INVESTIGATE THE ROLE OF AMPAR MOBILITY IN SYNAPTIC TRANSMISSION PROPERTIES

Hanna ZIEGER; Eric HOSY; Daniel CHOQUET;

CNRS, Interdisciplinary Institute for Neuroscience, UMR 5297, 33000 Bordeaux, France

Lateral diffusion of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) at the surface of the post-synapse is an important mechanism involved in

both short term synaptic plasticity by favouring the replacement of desensitised receptors, and long term synaptic plasticity by providing new exocytosed receptors to their trapping sites. Impairment of AMPAR diffusion by various surface cross-linking techniques has been reported as affecting for example the early phase of long term potentiation (eLTP) and contextual learning. Here we report the development of a knock-in mouse presenting a small acceptor peptide (AP) tag at the extracellular part of the receptor. This tag can be endogenously biotinylated when a biotin ligase retained in the endoplasmic reticulum (ER) is expressed by viral infection. Local application of neutravidin is able to crosslink AMPARs both in cell culture, brain slices and even in vivo.
Our aim is to investigate how crosslink, in our model, impairs the basic properties of synaptic transmission and AMPAR nanoscale organization. Using direct STochastic Optical Reconstruction Microscopy (d-STORM) we determined changes in cluster size and nano-organisation of AMPARs, and lateral mobility of single receptors is assessed by universal Point Accumulation In Nanoscale Topography (u-PAINT) technique. In order to further characterise this mouse line we will also carry out electrophysiology experiments to explore the effects of AMPAR immobilisation on short term potentiation (STP) as well as on long term potentiation (LTP).

P20- EVIDENCE FOR A FAST NON-SELECTIVE LOW VOLTAGE-ACTIVATED CATIONIC CHANNEL IN RAT PULMONARY VEIN CARDIOMYOCYTES

Claire o MALECOT;

Lab Signalisation et Transports Ioniques Membranaires, EA7349, Faculté des Sciences, Parc de Grandmont, F-37200 TOURS

Ectopic foci in pulmonary veins (PV) myocardial sleeves are involved in the onset of atrial fibrillation. Although T-type Ca channels exist in rat PV cardiomyocytes (CM), we found an additional fast low voltage-activated (LVA) Ca current, susceptible to trigger ectopic foci.
PVCM were enzymatically isolated from the rat main PV. Whole-cell ICa (2 or 5 mM extracellular Ca) was recorded by voltage steps from -100mV with classical Na- and K-free bath solutions. A fast LVA ICa (FLVA-ICa) was observed with voltage steps to between ~-55 to ~-30 to -20 mV in 53% of PVCM. Increasing bath Ca concentration from 2 to 5 mM almost doubled its amplitude (from -0.58±0.07, n=63, to -1.55±0.20 pA/pF, n=45) as well its ratio to maximum peak ICaL (from 13.5% to 26%) recorded at either +15 or +20 mV, respectively. FLVA-ICa was blocked by 10 µM TTX, suggesting it corresponds to the previously reported ICa(TTX). However, FLVA-ICa was markedly increased by bath addition of not only NaCl (1 or 3 mM), but also KCl (5 or 10 mM). Permeability ratios P'Ca/PNa and P'Ca/PK calculated with a modified GHK equation for bi-ionic conditions were respectively 2.25±0.51 (n=6) and 1.88±0.25 (n=14), and not different from a value of 2. Norepinephrine (10 µM, n=13) increased FLVA-ICa and negatively shifted its activation threshold, an effect partially reversed by 5 µM Atenolol. It was partially blocked by 5 µM Nifedipine (n=5) and increased by 300 nM BayK8644 (n=6), but not blocked by Ranolazine (10 µM, n=6), NiCl2 (40 µM, n=9) or TTA-A2 (10 or100 nM, n=11). Neither Ba nor Sr alone could permeate the FLVA channel or block Ca influx.
Our results suggest that the FLVA channel is neither a Na channel (because of its permeability to K) nor a Ca channel (lack of Ba or Sr permeation) but more likely a fast voltage-gated non-selective cationic channel with a dihydropyridine binding site and unique properties.

P21- NOVEL REGULATION OF GIRK POTASSIUM CHANNELS BY δ-OPIOID RECEPTORS

Ana sofia ERIA OLIVEIRA; K. HASPEL; K. LANGER; J. REVILLOUD; M. FOLACCI; M. VIVAUDOU ;

Channels, IBS

G protein-gated potassium channels (GIRK or Kir3 channels) exist in most excitable cells where they regulate cellular excitability under the control of G Protein-Coupled Receptors (GPCR). That is, GIRK channels are activated by direct binding of the Gβγ subunits that are released upon GPCR activation. Remarkably, opioid receptors belong to the GPCR family and interact with GIRK channels to modulate pain perception.
We have studied the regulation of GIRK channels by opioid GPCRs by coexpressing them in Xenopus oocytes and measuring channel activity using electrophysiological techniques.
We discovered that opioid agonists SNC80, DALE and DADLE, acting through δ-opioid receptors, activate GIRK channels at nM concentrations but inhibit them at higher concentrations. Notably, inhibition of the GIRK channels was only observed at high levels of expression of the δ-opioid receptor. This observation suggests that inhibition is dependent on receptor density, possibly by impacting receptor oligomerization.
While activation of the channels followed the classical Gβγ pathway and was consequently blocked by pertussis toxin, inhibition remained unaffected, disclosing that these are two independent pathways. Applying the Gq blocker YM-254890 did not affect inhibition, suggesting that it is not Gq-dependent either.
Control experiments performed with the closely related μ-opioid receptor in the same conditions did not reveal any sign of inhibition. Inhibition appears to be specific to δ-opioid receptors. However, by constructing a chimeric protein, we were able to confer inhibitory capability to the μ-opioid receptor by substituting its intracellular loops by those of the δ-opioid receptor. By further exploring the chimeric approach, we intend to pinpoint the residues of the δ-opioid receptor that are involved in the inhibition, which in turn should allows us to investigate and identify the pathway involved.
Our data therefore disclose an unreported phenomenon specific to the δ-opioid receptors where receptor monomers and oligomers potentially activate different signalling pathways. These observations uncover a novel, complex regulation with mechanistic and physiological implications that remain to be fully elucidated.

P22- N-3,4-DIMETHOXYCINAMOYL ANTHRANILIC ACID (TRANILAST) VALIDATION AND OPTIMIZATION AS PHARMACOLOGICAL TOOL TO TARGET TRPV2 PRO-METASTATIC ACTION.

Christine BARRAULT[1]; Samia ACI-SECHE[2]; Paul BUSCAGLIA[3]; Stéphane DAYOT[4]; Victoire PATEAU[4]; Olivier MIGNEN[3]; Pascal BONNET[2]; François CARREAUX[5]; Aubin PENNA[1];

1: 4CS team, STIM laboratory, CNRS ERL 7003, Poitiers
2: SB&C team, ICOA, CNRS UMR7311, Orléans
3: LBAI team, Inserm U1227, Brest

4: SMS team, IRSET, Inserm U1085, Rennes
5: CORINT team, ISCR UMR CNRS 6226, Rennes

TRPV2 channel aberrant expression promotes cancer cells invasive capacities and has been associated with a bad prognosis in several cancers. Hence, impeding TRPV2-dependent cancer cells dissemination appears as a promising way to prevent metastatic spread and related mortality. However, the perspective of new treatment based on the TRPV2 drug target is currently limited by the lack of highly selective and fully validated pharmacological tools. Tranilast (TR) is a well-tolerated marketed drug prescribed for allergic and fibrotic diseases, whose anti-tumor potential has recently aroused considerable interest. TR molecular targets are poorly characterized but this compound is commonly used as TRPV2 “specific” inhibitor. Yet, TR has never been fully validated as direct TRPV2 blocker nor designed or optimized for such effects. Using HEK293 cells stably overexpressing recombinant TRPV2 channels, we have demonstrated that TR dose-dependently inhibits agonist-induced rat and human TRPV2 activities with species-dependent differences in potency. Further analyses on closely related human channels evidenced TR selectivity for TRPV2 inhibition. Based on these results, we have evaluated TR ability at inhibiting cancer cells endogenous TRPV2 channel dependent Ca2+-transport activity and migration. In order to develop TR derivatives with better solubility and potency, we performed a pilot Structure-Activity Relationship (SAR) study. This revealed important TR chemical functions for TRPV2 inhibition and identified water-soluble TR derivatives without compromised biological activity. Molecular docking and structure-based virtual screening approaches are currently performed to address TR mechanism of action for TRPV2 target inhibition and to rationally design more potent derivatives.

P23- EFFECTS OF ENDOGENOUS LYSOLIPIDS ON ACID-SENSING ION CHANNEL 3 (ASIC3)

Delanoe KEVIN[1]; Lingueglia ERIC[1]; Vandier CHRISTOPHE[2]; Deval EMMANUEL[1];

1: IPMC UMR7275 - 660 Route des Lucioles, 06560 Valbonne
2: UMR INSERM U 1069 - 10 Boulevard Tonnellé 37032 TOURS

Acid-sensing ion channels (ASIC) are cationic channels expressed both in the central and peripheral nervous systems and involved in many aspects of nociception. Extracellular pH variations are seen as the principal endogenous signal that triggers activation of ASIC, which are basically considered as proton sensors. In recent years lysophosphatidylcholine (LPC) and arachidonic acid (AA) have also been shown to positively modulate ASIC3 channels, with strong potentiations of the acid-evoked currents and/or activations in the absence of extracellular acidification. To further investigate the effect of lipids on ASIC3 channels, we tested here structural analogues of LPC, focusing on endogenous alkyl-ether lipids such PAF (Platelet-Activating Factor) and lysoPAF and also synthetic lipids with therapeutic potential in cancer. We show that, together with LPC, lysoPAF and PAF are the most efficient to activate and potentiate ASIC3 channels. We report that the effect of LPC, lysoPAF and PAF is not sensitive to the PAF receptor inhibitor Ginkgolide B, strongly suggesting that the lipids act on ASIC3 channels through a more direct way. Furthermore, we correlated the amplitude of ASIC3 current recorded at pH7.0 with the amplitude of the current activated by lipids, further demonstrating that the effects of lipid are mediated by ASIC3 channels. LPC, lysoPAF and PAF act on ASIC3 with similar kinetics, which suggest a

common mechanism of action on the channel. These findings open the way to new roles for ASIC3 channels and lipids in physiological functions and responses to pathophysiological states.

P24- EFFECT OF CUTANEOUS LPC INJECTIONS ON DIFFERENT SPINAL CORD NEURONS.

