ASSOCIATION « CANAUX IONIQUES »

PROGRAMME
&
RESUMES

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Erratum

Erratum 1

Symposium 2 Plant ion channels and their regulation

17:00 Anne-Alienor Very (Montpellier, France) Functional diversity of cation channels and transporters in rice

17:30 Markus Gierth (Cologne, Germany) Potassium Channel and Transporter Proteins Involved in Nutrient Acquisition and Homeostasis

18:00 Rainer Hedrich (Würzburg, Germany) Excitement and movement in plants

18:30 Daniel Tran (Gif sur Yvette, France) Role in symbiosis of medicago root hair mechanosensitive msls channels and clcs anion transporters

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Erratum 2

Symposium 4 Molecular and functional diversity of ionotropic glutamate receptors

Organized by David Stroebel, Paris

17:00 Christophe Mulle (Bordeaux, France) Activity-dependent plasticity of synaptic NMDA receptors

17:30 Andrew Plested, (Berlin, Germany) Glutamate receptors: fast, loose and full of surprises

18:00 Indira Shivastava (Pittsburg, USA) Structural Dynamics and Allosteric Potential in Ionotropic Glutamate Receptors.

18:30 Abdessattar Khlaifia (Marseille, France) Presynaptic LTD in brainstem synapses involves anandamide

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Erratum 3

Affiliation of S-07 Excitement and movement in plants

Rainer Hedrich[1]; Felix Bemm[2]; Sönke Scherzer[1]; Elzbieta Krol[1]; Khaled Al-Rasheid[3]; Ines Kreuzer[1]; Erwin Neher[4]; Hubert Bauer[1]; Peter Ache[1]; **Geiger Dietmar[1]**

1: Julius-von-Sachs-Intstitute, University of Wuerzburg, Germany

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Erratum 4

POSTER P-01 Lactate Increases Trek1 Channel Activity (Swagata Ghatak) withdrawn and replaced by **Regulation of $Ca_V2.x$ Ca^{2+} channels by RhoA GTPase (P. Charnet)**

Abstract S06-Potassium Channel and Transporter Proteins Involved in Nutrient Acquisition and Homeostasis

Markus Gierth[1], Hans-Henning Kunz[2], Myeon Haeng Cho[3], Young Jae Pyo[3], Julian I. Schroeder[2]
1 Department of Botany, University of Cologne, Cologne, Germany
2 Division of Biological Sciences, UCSD, La Jolla, CA 92093-0116, USA
3 Department of Biology, Yonsei University, Seoul, Republic of Korea

Potassium is the most abundant cation in plants with functions in osmoregulation, enzyme activation and maintenance of the membrane potential. On average potassium accumulates in plant dry matter to approximately 10%. Thus it has to be absorbed by roots from the soil solution in large quantities and properly distributed throughout the plant body and within cells. Potassium concentrations in the soil solution can vary substantially from millimolar down to low micromolar concentrations. Therefore plants have developed strategies to maintain growth even at low potassium availability by employing high-affinity potassium uptake proteins.
Transcriptome analysis of potassium-starved Arabidopsis plants identified AtHAK5 as the inducible high-affinity component of root potassium uptake in addition to the constitutively expressed shaker-like potassium channel AKT1. Investigation of single and double mutants revealed that AKT1 and AtHAK5 represent the two major, physiologically relevant molecular entities mediating high-affinity potassium uptake into roots. Once incorporated into the plant and transferred to leaves, potassium exhibits important functions in chloroplast osmo- and pH regulation. Channels are often found to work in concert with transporters. However, the
molecular identity of members from either class for the chloroplast envelope membrane is still missing although earlier investigations on isolated plastids have clearly shown their presence. In recent, ongoing studies we indentified envelope-localized members of a potassium transporter family that are critical for K+ and H+ homeostasis of chloroplasts. Data on mutant analyses will be presented.