Ludivine PIDOUX; Eric LINGUEGLIA; Emmanuel DEVAL;

IPMC, CNRS UMR 7275

Acid sensitive ion channels (ASICs) are sodium channels activated by extracellular acidification. They are expressed along the sensory pathway within the peripheral and central nervous system. These channels are involved in many aspects of nociceptions, including acute and chronic pain. Recently, our lab showed that LPC (lysophosphatidylcholine) is a new lipidic endogenous modulator of ASIC3 that can activate the channel without any extracellular acidification. Single cutaneous injections of LPC in rodents also induce acute pain behaviors, which are dependent on ASIC3. Moreover, our recent data show that repetitive injections of LPC generate long-lasting pain behaviors in mice. If the activation of ASIC3 at the periphery appears to be an important process for the development of pain behaviors, the consequences of such activation on spinal cord neurons activity and sensitization are not known. To determine the effect of peripheral LPC injections on dorsal spinal cord neurons (DSCNs), we did in vivo recordings in rats and mice. LPC was injected subcutaneously at the level of hind paws, within the receptive fields of DSCNs. LPC injections in rats induce a significant increase of the basal activity of high threshold DSCNs (nociceptive DSCNs) . In addition, LPC injection potentiates the response of these neurons to nociceptive mechanical stimulation (pinching). These LPC effects on nociceptive spinal neurons last for 30-60 min. On the other hand, LPC injected subcutaneously also seems to have an effect on WDR neurons by increasing both their basal activity and their short term potentiation (windup) after nociceptive stimulation. We did not find any effect of LPC injection on low threshold DSCNs (non nociceptive neurons), strongly suggesting that LPC only affects the nociceptive pathway. Interestingly, similar results were observed in WT mice, whereas LPC injections in ASIC3-KO mice did not produce any changes in basal activity or in the response to high threshold DSCNs. This work demonstrates that cutaneous LPC injections activate and enhance the spinal cord nociceptive response, which is drived by peripheral ASIC3 channels.

P25- ROLE OF L-TYPE CAV1.3 CALCIUM CHANNELS IN DORMANT, DYSRHYTHMICALLY AND RHYTHMICALLY FIRING SINGLE PACEMAKER CELLS ISOLATED FROM MOUSE SINOATRIAL NODE

Julien LOURADOUR[1]; Isabelle BIDAUD[1]; Jean-yves LE GUENNEC[2]; Matteo MANGONI[1]; Pietro MESIRCA[1];

1: Institut de Génomique Fonctionnelle, Montpellier
2: Physiologie et Médecine Expérimentale du cœur et des muscles, Montpellier

Cardiac pacemaking relies on electrical activity of a group of specialized, spontaneously active myocytes called sinoatrial node cells (SANCs) located in the right

atrium. Automaticity in SANCs is the result of a complex interaction between membrane ion channels activity and intracellular calcium cycling and sets the rate for proper cardiac chamber contraction. However, when isolated, only a small fraction of SANCs exhibit rhythmic firing whereas most of isolated SANCs show dysrhythmic firing or remain dormant (i.e. without any apparent spontaneous activity). Here we studied the capability of L-type Cav1.3 calcium channels to initiate spontaneous electrical activity in dormant SANCs from a knock-in mouse strain in which the DHP-sensitivity in Cav1.2 α1 subunits was eliminated (Cav1.2DHP-/-).
We first performed voltage clamp experiments on isolated SANCs from Wild-type (WT) and Cav1.2DHP-/- mice. L-type Ca2+ current was completely blocked by 3μM nifedipine (L-type Ca2+ channel blocker) in WT cells, whereas Cav1.2DHP-/- SANCs showed a DHP-resistant L-type Ca2+ current attributed to Cav1.2. Accordingly, current clamp data showed that inhibitory effect of nifedipine on the rate of spontaneous action potential (AP) was stronger in WT than in mutant SANCs. As a whole, these data confirm Cav1.2DHP-/- mouse as a good model to pharmacologically study the role of Cav1.3 channels in cardiac automatism.
We then studied the effect of β-adrenergic stimulation (with isoprenaline, ISO) in dormant Cav1.2DHP-/- SANCs. On 21 dormant cells, 11 started firing after 100nM ISO perfusion (0 AP.min-1 before, 330 ± 47 AP.min-1 after; Coefficient of Variation-CoV=0.21 ± 0.1). Strikingly, in 8 out of 11, nifedipine totally stopped ISO-induced automaticity.
β-adrenergic stimulation converted dysrhythmic firing (95 ± 32 AP.min-1, CoV= 0.8 ± 0.23) to rhythmic (328 ± 11 AP.min-1, CoV = 0.11 ± 0.03) in n=4 cells. In these myocytes, subsequent perfusion of nifedipine ended spontaneous AP firing. In n=2 rhythmic firing SANCs, ISO strongly accelerated the frequency of AP which was strongly decelerated by nifedipine application (463 AP.min-1, CoV= 0.03 before vs 234 AP.min-1, CoV=0.97 after).
Taken together these results tend to indicate that Cav1.3 channels may be sufficient to trigger cardiac pacemaking in mice.

P26- FUNCTIONAL ROLE FOR THE GIRK1/4, CAV1.3 AND HCN4 CHANNELS IN MUSCARINIC REGULATION OF HEART RATE

Leïla TALSSI; Isabelle BIDAUD; Matteo elia MANGONI; Pietro MESIRCA;

IGF, Univ. Montpellier, CNRS, INSERM, Montpellier, France

Parasympathetic (cholinergic) stimulation slows the heart-rate (HR) by decreasing the spontaneous pacemaker activity of the sinoatrial-node (SAN). Cholinergic regulation in SAN pacemaker cells is mediated by the acetylcholine(ACh)-dependent activation of M2-receptors. The M2-receptors are coupled to a G-protein. Once muscarinic receptors are activated, the βγ-subunit of G-protein directly activates the GIRK1/4 channels (responsible for IKACh) while the αi-subunit inhibits the activity of the adenylate cyclase (AC). AC inhibition decreases the intracellular cAMP production thus affecting the voltage-dependent ion channels involved in cardiac pacemaking, in particular HCN4 and L-type Ca channels (notably Cav1.3-subunit).The objective of my work is to determine the differential role of GIRK1/4, HCN4 and Cav1.3 channels in cholinergic regulation of HR. To that, we studied the role of GIRK1/4, HCN4 and L-type Ca channels after ACh perfusion using electrocardiograms recorded in isolated hearts from five genetically modified mouse models: Girk4KO (no IKACh), HCN4-CNBD (no HCN4 cAMP-regulation), Cav1.3KO (no Cav1.3 L-type current), Girk4KO/HCN4-CNBD and Girk4KO/Cav1.3KO.In all the mutants studied ACh 300nM did not have any effect on the HR. Perfusion of 1μM ACh statistically reduced HR in

control (22%), Girk4KO (13%), HCN4-CNBD (21.7%) animals but it did not have any effect on Cav1.3KO (6.57%), Girk4KO/HCN4-CNBD (8.8%) and Girk4KO/Cav1.3 (5.38%) mice. 3µM ACh reduced HR in all the strains tested except for Girk4KO/Cav1.3KO animals. Preliminary data indicate that concomitant ablation of GIRK1/4 and Cav1.3 channels strongly impaired cholinergic regulation in mice isolated heart.

P27- MANIPULATIONS OF GSH CONTENT MODULATE [CA2+]I HOMEOSTASIS IN ASTROGLIOMA CELLS AND CORTICAL NEURONS

Nawfel MOKRANE; Julien ROUSSEL; Elodie AREL; Mahira KAABECHE; Catherine COHEN SOLAL; Janique GUIRAMAND; Thierry CENS; Michel VIGNES;

IBMM, CNRS UMR-5247, Université Montpellier, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France

Introduction: Oxidative stress is an aggravating factor of all neurological disorders, including neurodegenerative diseases. It is due to an exaggerated accumulation of reactive oxygen species (ROS) concomitant to decreased reduced glutathione (GSH) levels; the main antioxidant of brain cells produced by astrocytes in large amounts. Cell death triggered by oxidative damage results from intracellular calcium overload which is deleterious to cell activity. The mechanisms involved in these calcium increases are not fully understood. Here, we have thus investigated whether GSH could protect neurons against oxidative stress through the modulation of calcium homeostasis using manipulations of GSH cell content.
MM and results: In rat astroglioma cells (C6) and primary cultured neurons (cortex and hippocampus), we observed that the increases in intracellular calcium concentration during oxidative stress elicited by tert-butyl hydroxide (tBuOOH) require first intracellular calcium mobilization from endoplasmic reticulum (ER), and secondly an entry from the extracellular medium.
To investigate the link between GSH content and calcium homeostasis, we pre-treated cells with sulforaphane (SFN) to increase intracellular GSH levels, or buthionine sulfoximine (BSO) to decrease them, during 24 hours for both treatments.
We show using calcium imaging and electrophysiological “patch-clamp” recordings that increasing GSH levels reduces the intracellular mobilization from IP3-sensitive intracellular calcium stores and the associated membrane conductance changes. In contrary, the BSO pre-treatment leads to an increased IP3R response, and the appearance of the capacitive calcium entry (CCE) phenomenon.
Conclusion: We conclude that GSH protects cells from the calcium homeostasis disturbance by decreasing first IP3R-mediated calcium release from internal stores and then calcium membrane conductance changes associated with oxidative stress. The identity of the calcium channels involved in these mechanisms is currently examined.

P28- MODELLING DMD ASSOCIATED DILATED CARDIOMYOPATHY USING PATIENT-SPECIFIC IPS-DERIVED CARDIOMYOCYTES.

Monia SOUIDI[1]; Yvonne SLEIMAN[2]; Pascal AMEDRO[3]; Pierre MEYER[4]; François RIVIER[5]; Alain LACAMPAGNE[6]; Albano MELI[7];

1: moniasouidi@yahoo.com
2: yvonne.sleiman@etu.umontpellier.fr
3: p-amedro@chu-montpellier.fr
4: pagmeyer@yahoo.fr
5: f-rivier@chu-montpellier.fr
6: alain.lacampagne@inserm.fr
7: albano.meli@inserm.fr

Introduction: Duchenne Muscular Dystrophy (DMD) is a X-linked degenerative pathology with a prevalence of 1/3500 boys due to absence of functional dystrophin in muscles. In a late stage of DMD, patients developed a dilated cardiomyopathy (DCM) which can lead to heart failure and premature death.
In the past, we showed that DMD (mdx) mice exhibit abnormal age-dependent intracellular calcium homeostasis and pathological remodelling of the ryanodine receptor / calcium-release channel (RyR2) leading to DCM. However, the mouse does not fully recapitulate the DMD progression in patients. We hypothesize that human pluripotent stem-cell derived-cardiomyocytes (hiPSC-CMs) are a powerful technology to model the DMD-associated DCM and to better understand the pathophysiological underlying mechanisms.

Objective: We aim at deciphering the functional and molecular impacts of dystrophin-deficiency and comparing with the clinical echocardiography obtained in DMD patients.

Methods: Based on the recently published Speckle tracking echocardiography results in a DMD patient cohort in Montpellier Hospital, 3 blood samples from DMD patients with different DCM degrees of severity and 3 from healthy control (HC) were collected, reprogrammed in hiPSC and differentiated into cardiomyocytes.

Results: Our preliminary data indicate that DMD hiPSC-CMs display an abnormal intracellular calcium homeostasis characterized by the presence of leaky diastolic calcium events compared to HC hiPSC-CMs suggesting a RyR2 dysfunction. The leaky RyR2 in DMD was confirmed by increased open probability when measuring the single channel RyR2 activity In DMD hiPSC-CMs, we also observe alterations in the contractile properties with reduced contraction as sign of hypocontractility, a major feature of DCM.

Conclusion: Our results support the fact that DMD-associated DCM can be modelled in the dish using patient-specific hiPSC-CMs. Such modelling may provide a better understanding of the pathophysiological mechanisms and the pharmacological treatment of the DMD- associated DCM.

P29- HEXOSAMINE PATHWAY INDUCES CARDIAC ARRHYTHMIA: ROLE OF BACKGROUND ION CHANNEL

Matthieu DOUARD; Fanny VAILLANT; Emma ABELL; Pierre DOS SANTOS; Fabien BRETTE;

INSERM U1045/IHU Liryc ;Site Hôpital Xavier Arnozan;Avenue du Haut-Lévêque ; 33604 Pessac ; France

BACKGROUND Growing evidences show an implication of cardiac energetic metabolism in arrhythmogenesis of hearts. Here, we focused on the role of an alternative pathway of glycolysis, the hexosamines biosynthetic pathway (HBP). This pathway usually represents less than 5% of metabolic pathways, however it has been shown to be increased in some cardiomyopathies. HBP leads to the production of a glycosylation, consisting in the O-linked attachment of a single monosaccharide (N-acetyl-D-glucosamine, O-GlcNAc) to serine and threonine of nuclear and cytosolic proteins. This postranslational protein modification, as phosphorylation / dephosphorylation events, may alter many processes, including protein activity & localisation.
METHODS We studied the arrhythmogenesis of acute HBP overactivation by glucosamine administration (30 min) on healthy working rat hearts perfused ex vivo (N=12). We performed cellular electrophysiology on left rat ventricular cells by using patch clamp technique. We measured action potential in perforated whole cell configuration (N=8). We also measured different K+ currents (Ito, Isus & IK1) in ruptured whole cell configuration (N=9).
RESULTS Glucosamine administration increases the probability of spontaneous arrhythmia events ex vivo notably ventricular tachycardia, ventricular fibrillation and atrial fibrillation ($p<0.05$). Glucosamine perfusion in vitro reduced action potential duration at 90% of repolarization (APD90, $p<0.001$) and depolarize the rest membrane potential (RMP, $p<0.001$). On K+ currents, glucosamine increases the background repolarizing currents Isus ($p<0.001$) whereas no modification was observed on Ito and IK1.
CONCLUSION Theses observations indicate that HBP participate in the regulation of cardiac electrical properties. Furthermore, these data suggest a potential implication of HBP over-activation during arrhythmias. However, further experiments remain, in particular to determine the molecular identity responsible of K+ current modification.

P30- LYSOPHOSPHATIDYLCHOLINE INDUCES LONG-LASTING JOINT PAIN IN MICE IN AN ASIC3-DEPENDANT MANNER

Bonnie LABRUM[1]; Thierry FERREIRA[2]; Véronique BREUIL[3]; Fabien MARCHAND[4]; Eric LINGUEGLIA[1]; Emmanuel DEVAL[1];

1: Université Côte d'Azur, Institut de Pharmacologie Moléculaire et Cellulaire (Valbonne), CNRS, LabEx « Ion Channel Science and Therapeutics »
2: STIM, Université de Poitiers, CNRS, France
3: CHU de Nice, Hôpital de l'Archet, Université Nice Sophia-Antipolis, France
4: Neuro-Dol, Université Clermont-Auvergne, INSERM, France

Chronic pain is a main trigger for patients to visit a physician. Because chronic joint pain pathophysiology is poorly understood, mainly due to a lack of specific molecular targets, analgesic treatments prescribed today are disappointing. Discovering new therapeutical targets is crucial to improve patient care.

Acid-Sensing Ion Channels (ASICs) have emerged as important candidates in nociception signaling. Until recently, ASICs channels were known to be only activated by extracellular acidosis. Interestingly, a lipid, lysophosphatidylcholine (LPC), found in patients' synovial fluids suffering from chronic joint pain, has been shown to strongly modulate ASIC3 channel activity.

Here, we report that LPC not only modulates ASIC3 but also ASIC1a, two members of the ASIC channel family highly expressed in peripheral sensory neurons. Both ASIC3 and ASIC1a currents are potentiated by LPC in response to extracellular acidosis. However, LPC only activates ASIC3 at physiological pH7.4, i.e., without extracellular acidification.
In vivo we show that two LPC intra-articular injections produce chronic pain in WT mice, lasting for at least a month. Mice injected once with LPC don't develop a pain state as strong as the twice-injected mice. Interestingly, ASIC3 knock-out mice appear to be protected from this LPC-induced chronic pain state.

These data demonstrate that a lysolipid found at high level in human patients (LPC) triggers a chronic pain state when injected in mice joints. This new joint pain model now needs to be characterized in more details but we can show that it is driven by ASIC3 expressed in peripheral sensory neurons.
Focusing on ASIC3 and its regulators, such as LPC, may enlighten chronic joint pain pathophysiology, and could offer new strategies to relieve patients.

P31- SODIUM LEAK CHANNEL NALCN CONTROLS METASTATIC BEHAVIOR IN PROSTATE CANCER CELLS

Antoine FOLCHER[1]; Oksana IAMSHANOVA[2]; Dimitri GORDIENKO[3]; Alexandre BOKHOBZA[4]; George SHAPOVALOV[5]; Pascal MARIOT[6]; Sebastien ROGER[7]; Arnaud MONTEIL[8]; Natalia PREVARSKAYA[9];

1: antoine.folcher@inserm.fr
2: oksana.iamshanova@inserm.fr
3: dimitri.gordienko@inserm.fr
4: alexandre.bokhobza@inserm.fr
5: george.shapovalov@inserm.fr
6: pascal.mariot@inserm.fr
7: sebastien.roger@univ-tours.fr
8: arnaud.monteil@igf.cnrs.fr
9: natacha.prevarskaya@univ-lille.fr

Deregulated ionic homeostasis has been observed in various pathologies, including tumourigenesis. Importantly, elevated total tissue Na+ concentration was even proposed as a highly specific in vivo indicator of malignant lesions in cancer patients. Indeed, altered Na+ homeostasis was already implemented in prostate tumourigenesis. Therefore, we invastigated possible link between prostate cancer and Na+ influx provided by the recently discovered Na+ leak channel NALCN.
In vitro cell culture assays were performed on prostate cancer cell lines with different metastatic potential. NALCN expression was altered due to the applications of siRNA, shRNA and overexpression by lentivirus infection. Effects of NALCN alteration were investigated due to Ca2+ and Na+ imaging and perforated patch-clamp techniques.
Our previous results have shown that NALCN is overexpressed in human prostate cancer tissues, and its expression was detected only in highly aggressive prostate

cancer (PCa) cell lines (PC-3 and PC3-M). We also demonstrated that NALCN controls PCa cell migration and invasiveness. Now, using perforated patch-clamp recordings we show that NALCN is functional in PCa cells and is regulated by Ca2+ entry via Store Operated Ca2+ Channels (SOCs). Respectively, Na+ influx via NALCN regulates SOCs and plays an important role in maintaining calcium oscillations, which, in turn, are required for initiation of cells invasion, and subsequent metastasis. Overall, our data provide evidence on NALCN contribution to increased metastatic potential of human prostate cancer cells. Therefore, NALCN could provide new perspective molecular target for the disease suppression, in particular at its advanced stages.

P32- DEVELOPMENT OF NEW INHIBITORS OF SK3 CHANNEL TO PREVENT METASTASIS OCCURRENCE

Romain FELIX[1]; Céline GARCIA[1]; Aurélie CHANTOME[1]; Séverine MARIONNEAU-LAMBOT[2]; Thibault OUILLE[3]; Carina RUA[1]; Marie POTIER-CARTEREAU[1]; Frédéric BURON[4]; Sylvain ROUTIER[4]; Christophe VANDIER[1];

1: Inserm U1069 2°étage Bat Dutrochet 10 Bd Tonnellé 37032 TOURS
2: Université de Nantes : FED 4203 / Ums_S 016 / Ums_C 3556 SFR Santé
3: UMR 1235 TENS, Université de Nantes
4: Institut de Chimie Organique et Analytique, Université d'Orléans, UMR CNRS 7311

Currently, there is no treatment able to prevent bone metastasis. The abnormal expression of the SK3 channel by cancer cells promotes cancer cell migration and bone metastasis development and its suppression reduces them. Here, we propose to develop SK3 channel inhibitors as a new class of anti-metastatic drugs in targeted and personalized cancer therapy.
Lead compound NS8593 is currently one of the SK inhibitor. Our team is developing new synthetic strategies in order to provide novel polyfunctionalized pyridopyrimidines to explore structure-activity relationships. To achieve these objectives, we have developed efficient and modular strategies using SNAr and palladium-catalyzed coupling reactions to modulate the main scaffold.
Among all the 25 compounds tested using patch-clamp technique we identified GF495, a chiral compound, as strong inhibitor of SK3 channel with an IC50 = 18.4 nM. This compound inhibits also the SK2 channel with an IC50 of around 1nM. In vitro experiments showed that GF495 significantly reduces the migration of five cancer cell lines, expressing SK3 channel including the MDA-MB435s.
In vivo experiments showed that GF495 was not toxic until 20 mg/Kg (i.p. 5 times a week for 2 weeks). Finally, GF495 was tested on a murine model of metastatic breast cancer (i.p.1 mg/kg, 3 times a week for 15 weeks). GF495 treatment reduces dramatically bone metastasis and suppresses uterine and ovarian metastases.
To conclude, GF495 is a new and potent inhibitor of SK3 channel, that show a capacity to reduce the development of metastases. These promising results encourage us to develop analogues of GF495, with at least a better selectivity (no effect on SK2 channel). In addition, it seems necessary to characterize the role of SK2 channel in cancer cell biology.