Abstract P01 Regulation of $Ca_V2.x$ Ca^{2+} channels by RhoA GTPase

P. Charnet[1], M Rousset[1], V Limoges[1], M. Bellis[1], F. Scamps[2], T. Cens[1]
1:CRBM, UMR5237, CNRS, UM2 ; 1919 route de Mende, 34293 Montpellier, France
2: INM, INSERM U1051, 80 rue Augustin Fliche, 34091 Montpellier, France

RhoA GTPases are known to regulate LVA T-type Ca channels, via phosphorylation of the channel by the RhoA effector Rock. However, regulation of HVA Ca_V2 channels by these channels has been less studied. We, and others, have recently analyzed the effects of another small GTPase of the RGK families (Gem/Rem/Rem2) on the P/Q-type, CaV2.1 Ca^{2+} channels expression and activity and demonstrated the necessary role of the Ca_V□ subunit. These GTPases are also regulators of the RhoA pathway suggesting that these two signalling events may converge on Ca channels. To further decipher these complex regulations we have started to characterize the regulation of the CaV2 channels by RhoA and Rock in expression system, and their regulation by Gem.

We show, in this preliminary study that $Ca_V2.1$; $Ca_V2.2$ and $Ca_V2.3$ Ca^{2+} channels, are down-regulated by RhoA, in a □-subunit independent manner, and without modification in the biophysical parameters other than current amplitude. This effect is mimicked by co-expression of ROCK, a downstream effector of RhoA, and is blocked by perfusion of Y27632 (2µM), an inhibitor of ROCK. Morevover inhibition of current amplitude by RhoA is neither antagonized, nor cumulative, with the effects of the coexpression of Gem. Biochemical studies suggested that albeit the number of channels expressed at the plasma membrane is decreased by RhoA, the global expression of the channels, including the auxiliary subunits, is not affected. Consistently, the down-regulation of channel activity by RhoA was not affected by incubation of MG132, a proteasome inhibitor.

The mechanism responsible of this inhibition is now under investigation, and preliminary data suggest that channel targeting toward the plasma membrane could be affected.

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Comité d'organisation / Organizing Committee

PRESIDENTS :

Eric Boué-Grabot
Institut des Maladies Neurodégénératives (IMN), CNRS UMR 5293, Université de Bordeaux, Bordeaux, France
Tel: 05 57 57 57 63 - Mobile : 07 78 84 15 37
eric.boue-grabot@u-bordeaux2.fr

Guillaume Sandoz
institut de Biologie Valrose (iBV), UMR CNRS 7277/INSERM 1091/Université Nice Sophia Antipolis, Nice, France
Tel: 04 92 07 68 04 - sandoz@unice.fr

Thomas Boulin
Genetics and Neurobiology of *C. elegans*, École Normale Supérieure – IBENS INSERM U1024 / CNRS UMR 8197, Paris, France
Tel : 01 44 32 23 09 -boulin@biologie.ens.fr

Sylvain Feliciangeli
Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UMR7275, Université de Sophia-Antopolis, Valbonne, France
Tel: 04.93.95.77.32 - E-mail: feliciangeli@ipmc.cnrs.fr

Thomas Grutter
Laboratoire de Conception et Application de Molécules Bioactives, UMR 7199 CNRS, Université de Strasbourg, Strasbourg, France
E-mail: grutter@unistra.fr - Tel: 03 68 85 41 57

Carole Levenes
Laboratory of Neurophysique and Physiologie, CNRS UMR 8119, université Paris Descartes, Paris, France
Tel: 0142864157 - carole.levenes@gmail.com

Christian Mazars
LRSV - UMR CNRS-UPS N° 5546, Université Toulouse III Paul Sabatier, Toulouse, France
Tél: 05 34 32 38 36 - mazars@lrsv.ups-tlse.fr

Julie Perroy
Institut de Génomique Fonctionnelle ,UMR 5203 CNRS - U 661 INSERM - Univ. Montpellier I & II, Montpellier, montpellier
Tel: 0434 35 9210 - julie.perroy@igf.cnrs.fr