P33- EVALUATION OF THE SPIDER (PHLOGIELLUS GENUS) PHLOTOXIN 1 AND SYNTHETIC VARIANTS AS ANTINOCICEPTIVE DRUG CANDIDATES

Evelyne BENOIT ; Tânia c. GONçALVES ; Pierre LESPORT ; Sarah KUYLLE ; Enrico STURA ; Justyna CIOLEK; Gilles MOURIER; Denis SERVENT; Emmanuel BOURINET ; Nicolas GILLES ;

Over the two last decades, venom toxins have been explored as alternatives to opioids to treat chronic debilitating pain. Actually, approximatively 20 potential analgesic toxins, mainly from spider venoms, are known to inhibit with high affinity the NaV1.7 subtype of voltage-gated sodium (NaV) channels, the most promising genetically validated antinociceptive target identified so far. The present study aimed to consolidate the development of phlotoxin 1 (PhlTx1), a 34-amino acid and 3-disulfide bridge peptide of a Phlogiellus genus spider, as an antinociceptive agent by improving its affinity and selectivity for the human (h) NaV1.7 subtype. The synthetic homologue of PhlTx1 was generated and, as the natural peptide, equilibrated between two active forms on reverse-phase liquid chromatography and exhibited potent analgesic effects in a mouse model of inflammatory pain. The effects of PhlTx1 and 8 successfully synthetized alanine-substituted variants were studied (by automated whole-cell patch-clamp electrophysiology) on cell lines stably overexpressing hNaV subtypes, as well as two cardiac targets, the hCaV1.2 and hKV11.1 subtypes of voltage-gated calcium (CaV) and potassium (KV) channels, respectively. PhlTx1 and D7A-PhlTx1 were shown to inhibit hNaV1.1-1.3 and 1.5-1.7 subtypes at hundred nanomolar concentrations, while their affinities for hNaV1.4 and 1.8, hCaV1.2 and hKV11.1 subtypes were over micromolar concentrations. Despite similar analgesic effects in the mouse model of inflammatory pain and selectivity profiles, the affinity of D7A-PhlTx1 for the NaV1.7 subtype was at least 5 times higher than that of the wild-type peptide. Computational modelling was performed to deduce the 3D-structure of PhlTx1 and to suggest the amino acids involved in the efficiency of the molecule. In conclusion, the present structure-activity relationship study of PhlTx1 results in a low improved affinity of the molecule for the NaV1.7 subtype but without any marked change in the molecule selectivity against the other studied ion channel subtypes. Further experiments are therefore necessary before considering the development of PhlTx1 or synthetic variants as antinociceptive drug candidates.

P34- NEW CYCLIC TOXINS AS NOVEL TOOLS TO INVESTIGATE THE MECHANISM(S) SUPPORTING THE TRAIN-OF-FOUR FADE AT THE SKELETAL NEUROMUSCULAR JUNCTION

Muriel AMAR[1]; Julien GIRIBALDI [2]; Sebastien DUTERTRE [1]; Jordi MOLGO[1];

1: (1) Institut des sciences du vivant Frédéric Joliot, Laboratoire de Toxinologie Moleculaire et Biothechnologies, Service d'Ingénierie Moléculaire des Protéines (SIMOPRO), CEA Saclay, 91190 Gif sur Yvette.
2: (1) Institut des Biomolécules Max Mousseron, UMR 5247, Université de Montpellier-CNRS 34095 Montpellier.

Neuromuscular relaxants are widely used in intensive care units. The evaluation of myoneural function recovery immediately after an operation is essential as the residual paralysis induced by the relaxants increases the morbidity and mortality rates. Tests are used to evaluate the recovery of the function of the respiratory muscles to ensure the adequacy of spontaneous reactions. The "train of four" technique is an additional method of quantitative calculation measuring the weakening of the muscle during

repetitive nerve stimulation. It involves delivering a short train of four supra maximal stimuli to the nerve at a frequency of 2 Hz, and a train rate of 0.033 Hz. The ratio of the amplitude of the fourth to the amplitude of the first nerve-evoked contraction provides a significant index of the degree of neuromuscular blockade (TOF fade).

At the molecular level, different groups have tried to explain this TOF fade. One of the theories retained is that at the level of the neuromuscular junction, presynaptic α3β2 nicotinic receptors would increase the release of acetylcholine, via a positive feedback mechanism, to maintain the contraction at the same level following repeated nerve stimulation. The inhibition of these presynaptic receptors by myorelaxants has been implicated as one of the causes of an attenuated release of acetylcholine leading to nerve-evoked muscle contraction fade (1,2).

α-conotoxin CIA from the venom from Conus catus and cyclic analogs, block with high affinity the neuronal α3β2 (IC_{50}: 2,6 to 72,4 nM) and the rat muscle $\alpha1_2B\gamma\delta$ (IC_{50}: 3.45 to 8.55 nM) type nAChRs expressed in Xenopus laevis oocytes. Next, α-CIA and analogs were tested in vitro on isolated mouse phrenic nerve-hemi-diaphragm muscles stimulated by the motor nerve. The IC50 deduced from the concentration-response curves were comprised between 8, to 13 nM, demonstrating a highly specific and potent inhibitory action on $\alpha1_2B\varepsilon;\delta$ adult muscle-type nAChR of the neuromuscular junction.

In this context, it was of interest to determine whether α-CIA and analogs were able to produce TOF fade. Interestingly, when nerve-evoked contraction was inhibited about 76 % by α-CIA or analogs no significant TOF fade was observed, while a marked TOF fade was observed (TOF fade ratio: 0.46 ± 0.07; n=3) with the highly selective αC-Conotoxin PrXA peptide that blocks selectively the skeletal muscle-type nAChR, even when there was just about 45 % of neuromuscular block.

These results suggest that the positive feedback induced by the presynaptic α3β2 nAChR is not the only mechanism participating to the TOF fade.
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P35- STUDYING THE INTERACTOME OF K2P CHANNELS

Stephanie HäFNER; Perrine ROYAL; Pablo AVALOS PRADO; Brigitte WDZIEKONSKI; Guillaume SANDOZ;

iBV – Institut de Biologie Valrose, Laboratoire d'Excellence, Biology of Ion Channels, Université de Nice Sophia Antipolis, Sciences Naturelles, Parc Valrose, 06108 Nice Cedex 2

Potassium channels serve as a hub for generation and regulation of the negative membrane potential and neuronal excitability. One important superfamily of K+ channels, the K2P channels, represents a family of 15 members which are notably involved in depression or migraine. Based on sequence homology they can be further classified into the six subfamilies of TREK, TASK, TALK, TWIK, THIK and TRESK that also reflect their biophysical properties.
Despite the fact that K2P channels share low sequence identity, even between members of the same subfamily, we demonstrated that heteromerization is possible between different K2P isoforms. The TREK channel members, TREK1, TREK2 and TRAAK are able to physically heteromerize with each other to generate channels with

new properties increasing functional diversity (Levitz et al., 2016). We further extended this by showing that two distantly related family members, namely TREK1 and TRESK, which share only 19 % of homology outside the P loop, are also capable of functional heteromer assembly. Physiologically, we demonstrated that heteromerization allows the formation of specific channels and that the misregulation of them can lead to severe human diseases such as migraine (Royal et al., 2019). This provides motivation for a deeper understanding of K2P assembly.
We decided to generalize the study of the heteromerization of K2P channels. With the 15 genes encoding for human K2P channels, 120 combinations of channel complexes are conceivable, which in theory each of them would show different functional properties. To study further possible interactions between the K2P channel members, we have developed and use the single-molecule pull-down ("SiMPull") assay. The combination of biochemical immunoprecipitation with single molecule fluorescence microscopy enables the determination of protein interactions as well as the stoichiometry within the individual protein complexes by observing fluorophore bleaching steps. Our results demonstrate that subunit recognition cannot occur between all K2P members and that the sequence homology cannot be used to predict the ability of two subunits to coassemble. We are currently determining the general rules of subunit recognition that allows the formation of functional K2P heterodimers.

P-36 ORAI3 EXPRESSION INCREASES DURING CHEMOTHERAPY IN LUNG ADENOCARCINOMA AND IS INVOLVED IN RESISTANCE TO CHEMOTHERAPY

Hiba ABOU DAYA; Frédéric HAGUE; Halima OUADID-AHIDOUCH;

LPCM-EA46-67, Université de Picardie Jules Verne, Amiens, France.

Since only 15% of lung cancer cases are operable, chemotherapy is generally considered. Cisplatin-based chemotherapy regimens are used as a front-line therapy in the treatment of non-small cell lung cancer. Unfortunately, such treatment often leads to chemoresistance and hence therapy failure. Cancer cells are known to exhibit a high rate of proliferation, migration ability and resistance to apoptosis. These processes are proved to be controlled by calcium and calcium-permeable ion channels. Store Operated Calcium Channels (SOCs) represent a major calcium entry pathway in non-excitable cells and are shown to be implicated in chemoresistance. Recently, we showed that Orai3 is overexpressed in lung adenocarcinoma, correlated to high tumor grade and controls cell proliferation via Akt pathway (Ay et al., 2013). We also showed that Orai3 constitutes a predictive marker of metastasis and survival in resectable lung adenocarcinoma (Benzerdjeb et al., 2016). Thus, we aimed to investigate Orai3 involvement in resistance to chemotherapy in two lung adenocarcinoma cell lines (A549 and H23).
The expression of SOC actors was studied using RT-qPCR and confirmed by Western-Blot. The inhibition of the expression of SOC actors was done via transfection using (siOrai1, siOrai3 and siStim1) vs. siControl (electroporation, AMAXA). Cellular viability and mortality were assessed (48 hours or 4 days after treatment with Cisplatin) using MTT and Trypan blue methods. Calcium imaging experiments were also conducted to measure the functional effect of the treatment obtained at the level of the channels. We found that Cisplatin treatment increases Orai3 expression, SOC entry, and favors cell survival in both cell lines. However, in contrast to H23 cells where Orai3 constitutes a native SOC channel, in A549 cell line, Orai3 doesn't contribute to SOC entry but upon the treatment with Cisplatin, it becomes a SOC channel. Moreover, we found that the long term treatment with Cisplatin leads to morphological change of A549 cells along with increased stem cell marker expression (Nanog) suggesting a stem cell

enrichment process.
We demonstrated that Orai3 channel becomes overexpressed and contributes to Cisplatin resistance in lung adenocarcinoma and the ability of conferring resistance is related to the SOC activity of the channel.

P-37 STORE OPERATED CALCIUM CHANNELS REGULATE CANCER STEM CELLS SELF-RENEWAL IN HUMAN GLIOBLASTOMA

Elodie TERRIE[1]; Nadine DELIOT[1]; Thomas HARNOIS[1]; Laëtitia COUSIN[1]; Yassine BENZIDANE[1]; Patrick BOIS[1]; Patricia ARNAULT[1]; François VALLETTE[2]; Bruno CONSTANTIN[1]; Valérie CORONAS[1];

1: STIM- Université de Poitiers, CNRS-ERL 7003, 83073 Poitiers cedex 09 2: CRCINA- UMR Inserm 892-CNRS 6299-Université de Nantes, 44007 Nantes Cedex 01

Glioblastomas are primary brain tumors and rank among the most lethal of all human cancers. The current standard therapy is safe maximal resection followed by concurrent radiotherapy and chemotherapy. Despite multimodal treatment, recurrence of the tumor occurs in more than 90 % of patients and the average life expectancy does not exceed 15 months. This poor prognosis has been related to the existence, within the tumor, of a small cell subpopulation called cancer stem cells that are resistant to radiation and chemotherapy and therefore may be responsible for the tumor relapse.

Store Operated Calcium Channels (SOC) are calcium channels that transduce signals from the microenvironment. In addition, these channels are also responsible for replenishing calcium stores. Previous studies have highlighted a critical role of these channels in several types of cancers. Transcriptomic analysis suggested a major role of calcium signaling in glioblastoma that also displayed overexpression of STIM1 (Stromal Interaction Molecule 1) protein, the calcium level sensor of the endoplasmic reticulum and activator of SOC.

We found that stem cells derived from glioblastomas of patients express the proteins that build-up SOC-type channels, and display store-operated calcium entries (SOCE). Pharmacological inhibition of SOCE reduced proliferation and stemness (assessed by the ability to form spheres) of glioblastoma cancer stem cells.

Collectively, our study assigns to SOC a key role in the regulation of glioblastoma stem cells self-renewal and might pave the way for a strategy to target the cells that convey resistance to cancer treatment.

P38-OPTICAL CONTROL OF GLUN2B-NMDA RECEPTOR

Chloé Geoffroy;

Institut de Biologie de l'Ecole Normale Superieure, Paris

NMDA receptors (NMDARs) are a family of excitatory ionotropic receptors activated by glutamate, the major excitatory neurotransmitter in the brain. They play a central role in synaptic transmission and plasticity. They are also involved in many neuropsychiatric disorders including stroke, mental retardation or schizophrenia. At the molecular level, NMDARs are tetramers usually composed of two GluN1 and two GluN2 subunits. There are four GluN2 subunits (GluN2A-D), resulting in a large number of receptor subtypes having distinct anatomical, biophysical, pharmacological and signaling properties. Understanding the functional role of these individual subtypes in the brain is fundamental to develop new strategies to counteract the deleterious effects of NMDAR deregulation. However, this understanding is limited by the lack of specificity and the low spatio-temporal resolution of the currently available tools. Taking advantage of the high spatio-temporal resolution of light, optopharmacology allows overcoming these limits. My project consists in developing this approach to selectively inhibit NMDA receptors containing the GluN2B subunit (GluN2B-NMDARs). We developed inhibitors whose activity can be reversibly controlled by light (OptoNAMs). Photosensitivity was conferred by incorporating within the molecular scaffold of well-known GluN2B-NMDAR inhibitors, an azobenzene moiety that can alternate between a trans and a cis configuration depending on the illumination wavelengths. By studying the activity and photo-modulation properties of these OptoNAMs, we found that they exerted an inhibition specific for GluN2B-NMDARs, with a greater activity for the trans isomer. Our plan is then to use in silico modeling (docking) to understand the binding modes of the cis and trans configurations, in order to increase the difference of activity between the two isomers. This work should further advance our understanding of GluN2B-NMDAR physiology.

ATTENDEES: CONTACT INFO

ABOU DAYA Hiba
LPCM
33 rue saint leu
80000 Amiens - France
Tel: 695750824 - Fax:
hibaaboudaya@hotmail.com

AMAR Muriel
CEA SACLAY
Laboratoire de toxinologie moléculaire et biotechnologies IBiTec-S / SIMOPRO / LTMB / Bât 152
91191 GIF SUR YVETTE - France
Tel: 0612503755 - Fax:
muriel.amar@cea.fr

ARCANGELI Annarosa
University of Florence
University of Florence Department of Experimental and Clinical Medicine
Viale GB Morgagni, 50
50134 Firenze - Italy
Tel: +39 (0) 55 2751283 - Fax:
annarosa.arcangeli@unifi.it

ARHATTE Malika
IPMC/CNRS
660 ROUTE DES LUCIOLES
06560 VALBONNE - FRANCE
Tel: 0493957723 - Fax:
arhatte@ipmc.cnrs.fr

BECQ Frédéric
Laboratoire STIM
Université de Poitiers
86073 Poitiers - France
Tel: 0678734383 - Fax: 0549454014
Frederic.Becq@univ-poitiers.fr

BENOIT Evelyne
CEA
CEA de Saclay, SIMOPRO, bat. 152
91191 Gif-sur-Yvette - France
Tel: 0169085685 - Fax: 0169089071
evelyne.benoit@cea.fr

BERROU Mikael
World Precision Instruments
1 Hunting gate
SG4 0TJ Hitchin - Royaume-Uni
Tel: +33 9 70 44 90 00 - Fax:
mberrou@wpi-europe.com

BERTAUD Anaïs
IBMM - UMR 5247
Faculté des Sciences de Montpellier
Place Eugène Bataillon
34095 Montpellier - France
Tel: 0634459717 - Fax:
bertaudanais@hotmail.com

BICHET Delphine
IPMC-CNRS
660 route des Lucioles
06560 VALBONNE - FRANCE
Tel: 04 93 95 77 31 - Fax: 04 93 95 77 08
bichet@ipmc.cnrs.fr

BIDAUD Isabelle
Institut de Génomique Fonctionnelle
141 rue de la Cardonille
34094 Montpellier cedex 5 - France
Tel: 0434359245 - Fax: 04 67 54 24 32
isabelle.bidaud@igf.cnrs.fr

BIGNUCOLO Olivier
University of Lausanne
rue du Bugnon, 27
1011 Lausanne - Switzerland
Tel: +41 21 692 5408 - Fax:
olivier.bignucolo@unil.ch

BLONDE Anne
DOMICONUS
13 B rue principale
68130 OBERMORSCHWILLER - France
Tel: 0622134712 - Fax: 622134712
a.blonde68@gmail.com

BOIS Patrick
Poitiers universite
Equipe Transferts Ioniques et Rythmicité Cardiaque (TIRC)
Laboratoire Signalisation et Transports Ioniques Membranaires (STIM), ERL CNRS 7368 Université de Poitiers, UFR SFA Pôle Biologie Santé, Bâtiment B36/B37 1 Rue Georges Bonnet, TSA 51106 86073 POITIERS Cedex 9
86073 Poitiers - France
Tel: 0664979785 - Fax:
Patrick.bois@univ-poitiers.fr

BOSSU Jean louis
CNRS
INCI 5 rue Blaise Pascal
67250 Strasbourg - France
Tel: 0614296610
jlbossu@inci-cnrs.unistra.fr

BRETTE Fabien
INSERM U1045/IHU Liryc
Site Hôpital Xavier Arnozan Avenue du Haut-Lévêque
33604 Pessac - France
Tel: (+33) 5 35 38 19 11 - Fax:
fabien.brette@inserm.fr

BULTYNCK Geert
University of Leuven
Laboratory Molecular and Cellular Signaling Department Cellular and Molecular Medicine Campus Gasthuisberg O/N-I bus 802 Herestraat 49
BE-3000 Leuven - Belgium
Tel: +32 16 33 02 15 - Fax:
geert.bultynck@kuleuven.be

CAMUS Côme
IINS UMR 5297
Batiment Broca 146 RUE LEO SAIGNAT CS 61292
33076 BORDEAUX - France
Tel: 0619321686 - Fax:
comecamus6@yahoo.fr

CANTELMO Anna rita
Inserm U1003, University of Lille
Avenue Paul Langevin Cité Scientifique
59655 Villeneuve d'Ascq - France
Tel: +33 (0) 320 43 40 77 - Fax: +33 (0) 320 43 40 66
anna-rita.cantelmo@univ-lille.fr

CHADET Stéphanie
Université de Tours
10 boulevard Tonnellé
37000 Tours - France
Tel: 02 47 36 61 22 - Fax:
stephanie.chadet@univ-tours.fr

CHAHINE Mohamed
Laval University
256 rue Pellan
G2G 2K1 Québec - Canada
Tel: 14186635747 #4723 - Fax: 14186635747
mohamed.chahine@phc.ulaval.ca

CHARNET Pierre
ibmm umr 5247 cnrs
Univ Montpellier Bat 24, 4et. Place Eugene Bataillon
34293 Montpellier - france
Tel: 0467143966 - Fax:
pierre.charnet@cnrs.fr

CHATELAIN Franck
IPMC-CNRS
660 route des Lucioles
06560 VALBONNE - FRANCE
Tel: 04 93 95 77 32 - Fax: 04 93 95 77 08
chatelain@ipmc.cnrs.fr

CHATELIER Aurélien
Université de Poitiers
Pôle Biologie Santé, Bâtiment B36/B37
1 Rue Georges Bonnet, TSA 51106 Cedex 9
86073 Poitiers - France
Tel: +33(0) 549 453 747 - Fax: +33(0) 549 454 014
aurelien.chatelier@univ-poitiers.fr

CHERUBINI Enrico
European Brain Institute
via le regina Elena 293-295
00161 Roma - Italy
Tel: 393333836197 - Fax:
cher@sissa.it

CHINIGÒ Giorgia
University of Turin
Via Oslavia 20/A
10153 Torino - Italy
Tel: 348 2647712 - Fax:
giorgia.chinigo@unito.it