David Stroebel
École Normale Supérieure – IBENS
INSERM U1024 / CNRS UMR 8197, Paris, France
Tel: 0144323892 - Mobile : 0628250129 **-** David.stroebel@ens.fr

Fabien Vanden Abeele
INSERM U1003, Laboratoire de Physiologie Cellulaire, Université Lille1, Villeneuve d'Ascq, France
Tel : 03 20 33 70 78 - fabien.vanden-abeele@inserm.fr

Caroline Strube (Présidente de l'association)
Faculté de Médecine Secteur Nord, CRN2M-UMR6231, Marseille

Cyril Sarrauste de Menthière (Développeur Informatique)
IGH, CNRS UPR1142, Montpellier
Tel : 04 34 35 99 81 - cyril.sarrauste@igh.cnrs.fr

Sponsors

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We thank for their support :

PROGRAM Oléron 2013

Sunday, september 15th

16:00 - 20:00 Welcome of the meeting attendees

20:15 « Oleron Oysters » cocktail

21:15 Dinner

Monday, september 16th

8:20 Meeting opening

8:30 Symposium 1. Chemical Engineering of Ion Channels

Organized by Thomas Grutter, Illkirch

Dennis Dougherty (Pasadena, USA) Unnatural amino acid mutagenesis on pentameric receptors: what have we learned?

Dirk Trauner (München, Germany) Optochemical Genetics

Klaus Benndorf (Jena, Germany) Unravelling the intricate subunit cooperativity in the cAMP-induced gating of HCN2 channels

Shixin Ye (IBENS, Paris) Genetically encoding a light-switch in ionotropic glutamate receptors

10:15 A talk from Explora Nova : Nicholas Mellen (Louisville, USA) From the forest to the trees and back again: online Ca2+ imaging analysis as a tool for linking cellular and network function.

10:30 Coffee break

11:00-12:00 Oral communication 1. Nervous System Ragtime

Organized by Julie Perroy, Montpellier

Iulia Blesneac (Montpellier, France) Profiling the phosphorylation status of T-type calcium channels reveals great complexity and fine regulation

Lik-Wei Wong (Melbourne, Australia) The N-Terminal α-helix and preceding residues in homomeric $\rho 1$ GABAc and heteromeric $\alpha 1\beta 2\gamma 2$ GABAa recepotors play an important role in receptor assembly and function.

Damien Lemoine (Strasbourg, France) A light-gated P2X receptor

Simone Temporal (Marseille, France) Neuromodulation maintains mRNA channel correlations in the stomatogastric ganglion pacemaker cell

12:15 Wine tasting : « Pineau des charentes » of Vincent vineyard

13:00 Lunch

15:00-17:00 Poster session 1 (numéros impairs / odd numbers)

16:30 coffee break

17:00 Symposium 2. Plant ion channels and their regulation

Organized by Christian Mazars, Toulouse

Anne-Alienor Very (Montpellier, France) Functional diversity of cation channels and transporters in rice

Julian Schroeder (La Jolla, USA) Guard Cell Ion Channels: Abscisic Acid and Calcium Signal

Rainer Hedrich (Würzburg, Germany) Excitement and movement in plants
Selected speaker from abstract

Daniel Tran (Gif sur Yvette, France) Role in symbiosis of medicago root hair mechanosensitive msls channels and clcs anion transporters

18:45 break

19:00 Plenary lecture

John Wood (London, UK) Pain pathways; modality-specific wiring and ion channels

20:00 Dinner

Tuesday september 17th

8:45 Symposium 3. The family of Pannexin proteins: structure and functions of a new class of channels

Organized by Carole Levenes, Paris

Dale Laird (London, Canada) The emergence of pannexins as a new class of channels"

Isabelle Couillin (Orléans, France) Role of ATP release through connexin/pannexin channels in Nlpr3 inflammasome activation

Panagiotis Bargiotas (Lubeck, Germany) Channel forming proteins in cerebral ischemia

Sarah Lalisse (Montpellier, France) Organotypic hippocampal slice cultures and microglia

10 :30 Coffee break

11:00-12 :00 Oral communication 2. Ion Channel Melting Pot

Organized by Fabien Vanden Abeele, Villeneuve d'Ascq

Rémi Peyronnet (London, UK) Mechanoprotection by polycystins against apoptosis is mediated through the opening of stretch-activated K2P channels.