CONSTANTIN Bruno
STIM laboratory - CNRS ERL 7003 - Poitiers University
Pôle Biologie Santé Bâtiment B36 1 Rue Georges Bonnet TSA 51106
86073 POITIERS Cedex 9 - France
Tel: 33(0)5 49 45 35 27 - Fax: 33(0)5 49 45 40 14
bruno.constantin@univ-poitiers.fr

CORONAS Valérie
Université de Poitiers-CNRS
Laboratoire STIM Université de Poitiers, CNRS ERL 7003 Pôle Biologie Santé, Bat B36 1 rue Georges Bonnet, TSA 51106
86073 Poitiers - France
Tel: 33 (0)5 49 45 36 55 - Fax: 33 (0)5 49 45 40 14
valerie.coronas@univ-poitiers.fr

COUGNON Marc
LP2M CNRS UMR7370
Faculté de Médecine tour Pasteur 28 ave vallombrose, 3em étage,
06107 Nice - France
Tel: 0493377036 - Fax:
cougnon@unice.fr

DE WAARD Michel
Inserm
Inserm U1087 / CNRS UMR 6291
Université de Nantes l'institut du thorax 8 quai Moncousu 8
44007 Nantes - France
Tel: 0228080076 - Fax:
michel.dewaard@univ-nantes.fr

DEJOS Camille
Inserm U1003, Universitté de Lille
Avenue Paul Langevin Cité Scientifique
59655 Villeneuve d - France
Tel: +33(0)320434077 - Fax: +33(0)320434066
camille.dejos@univ-lille.fr

DELANOE Kevin
IPMC
660 Route des Lucioles
06560 Valbonne - France
Tel: 04 93 95 77 77 - Fax:
kevin.delanoe@ipmc.cnrs.fr

DEVAL Emmanuel
IPMC - UMR 7275 CNRS/Université Côte d'Azur
660 route des Lucioles Sophia Antipolis
06560 Valbonne - France
Tel: 0493953429 - Fax:
deval@ipmc.cnrs.fr

DIOCHOT Sylvie
CNRS UMR7275
IPMC, 660 Route des Lucioles, Sophia Antipolis
06560 VALBONNE - France
Tel: 33 4 93 95 34 22 - Fax: 33 4 93 95 77 08
diochot@ipmc.cnrs.fr

DORAY Adélaïde
EA4245 - Transplantation, Immunologie, Inflammation
UFR de Médecine 10 boulevard Tonnellé
37032 Tours cedex - France
Tel: +33 2 47 36 61 28 - Fax: +33 2 47 36 61 47
adelaide.doray@etu.univ-tours.fr

DOUARD Matthieu
INSERM U1045/IHU Liryc
Site Hôpital Xavier Arnozan Avenue du Haut-Lévêque
33604 Pessac - France
Tel: (+33) 5 35 38 19 11 - Fax:
matthieu.douard@ihu-liryc.fr

DUBOIS Charlotte
Laboratoire Physiologie cellulaire INSERM U1003
Bat. SN 3, UFR de Biologie, Université Lille 1
59655 Villeuneve d'ascq - FRANCE
Tel: +33 320 33 64 23 - Fax:
charlotte.dubois@inserm.fr

DUCREUX Sylvie
Laboratoire CaRMeN - Université de Lyon
Laboratoire CarMeN Equipe 5 Cardioprotection Groupement Hospitalier Est Bâtiment B13 59 boulevard Pinel
69500 BRON - FRANCE
Tel: 0478777047 - Fax:
sylvie.ducreux@univ-lyon1.fr

DUCROZET Mallory
CarMeN U1060
59 bd Pinel
69500 Bron - France
Tel: 04.78.77.71.75 - Fax:
mallory.ducrozet@univ-lyon1.fr

DURANTON Christophe
LP2M CNRS UMR 7370
Université côte d'azur, CNRS-UMR 7370, LP2M, LabEx ICST, faculté de médecine, 28 av Vallombrose
06107 Nice - France
Tel: 0493377036 - Fax: 0493377024
duranton@unice.fr

EL HASSAR Lynda
iBV - CNRS
Parc Valrose
06108 cede Nice - France
Tel: +33604677177 - Fax:
Lynda.EL-HASSAR@univ-cotedazur.fr

ELBINI DHOUIB Ines
Pasteur Institute of Tunisia
13, Place Pasteur Tunis- Belvédère
1002 Tunis - Tunisia
Tel: +216 52 188 717 - Fax: +216 71 791 833
ines.bini@pasteur.tn

ERIA OLIVEIRA Ana sofia
IBS
71 Avenue des Martyrs, 38000 Grenoble
38000 Grenoble - France
Tel: 0769775330 - Fax:
as.eriaoliveira@gmail.com

FAGNI Laurent
CNRS
294 RUE DES OLIVIERS
34000 MONTPELLIER - FRANCE
Tel: 0631138706 - Fax:
laurent.fagni@igf.cnrs.fr

FELICIANGELI Sylvain
IPMC-CNRS
Institut de Pharmacologie Moléculaire et Cellulaire 660 route des lucioles
06560 Valbonne - France
Tel: 0493957732 - Fax: 0493957708
feliciangeli@ipmc.cnrs.fr

FELIX Romain
Inserm U1069, N2C
Faculté de Médecine Bat Dutrochet 10 Bd Tonnellé
37032 Tours - France
Tel: 0671955555 - Fax:
romain.felix37@gmail.com

FELTZ Anne
CNRS
IBENS 46 Rue d'Ulm
75005 Paris - France
Tel: 0631138706 - Fax: 0631138706
feltz@biologie.ens.fr

FIORIO PLA Alessandra
University of Torino/INSERM U1003 Lille
Via Accademia Albertina 13
10123 Torino - Italy
Tel: 00390116704660 - Fax:
alessandra.fiorio@unito.it

FLOTA Dominique
DomiConus
13B rue principale
68130 Obermorschwiller - France
Tel: 0607904888 - Fax:
d.flota@domiconus.com

FOLACCI Mathilde
Institut de Biologie Structurale
71 Avenue des Martyrs
38000 Grenoble - France
Tel: 0457428710 - Fax:
mathilde.folacci@ibs.fr

FOLCHER Antoine
INSERM U1003
Laboratoire de physiologie cellulaire
Université de Lille UFR biologie
Bâtiment SN3 -2eme étage
59655 Villeneuve d'ascq cedex - France
Tel: 0320336423 - Fax: 0320434066
antoine.folcher@inserm.fr

GEOFFROY Chloé
Institut de Biologie de l'Ecole Normale Superieure (IBENS)
46 rue d'Ulm
75005 Paris - France
Tel: +33 (0)1 44 32 37 53 - Fax:
cgeoffro@biologie.ens.fr

GHESQUIER Aline
INSERM
Bâtiment SN3, 2ème étage, Cité Scientifique
59655 Villeneuve d'Ascq - France
Tel: 0320337078 - Fax:
ghesquier.aline@gmail.com

GIERÉ Clémence
DomiConus
34 Rue Titien
67200 Strasbourg - France
Tel: 0680812972 - Fax:
clemence.giere@gmail.com

GKIKA Dimitra
Univerity of Lille / Inserm
University of Lille - Sciences and Technologies Cell Physiology lab, Inserm U1003
59650 Villeneuve d - France
Tel: +33320436838 - Fax:
dimitra.gkika@univ-lille.fr

GUERINEAU Nathalie
Institute of Functional Genomics
141 rue de la Cardonille
34094 Montpellier - FRANCE
Tel: 33434359250 - Fax:
nathalie.guerineau@igf.cnrs.fr

HÄFNER Stephanie
Université Côte d'Azur - iBV
Université de Nice Sophia Antipolis
Sciences Naturelles 28 Av. de Valrose
06108 Nice - France
Tel: +33 489150818 - Fax:
Stephanie.HAFNER@univ-cotedazur.fr

HAGUE Frederic
UNIVERSITE DE PICARDIE JULES VERNE
33 RUE SAINT LEU LPCM
80039 AMIENS - FRANCE
Tel: 0322827643 - Fax:
frederic.hague@u-picardie.fr

HAUSTRATE Aurélien
Laboratory of Cell Physiology, INSERM U1003
Cité Scientifique
59650 Villeneuve d'ascq - France
Tel: 0320337078 - Fax:
haustrateaurelien@gmail.com

HILAIRE Cecile
INM / INSERM
Institut des neurosciences 80 rue augustin flich
34000 montpellier - france
Tel: 0499636033 - Fax:
cecile.hilaire@umontpellier.fr

HIRBEC Helene
IGF, CNRS UMR5203
141 rue de la cardonille
34094 Montpellier - France
Tel: 0434359304 - Fax: 0434359304
helene.hirbec@igf.cnrs.fr

HOSY Eric
CNRS, University of Bordeaux
9 rue paul doumer
33140 villenave d'ornon - France
Tel: 0624212101 - Fax:
eric.hosy@u-bordeaux.fr

HU Hongzhen
Washington University School of Medicine in St. Louis
Department of Anesthesiology The Center for the Study of Itch St. Louis Campus Box 8054 660 South Euclid Avenue
63110-1093 St. Louis - USA
Tel: 1-314-747-4317 - Fax:
huh@anest.wustl.edu

IMPHENG Hathaichanok
Institut de Génomique Fonctionnelle
141, rue de la Cardonille
34094 Montpellier - France
Tel: (33) 4.34.35.92.50 - Fax:
hathaichanok.impheng@igf.cnrs.fr

INQUIMBERT Perrine
INCI CNRS UPR3212/ Université de Strasbourg
8 allée du Général Rouvillois
67000 strasbourg - France
Tel: +33 (0)3 88 45 66 62 - Fax: +33 (0)3 88 45 66 54
inquimbert@unistra.fr

KELLENBERGER Stephan
University of Lausanne
Département de Pharmacologie et de Toxicologie UNIL Rue du Bugnon 27
1011 Lausanne - Switzerland
Tel: +41216925422 - Fax:
+41216925355
stephan.kellenberger@unil.ch

KHOUBZA Lamyaa
IPMC
660 route des lucioles
06560 valbonne - france
Tel: 0493957731 - Fax:
khoubza@ipmc.cnrs.fr

LABRUM Bonnie
IPMC
660, route des Lucioles
06560 Valbonne - France
Tel: 0493953418 - Fax:
labrum@ipmc.cnrs.fr

LEGENDRE Claire
MitoVasc Laboratory
3 Rue Roger amsler
49100 Angers - France
Tel: 02 44 68 82 86 - Fax:
claire.legendre@univ-angers.fr

LEGROS Christian
MitoVasc Laboratory
3 Rue Roger Amsler
49100 Angers - France
Tel: 02 44 68 82 73 - Fax:
christian.legros@univ-angers.fr