Karima Habbout (Nice, France) A novel skeletal muscle sodium channel mutation underlays congenital myasthenic syndrome.

Michel Ronjat (Grenoble, France) CACNB4 subunit of voltage-gated calcium channels promotes the formation of a nuclear protein platform that controls gene expression.

Ludovic Bannwarth (Paris, France) Gating of KIRBAC3.1 channels: structural investigation of mutants.

12:00-12:05 A word from Molecular devices

12:15 Lunch

15:00-17 :00 Poster session 2 (Numéros pairs – even numbers)

15:30 – 16:30 Workshop of Molecular Devices

16:30 Coffee break

17:00 Symposium 4. Molecular and functional diversity of iGluRs

Organized by David Stroebel, Paris

Christophe Mulle (Bordeaux, France) Activity-dependent plasticity of synaptic NMDA receptors

Andrew Plested, (Berlin, Germany) Glutamate receptors: fast, loose and full of surprises

Ivet Bahar (Pittsburg, USA) Allosteric dynamics and druggability of iGluRs N-terminal Domains: Insights from Computations

Abdessattar Khlaifia (Marseille, France) Presynaptic LTD in brainstem synapses involves anandamide

18:45 Annual meeting of the association Canaux ioniques

20:15 Special Dinner « Menu Boyard »

21:30 Poster and Oral communication prizes and « Mort Subite » session

22:30 Evening Party

Wednesday, September 18th

9:45 Symposium 5. Ion channels, from physiology to pathology

Organized by Sylvain Feliciangeli, Valbonne

Haijun Chen (Albany, USA) Dynamic ion selectivity of K2P channels: physiology and pathophysiology

Daniela Pietrobon (Padova, Italy) Neuronal calcium channel dysfunction and migraine

Stephane Hatem (Paris, France) Up and down of potassium channels in cardiac myocytes

Anne Baron (Valbonne, France) Mamba toxins reveal new roles for Acid-Sensing Ion channels in pain

11:30 Meeting closure

11:45 Lunch

13:00 Shuttle departure

RESUMES DES SYMPOSIA ET DES COMMUNICATIONS ORALES

SYMPOSIA AND ORAL COMMUNICATION ABSTRACTS

Chemical Engineering of Ion Channels

Organized by Thomas Grutter (Illkirch, France)

Monday, september 16th

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8:30 Dennis Dougherty (Pasadena, USA)
Unnatural amino acid mutagenesis on pentameric receptors: what have we learned?

9:00 Dirk Trauner (München, Germany)
Optochemical Genetics

9:30 Klaus Benndorf (Jena, Germany)
Unravelling the intricate subunit cooperativity in the cAMP-induced gating of HCN2 channels

10:00 Shixin Ye (IBENS, Paris)
Genetically encoding a light-switch in ionotropic glutamate receptors

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S-01

Unnatural Amino Acid Mutagenesis on Pentameric Receptors: What Have We Learned?

Dennis Dougherty;
Caltech, Pasadena, CA 91125 USA

As more structural information appears on related systems (GluCl, ELIC, GLIC, AChBPs …), the question arises as to what is the role of structure-function studies on full Cys-loop receptors. For some time we have been performing such studies on nAChRs, 5HT3 receptors, and other systems, emphasizing the high-precision, "chemical-scale" information we can obtain from unnatural amino acid mutagenesis. Here we will describe several studies of functional behaviors of mammalian receptors that are designed to test predictions based on structural studies of model systems. A general finding is that, even among closely related systems, broad variations in functional behaviors can be seen. The studies reveal the undeniable value of model structures, but also the danger of extrapolating all features to the functional behavior of a mammalian receptor.