LEHENKYI Vyacheslav
Laboratoire de Physiologie Cellulaire INSERM U1003
Département de Biologie Faculté des Sciences et Technologies Université de Lille Bâtiment SN3, 2ème étage, porte 223
59655 VILLENEUVE D asq - france
Tel: +33 (0) 3 20 33 70 7 - Fax:
vyacheslav.lehenkyi@univ-lille1.fr

LESAGE Florian
IPMC CNRS
660 route des lucioles
06560 Valbonne - France
Tel: 0677791434 - Fax:
lesage@ipmc.cnrs.fr

LINGUEGLIA Eric
IPMC- CNRS/UCA Sophia Antipolis
Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), CNRS UMR7275
Université Côte d'Azur, 660 Route des Lucioles, Sophia Antipolis
06560 Valbonne - France
Tel: 04 93 95 34 23 - Fax:
lingueglia@ipmc.cnrs.fr

LIZOT Guénaëlle
Laboratoire STIM / Université de Poitiers
40 boulevard François Albert
86000 Poitiers - France
Tel: 0676513892 - Fax:
guenaelle.lizot@univ-poitiers.fr

LLINARES Jodie
Laboratoire STIM
Pôle Biologie Santé B36 1 rue Georges Bonnet
86073 Poitiers - France
Tel: +33(0)5.49.45.37.44 - Fax:
jodie.llinares@univ-poitiers.fr

LOPEZ-CHARCAS Osbaldo
Université de Tours
10 Boulevard Tonnellé,
37032 Tours - France
Tel: 0247366128 - Fax: 0247366147
osbaldo.lopez@univ-tours.fr

LORY Philippe
IGF CNRS Montpellier
141, rue de la Cardonille
34094 Montpellier - France
Tel: 0434359250 - Fax:
philippe.lory@igf.cnrs.fr

LOURADOUR Julien
Institut de Génomique Fonctionnelle (IGF)
141 Rue de la Cardonille
34000 Montpellier - France
Tel: 0434359246 - Fax:
julien.louradour@igf.cnrs.fr

MALECOT Claire
Laboratoire STIM, EA7349
Faculté des Sciences, Parc de Grandmont
37200 TOURS - FRANCE
Tel: 02-47-36-70-09 - Fax:
claire.malecot@univ-tours.fr

MALECOT Lionel
MICRO MÉCANIQUE
101 rue Pereire Parc Pereire Bât EH
78100 SAINT GERMAIN EN LAYE - FRANCE
Tel: 0672364541 - Fax:
malecot.l@micromecanique.fr

MANGONI Matteo
CNRS
Institut de Génomique Fonctionnelle (IGF) 141, rue de la cardonille
34094 Montpellier - France
Tel: +33633181463 - Fax:
matteo.mangoni@igf.cnrs.fr

MANTEGAZZA Massimo
IPMC/CNRS UMR7275
660 Route des Lucioles
06560 Valbonne - France
Tel: 0493953425 - Fax:
mantegazza@ipmc.cnrs.fr

MEDYNA Igor
INSERM
INMED Parc Scientifique de Luminy
163 Avenue de Luminy
13273 MARSEILLE - France
Tel: 0491828107 - Fax:
igor.medyna@inserm.fr

MELI Albano
Inserm U1046
371 Avenue du Doyen Gaston Giraud
Batiment Crastes de Paulet
34295 Montpellier - France
Tel: 0467415244 - Fax:
albano.meli@inserm.fr

MESIRCA Pietro
IGF-CNRS
141, Rue de la Cardonille
34094 Montpellier - France
pietro.mesirca@igf.cnrs.fr

MOHA OU MAATI Hamid
IGF CNRS INSERM UMR 5203
141 rue de la cardonille
34095 Montpellier Cedex 5 - france
Tel: 04.34.35.92.53 - Fax:
hamid.moha@igf.cnrs.fr

MOKRANE Nawfel
UMR5247 IBMM
University of Montpellier - Science Faculty Place Eugène Bataillon CC-090
34095 Montpellier - France
Tel: 0467143699 - Fax:
nawfel.mokrane@umontpellier.fr

MONTEIL Arnaud
Institut de Genomique Fonctionnelle
141 rue de la Cardonille
34094 Montpellier - France
Tel: (33) 4.34.35.92.50 - Fax:
arnaud.monteil@igf.cnrs.fr

MORDMUANG Auemphon
Institut de Génomique Fonctionnelle
141 rue de la Cardonille
34094 Montpellier Cedex 5 - France
Tel: (33) 4.34.35.92.50 - Fax:
auemphon.mordmuang@igf.cnrs.fr

MORONI Anna
Università degli Studi di Milano
Dipartimento di Bioscienze Via Celoria, 26
20133 Milano - Italy
Tel: 0 - Fax: 0
anna.moroni@unimi.it

MULLE Christophe
CNRS
IINS 146 rue Léo-Saignat CS 61292
CASE 130
33076 Bordeaux - France
Tel: 0631138706 - Fax: 0631138706
mulle@u-bordeaux2.fr

NICOLE Sophie
Institut Génomique Fonctionnelle
141 rue de la Cardonille
34094 Montpellier - France
Tel: 33434359250 - Fax:
sophie.nicole@igf.cnrs.fr

OANCEA Elena
Department of Molecular Pharmacology, Physiology and Biotechnology Brown University Department of Molecular Pharmacology, Physiology and Biotechnology Brown University Box G-B3
02912 Providence - USA
Tel: 401-863-6116 - Fax:
elena_oancea@brown.edu

PAOLETTI Pierre
CNRS
IBENS, 46 rue d'Ulm
75005 Paris - France
Tel: 0631138706 - Fax: 0631138706
pierre.paoletti@ens.fr

PAPAZIAN Diane
UCLA
University of California
David Geffen School Of Medicine
University Of California Box 951751
Los Angeles CA - USA
Tel: (310)206-7043 - Fax:
Papazian@mednet.ucla.edu

PARK Joohee
MitoVasc Laboratory
3 Rue Roger Amsler
49100 Angers - France
Tel: 02 44 68 82 66 - Fax:
joohee.park@univ-angers.fr

PENG Zhong
university of Lausanne
Rue du Bugnon 27
1011 Lausanne - Switzerland
Tel: +41-786375206 - Fax:
zhong.peng@unil.ch

PENNA Aubin
CNRS ERL 7003
Laboratoire Signalisations et Transports Ioniques Membranaires (STIM) CNRS ERL 7003 Université de Poitiers UFR SFA Pôle Biologie Santé Bâtiment B36/B37 1 Rue Georges Bonnet TSA 51106
86073 Poitiers - France
Tel: 0622421639 - Fax:
aubin.penna@univ-poitiers.fr

PIDOUX Ludivine
CNRS UMR 7275
IPMC 660 route des lucioles
06560 Valbonne - France
Tel: 0493953422 - Fax:
pidoux@ipmc.cnrs.fr

POET Mallorie
LP2M UMR CNRS 7370
Université côte d'azur Faculté de Médecine 28 av. Valombrose Tour Pasteur, 3eme étage
06107 Nice cedex2 - FRANCE
Tel: 00 33 4 93 37 70 46 - Fax:
Mallorie.Poet@univ-cotedazur.fr

POISSON Lucile
Université de Tours, EA4245 Transplantation, Immunologie et Inflammation
Université de Tours, EA4245 Transplantation, Immunologie et Inflammation 10 Boulevard Tonnellé, 37032 Tours, France
37032 Tours - France
Tel: 0630957338 - Fax:
lucile.poisson@etu.univ-tours.fr

PREVARSKAYA Natalia
laboratoire de physiologie cellulaire inserm u1003
Université de Lille - Sciences et Technologies Laboratoire de Physiologie Cellulaire INSERM U.1003
Av. Paul Langevin Bât. SN3 2ème étage
59655 villenueve d'ascq - france
Tel: 0320336018 - Fax:
natacha.prevarskaya@univ-lille.fr

QUIGNARD Jean-francois
CRCTB INSER U1045 Université de Bordeaux
CRCTB , U1045 PTIB Hopital Xavier Arnoson Av Haut Leveque
33600 Talence - France
Tel: 05 47 30 27 51 - Fax:
jean-francois.quignard@u-bordeaux.fr

RAPETTI-MAUSS Raphaël
Institut de Biologie Valrose
Institut de Biologie Valrose (iBV) UMR CNRS 7277- UMR INSERM 1091
Université de Nice Sophia-Antipolis
Faculté des sciences de Valrose
Bâtiment de sciences naturelles
06108 Nice - France
Tel: 0492076553 - Fax:
rrapettima@unice.fr

RÉTHORÉ Léa
Institut MitoVasc UMR CNRS 6015 - INSERM U1083
3, rue Roger Amsler
49100 ANGERS - FRANCE
Tel: 0244688286 - Fax: 0244688286
claire_legendre@yahoo.fr

ROBERTSON Janice
Washington University
7241 Forsyth Blvd
63105 St. Louis - USA
Tel: 917-597-5535 - Fax: 617-208-2524
rajan.sah@wustl.edu

RODAT-DESPOIX Lise
Université Picardie Jules Verne -UFR des Sciences Laboratoire de Physiologie Cellulaire et Moléculaire-EA4667 33 rue Saint Leu
80039 Amiens - France
Tel: 0322827647 - Fax: 0322824645
lise.despoix@u-picardie.fr

ROGER Sébastien
University of Tours, EA4245 T2I
EA4245 Transplantation, Immunology, Inflammation 10 Boulevard Tonnellé, Faculté de médecine de Tours
37032 Tours - France
Tel: 0033 2 47 36 61 30 - Fax:
sebastien.roger@univ-tours.fr

ROLLAND Laura
IGF
141 rue de la Cardonille
34094 Montpellier Cedex 5 - France
Tel: 0434359253 - Fax:
laura.rolland@igf.cnrs.fr

RONJAT Michel
CNRS
Inserm U1087 / CNRS UMR 6291
Université Nantes l'institut du thorax 8 quai Moncousu
44007 Nantes - France
Tel: 0228080148 - Fax:
michel.ronjat@univ-nantes.fr

SAH Rajan
Washington University School of Medicine
Department of Internal Medicine, Cardiovascular Division Washington University School of Medicine
MO 63130 St. Louis - USA
Tel: 0 - Fax: 0
rajan.sah@wustl.edu

SANDOZ Guillaume
iBV - CNRS
Parc Valrose
06108 cede Nice - france
Tel: +33604677177 - Fax:
sandoz@unice.fr