S-02

Ion Channels and GPCRs: The Age of Enlightenment

Dirk Trauner;
Department of Chemistry, University of Munich, Butenandtstrasse 5-13, D-81377 Munich, Germany

Transmembrane receptors allow a cell to communicate with its environment in response to a variety of input signals. These can be changes in the concentration of ligands (e.g. hormones or neurotransmitters), temperature, pressure (e.g. via acoustic waves or touch), transmembrane potential, or light intensity. Many important receptors have now been characterized in atomic detail and our understanding of their functional properties has markedly increased in recent years. As a consequence, these sophisticated molecular machines can be reprogrammed to respond to unnatural input signals. For instance, voltage-gated and ligand-gated ion channels, as well as GPCRs, can be endowed with synthetic photoswitches. The resulting artificial photoreceptors can be used to optically control neurons with exceptional temporal and spatial precision. They work well in animals and have already found applications in the restoration of vision and the optical control of other sensations.

S-03

Unravelling the intricate subunit cooperativity in the cAMP-induced opening of HCN2 channels

Klaus Benndorf;
Institute of Physiology II, Jena University Hospital, 07740 Jena, Germany

Hyperpolarization activated cyclic nucleotide-modulated (HCN) channels generate the rhythmic electrical activity in specialized neurons and cardiomyocytes. Activation of these non-specific cation channels is enhanced by the binding of cAMP to cyclic nucleotide-binding domains (CNBD) in each of the four subunits. Using confocal patch-clamp fluorometry we studied in voltage-activated HCN2 channels in parallel the binding/unbinding of a fluorescent cAMP and the evoked activation/ deactivation. The time-dependent current and fluorescence data were globally fitted with a Markovian state model containing four binding steps in both the open and closed channel plus five closed-open isomerizations. The fit provided us a set of rate constants for all transitions which allowed us to calculate all equilibrium association constants, KA, and also all equilibrium constants of the closed-open isomerizations, E (Kusch et al., Nat. Chem. Biol. 8, 162-9, 2012). As result we obtained that states with zero, two, and four ligands are more stable than states with one or three ligands bound. To prove that the single channels work as independent molecules we performed single-channel analysis. Our results do not provide any evidence for a relevant interaction of neighboured channels and they show that the activating effect of cAMP is mediated by an increase of the open probability solely. Hence, the time courses of macroscopic current and ligand binding are appropriate to study the activity of a single channel. Based on these results we used the determined rate constants to analyze the probability fluxes within the favored Markovian model by following the transition path theory. The binding of the first, third and fourth ligand evoked robust channel opening whereas the binding of the second ligand obstructed channel opening, similar to the empty channel. The net probability fluxes revealed a notable hysteresis for the processes of channel activation and deactivation. These results provide insight into the complex cooperative interaction of the four HCN2 subunits and support the idea that the subunits work as functional dimers.

S-04

Genetically encoding a light-switch in ionotropic glutamate receptors

Shujia Zhu; Morgane Riou; Stephanie Carvalho; Pierre Paoletti; Shixin Ye;
Institut de Biologie de l'Ecole Normale Supe´rieure (IBENS), Ecole Normale Supe´rieure, CNRS UMR 8197, INSERM U1024, Paris, France

Genetic code expansion introducing chemical moieties naturally absent in proteins has strong potential for structure-function studies. Here we demonstrate a general strategy to design light-controlled ligand-gated ion channels (LGICs) by encoding photo-reactive unnatural amino acids (UAAs). Photo-cross-linking UAAs p-azido-phenylalanine (AzF) and p-benzoyl-phenylalanine (Bpa) were encoded in NMDARs, an important class of neuronal glutamate-gated ion channels, heterologously expressed in Xenopus laevis oocytes. We show that the incorporation of AzF in GluN1 subunit at the GluN1/GluN2B N-terminal domain (NTD) dimer interface, a region distal to the transmembrane gating machinery, leads to a specific and irreversible inhibition (a switch off) of receptor activity upon UV illumination. In contrast, when paring with the GluN2A subunit, light-dependent inactivation is completely absent. By combining electrophysiological and biochemical analyses, we identify the GluN1/GluN2 NTD dimer interface as a critical structural determinant that dictates UV-controlled inactivation. The subtype specificity suggests that the NTD dimer association in GluN1/GluN2A receptors has fewer structural constraints (i.e. forming a looser complex) than in GluN1/GluN2B receptors. Our work provides novel information on the NTD dimer interface regulating receptor activity in a subunit-specific manner. It also paves the way for the engineering of light-sensitive LGICs with subtype-specificity through the genetic code expansion.

A talk from Explora Nova : From the forest to the trees and back again: online Ca2+ imaging analysis as a tool for linking cellular and network function.

Nicholas Mellen;

KCHRI, University of Louisville, Louisville KY 40202 USA

Action potentials are accompanied by rapid increases in [Ca2+]i, which can be detected using Ca2+ indicators that emit photons as they bind Ca2+. The increasing sensitivity and dropping cost of CCD sensors, as well as the development of genetically encoded Ca2+ indicators, has made optical recording of Ca2+ transients an attractive modality for characterizing neuronal activity at the network level, at a spatial resolution that allows the experimenter to resolve individual neurons. An important limitation of this methodology is that currently, analysis of optical recordings is labor-intensive and error-prone, so that the time taken to extract and validate Ca2+ transients from an image series greatly exceeds the time taken to carry out the recordings. Here, an algorithm for extracting Ca2+ transients in real time is presented, with an emphasis on the novel experiments this technology enables. Real-time extraction of somatic Ca2+ transients allows the experimenter to identify neurons of interest, and to target these neurons using single-unit recording methods. In addition to standard protocols to characterize biophysical properties of the impaled neuron, connectivity can be characterized by stimulating the neuron while recording optically, and permits recorded neurons to be labeled for subsequent immunohistochemical analysis. These methods, as well as novel time-series analysis methods to infer coupling relations between optically recorded neurons will be presented, with an emphasis on describing how real-time signal processing enables interactive experiments to characterize neuronal networks, describing both the forest and the trees.

Nervous System Ragtime

Organized by Julie Perroy (Montpellier, France)

Monday, september 16th

11:00 Iulia Blesneac (Montpellier, France)

Profiling the phosphorylation status of T-type calcium channels reveals great complexity and fine regulation

11:15 Lik-Wei Wong (Melbourne, Australia)

The N-Terminal α-helix and preceding residues in homomeric $\rho 1$ GABAc and heteromeric $\alpha 1 \beta 2 \gamma 2$ GABAa recepotors play an important role in receptor assembly and function.

11:30 Damien Lemoine (Illkirch, France)

A light-gated P2X receptor

11:45 Simone Temporal (Marseille, France)

Neuromodulation maintains mRNA channel correlations in the stomatogastric ganglion pacemaker cell

O-01

Profiling the phosphorylation status of T-type calcium channels reveals great complexity and fine regulation

Iulia Blesneac; Sylvaine Huc-Brandt; Jean Chemin; Franck Vandermoere; Philippe Lory;
Institut de Génomique Fonctionnelle 141, rue de la Cardonille 34094 Montpellier Cedex 5, France

T-type calcium channels (Cav3) are voltage-gated calcium channels that play a key role in various physiological functions as cardiac and neuronal pacemaking, slow wave sleep, neuronal and muscle differentiation, neuronal excitability, hormone secretion and sensory processing. Dysfunctions in T-type calcium channel activity are linked to several neurological disorders including absence epilepsy and neuropathic pain. Hence, it is critical to understand the cellular mechanisms that control T-type calcium channel activity. Recent studies have highlighted that these channels are modulated by a variety of serine/threonine protein kinase pathways (for review see Huc et al BBA 2009). However, evidence for direct phosphorylation and a systematic analysis of T-type channels phosphorylation are still lacking.