SCAMPS Frédérique
INSERM
Institut des Neurosciences Montpellier
80 rue Augustin Fliche
34000 Montpellier - France
Tel: 04 99 63 60 33 - Fax:
frederique.scamps@inserm.fr

SERVANT Zoé
Institut de Génomique Fonctionnelle
141 rue de la Cardonille
34094 Montpellier Cedex 5 - France
Tel: (33) 4.34.35.92.50 - Fax:
zoe.servant@igf.cnrs.fr

SOUIDI Monia
PhyMedExp Laboratory, INSERM U1046
371 avenue du Doyen Gaston Giraud 34295
34295 Montpellier - France
Tel: 0651117603 - Fax:
moniasouidi@yahoo.com

SRAIRI-ABID Najet
Pasteur Institute of Tunisia
13, Place Pasteur Tunis Belvédère
1002 Tunis - Tunisia
Tel: +216 95 146 444 - Fax: +216 71 791 833
najet.abid@pasteur.rns.tn

TALSSI Leïla
Institut de Génomique Fonctionnelle
141 rue de la Cardonille
34090 Montpellier - France
Tel: 04 34 35 92 46 - Fax:
leila.talssi@igf.cnrs.fr

TERRIÉ Elodie
University of Poitiers, STIM Laboratory
1 rue Georges Bonnet Bat B36 PBS, laboratoire STIM
86000 Poitiers - France
Tel: 33 (0) 5 49 45 37 8 - Fax: 33 (0) 5 49 45 40 14
elodie.terrie@univ-poitiers.fr

TESSIER Nolwenn
CarMeN Laboratory, équipe 5
59 boulevard Pinel
69500 Bron - France
Tel: 0678240007 - Fax:
nolwenn.tessier@univ-lyon1.fr

THIEL Gerhard
TU-Darmstadt
Dept. of Biology B1|01 183
Schnittspahnstraße 3
64287 Darmstadt - Germany
Tel: +49 6151 16-21940 - Fax:
thiel@bio.tu-darmstadt.de

TRICOIRE Ludovic
CNRS
IBPS Campus Pierre et Marie Curie 7-9 quai Saint Bernard
75005 PARIS - France
Tel: 0631138706 - Fax: 0631138706
ludovic.tricoire@snv.jussieu.fr

VAITHIA NATHA SUBRAMANIAN Anand
University of Lausanne
Dept. Pharmacology and Toxicology,
Rue du bugnon 27,
1011 Lausanne - Switzerland
Tel: +41779664802 - Fax:
anand.vaithia@unil.ch

VIET Kerstin karina
Institute for Pharmacy and Biochemistry, Johannes Gutenberg-Universität Mainz
Johann-Joachim Becherweg 30
55128 Mainz - Germany
Tel: +49 (0) 6131 3925838
kviet@students.uni-mainz.de

VIGNES Michel
UMR5247 IBMM
University of Montpellier - Science Faculty Place E bataillon CC090
34095 Montpellier - France
Tel: 0467143699 - Fax:
michel.vignes@umontpellier.fr

VIOLETTE Aude
Alphabiotoxine Laboratory
Barberie 15
7911 Montroeul-au-Bois - Belgium
Tel: +32 471 51 26 17 - Fax:
aude.violette@alphabiotoxine.com

VIVAUDOU Michel
Institut de Biologie Structurale
71 Avenue des Martyrs
38044 Grenoble - FRANCE
Tel: +33 4 57 42 85 85 - Fax:
vivaudou@ibs.fr

WEICHBRODT Conrad
Nanion Technologies GmbH
Ganghoferstrasse 70a
80339 München - Germany
Tel: +49-(0)89-219095-062 - Fax: +49-(0)89-218997-960
conrad@nanion.de

WOLF Federica
Catholic University of Sacred Heart
Institute of General Pathology Gemelli Hospital
00168 Roma - Italy
Tel: + 6-30154914 / 4565 - Fax: + 6-3386446
federica.wolf@unicatt.it

YANAGIDA Takashi
Ono Pharma UK Ltd
MidCity Place, 71 High Holborn
WC1V 6EA London - UK
Tel: +44-(0)7920 460 869 - Fax:
t-yanagida@ono-uk.co.uk

YELANJIAN Tristan
SCOP PRO
25 Rue de la Vallée
91610 BALLANCOURT SUR ESSONNE - France
Tel: 0164939407 - Fax:
tristan@scop-pro.fr

ZIEGER Hanna
IINS Université de Bordeaux
Université de Bordeaux IINS - UMR 5297 - Centre Broca Nouvelle Aquitaine 146 rue Léo Saignat CS 61292 Case 130
33076 Bordeaux - FRANCE
Tel: 0557571450 - Fax:
hanna.zieger@u-bordeaux.fr

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

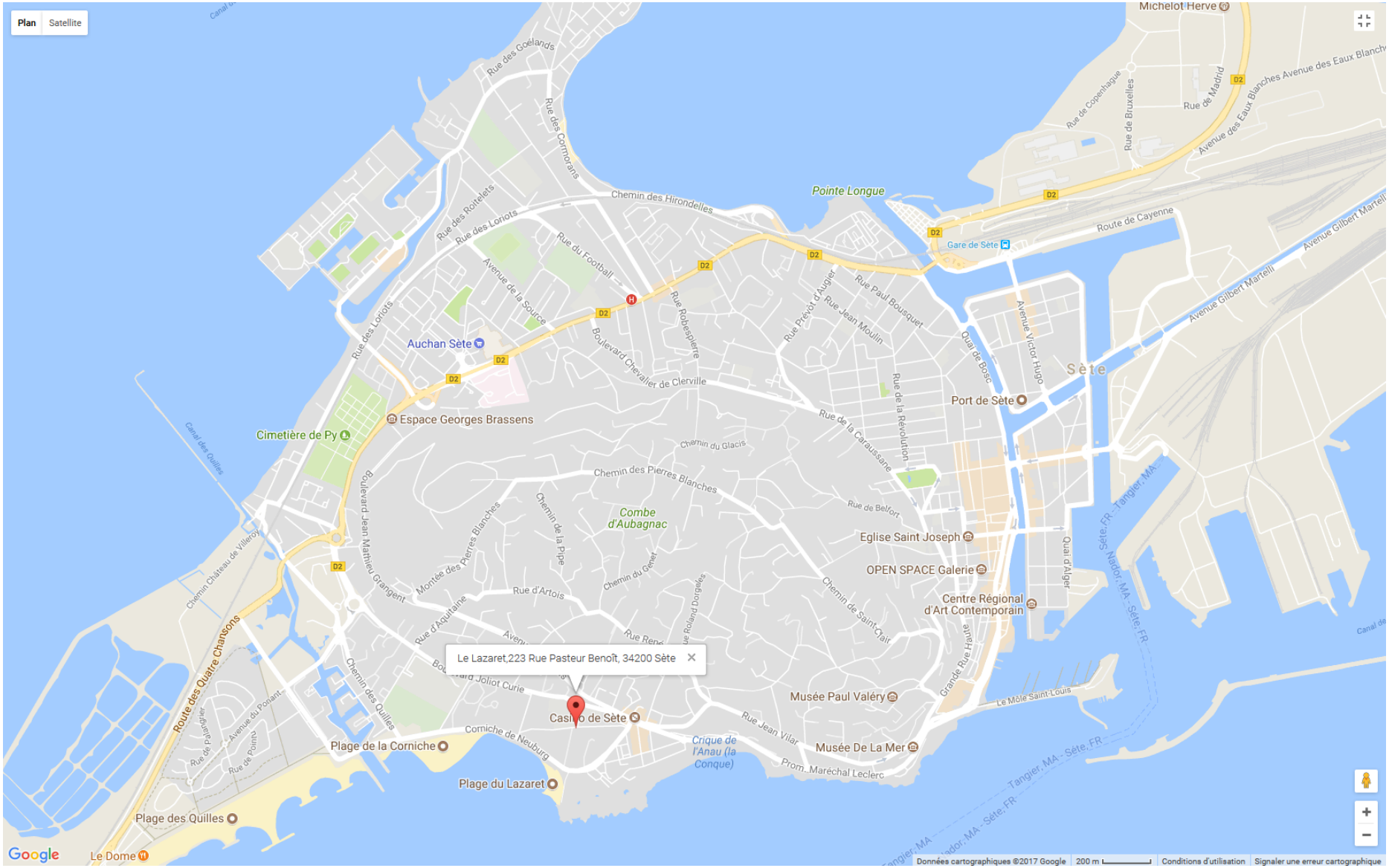
Taxi Valenti, +33 611 57 18 05, taxi.valenti@sfr.fr

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".


Plan
Satellite
Le Lazaret,223 Rue Pasteur Benoît, 34200 Sète
Pointe Longue
Auchan Sète
Espace Georges Brassens
Cimetière de Py
Combe d'Aubagnac
Port de Sète
Sète
Gare de Sète
Eglise Saint Joseph
OPEN SPACE Galerie
Centre Régional d'Art Contemporain
Musée Paul Valéry
Musée De La Mer
Plage de la Corniche
Plage du Lazaret
Plage des Quilles
Crique de l'Anau (la Conque)
Route des Quatre Chansons
Avenue Gilbert Martelli
Route de Cayenne
Avenue des Eaux Blanches
Rue de Madrid
Rue de Bruxelles
Rue de Copenhague
Rue des Goélands
Rue des Cormorans
Chemin des Hirondelles
Rue des Rotelets
Rue des Loriots
Rue du Football
Avenue de la Source
Boulevard Chevalier de Clerville
Rue Robespierre
Rue Prévot d'Augier
Rue Jean Moulin
Rue Paul Bousquet
Quai de Bosc
Avenue Victor Hugo
Rue de la Révolution
Rue de la Caraussane
Chemin du Glacis
Chemin des Pierres Blanches
Rue de Belfort
Chemin de la Pipe
Montée des Pierres Blanches
Boulevard Jean Mathieu Grangent
Chemin du Genet
Rue d'Artois
Rue d'Aquitaine
Chemin de Saint-Clair
Quai d'Alger
Grande Rue Haute
Le Môle Saint-Louis
Rue Jean Vilar
Corniche de Neuburg
Prom. Maréchal Leclerc
Chemin des Quilles
Chemin Château de Villeroy
Avenue du Ponant
Rue de Pont-Neuf
Rue de Pointu
Canal des Quilles
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Passage Le Dauphin
Épi d'Or
Centre Malraux
Jean Moulin
Mathieu Grangent
Le Vallon
Notre-Dame Souveraine du Monde
Sète

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