Using a mass spectrometry approach (immuno-purification of the channel protein from culture cells and animal tissues and phosphopeptide analysis by nanoflow liquid chromatography tandem mass spectrometry) we established the first phospho-map of T-type channels. We identified around ten phosphorylated residues in the Cav3.2 subunit of T-type channels in rat brain and more than forty in culture cells. Most of these phosphorylation sites were not even predicted by commonly used algorithms. Our results demonstrate that Cav3.2 channels are highly phosphorylated and suggest extensive modulation of channel activity. We are currently constructing phospho and de-phosphomimetic mutants in order to assess, through electrophysiological studies, the functional consequences of phosphorylation at the identified sites.

Huc S, Monteil A, Bidaud I, Barbara G, Chemin J, Lory P. (2009) Regulation of T-type calcium channels: signalling pathways and functional implications. Biochim Biophys Acta. 1793(6):947-52

Supported by ANR-10-BLAN ‘Phospho-Cav’

O-02

The N-Terminal α-helix and preceding residues in homomeric ρ1 GABAc and heteromeric α1β2γ2 GABAa recepotors play an important role in receptor assembly and function.

Lik-Wei Wong[1]; Han-Shen Tae[2]; Brett Cromer[2];
1: Department of Pharmacology and Therapeutics, University of Melbourne, Parkville VIC 3010 Australia
2: Health Innovation Research Institute, School of Medical Sciences, RMIT University, Bundoora VIC 3083 Australia

The GABAC and GABAA receptors are members of the pentameric ligand-gated ion channels (pLGICs) superfamily and mediate inhibitory fast synaptic transmission in the nervous system. Structural information shows that the agonist-binding domain is a β-sandwich, preceded by an N-terminal α-helix in eukaryotic structures. The N-terminal α-helix has been shown to be functionally essential in nicotinic acetylcholine receptors. Sequence analysis of GABAC and GABAA receptors predicts an α-helix in a similar position but preceded by 8-46 additional residues, which we term the N-terminal extension. Our aim was to test the role of the N-terminal extension and putative α-helix in ρ1 GABAC and α1β2γ2 GABAA receptors. We found that the N-terminal extension was not functionally essential in ρ1 GABAC receptors but its removal did reduce cell surface expression and agonist sensitivity. Conversely, the putative α-helix in ρ1 GABAC receptors was essential for functional cell-surface expression. In α1β2γ2 GABAA receptors, the effect of N-terminal deletions was greatest in the α1 subunit. Deletion of the α1 N-terminal extension alone or further deletion of the putative α-helix both dramatically reduced cell surface receptor expression. Further deletion of the putative α-helix in β2 and γ2 subunits did, however, reduced γ2 subunit incorporation, shown by increased zinc sensitivity and reduced benzodiazepine potentiation. Our results support a critical role for the N-terminal α-helix in pLGIC assembly, but with marked subunit differences. The role of the N-terminal extension was less critical but also very subunit-dependent. These results are consistent with both regions being involved in inter-subunit interactions.

O-03

A light-gated P2X receptor

Damien Lemoine[1]; Chloé Habermacher[1]; Adeline Martz[1]; Pierre-François Méry[2]; Federica Bertaso[2]; Fanny Diverchy[1]; Antoine Taly[3]; François Rassendren[2]; Alexandre Specht[1]; Thomas Grutter[1];
1: Laboratoire de Chimie et Neurobiologie Moléculaire, UMR 7199 CNRS, Conception et Application de Molécules Bioactives, Faculté de Pharmacie, Université de Strasbourg, 67400 Illkirch, France.
2: Département de Pharmacologie Moléculaire, Institut de Génomique Fonctionnelle, UMR 5203 CNRS, U661 INSERM, Universités Montpellier I et II, 141 rue de la Cardonille, 34094 Montpellier, France.
3: Laboratoire de Biochimie Théorique, UPR 9080 CNRS, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

Trimeric ATP-gated P2X receptors are widely expressed in tissues including the peripheral and central nervous systems. Optogenetics has revolutionized the neuroscience field by allowing the study of neural circuits with a spatiotemporal control of specific neurons provided by light. Of particular interest, optochemical genetics is a strategy that has already been successfully applied to many ion channels and receptors but so far not to P2X receptors. Here, we present an unsuspected mechanism that controls the P2X receptor function by light. Photosensitivity is conferred by a thiol reactive, positively charged molecule containing a photoisomerizable azobenzene moiety that is tethered to the channel. Moreover, we have associated to this tool a mutation that abolishes ATP binding. The result was a light-gated P2X receptor (LiP2XR) that was not sensitive to ATP. Our findings provide an original tool to delineate P2X receptor signaling in vivo.

O-04

Neuromodulation maintains mRNA channel correlations in the stomatogastric ganglion pacemaker cell

Simone Temporal[1]; David J. Schulz[2];
1: UMR 1072 (anciennement UMR 641) Unité de Neurobiologie des canaux Ioniques et de la Synapse (UNIS) Faculté de Médecine - Secteur Nord Université Aix Marseille 51, Bd Pierre Dramard 13015 Marseille
2: 218A Lefevre Hall, University of Missouri-Columbia, Columbia, MO 65211 USA

A single neuronal network requires neuromodulation to generate multiple patterns of output. The role of neuromodulation to network function is viewed to change from an essential component during development to an activating mechanism during the adulthood of an animal. Recent studies suggest that neuromodulation continues to be an essential component to proper network function throughout the lifespan of the animal. A recent stomatogastric ganglion (STG) study showed that these ion channel correlations are sensitive to the loss of neuromodulatory input but did not determine whether correlations were only dependent on this input. Since ion channel correlations have been shown to contribute to unique cellular output, one of the possible mechanisms neuromodulation may determine network function is through regulation of distinct ion channel correlations in specific cell types. It is not known whether relationships between ion channel types are dependent on neuromodulatory input, activity input or both. We used the pyloric network of the STG to determine whether or not correlated channel mRNA levels persists in the pyloric dilator (PD), one of the pacemaker cells of the STG under network preparations that do not have neuromodulation. We collected single PD neurons from different network preparations that decoupled neuromodulatory and activity inputs and quantified channel transcript levels with quantitative real-time RT-PCR. We found that intact neuromodulatory inputs maintained relationships between mRNA channels while activity input alone did not. Addition of muscarinic agonist and modulator, pilocarpine to a decentralized preparation maintained the same correlations as those found in preparations that only had neuromodulatory input. To determine whether these relationships are dependent on the same inputs uniformly across cell types, we repeated the network preparations for another STG cell type, lateral pyloric (LP) and found that the channel mRNA relationships in this cell type depended on different inputs from those of PD. These results indicate that neuromodulation maintains ion channel correlations but not uniformly across cell types. They also suggest a possible novel role of neuromodulation in the regulation of gene expression.

Plant ion channels and their regulation

Organized by Christian Mazars (Toulouse, France)

Monday, september 16th

17:00 Anne-Alienor Very (Montpellier, France)
Functional diversity of cation channels and transporters in rice

17:30 Julian Schroeder (La Jolla, USA)
Guard Cell Ion Channels: Abscisic Acid and Calcium Signal

18:00 Rainer Hedrich (Würzburg, Germany)
Excitement and movement in plants

18:30 Daniel Tran (Gif sur Yvette, France)
Role in symbiosis of medicago root hair mechanosensitive msls channels and clcs anion transporters

S-05

Functional diversity of cation channels and transporters in rice

Anne-Aliénor VERY;
B&PMP, UMR 5004 CNRS/386 INRA/SupAgro-M/UM2, Campus SupAgro-INRA, Montpellier, France

Potassium, which is the most abundant cation in the cytosol, plays essential roles in plants, being involved for instance in control of cell membrane electric polarisation and osmotic adjustments. Plant growth requires that large amounts of K+ ions are taken up by the roots and transported towards all the organs, but the availability of K+ in the soil is limiting in most ecosystems. Na+, in contrast, is not an essential macro-element in most plants and is even toxic at high concentration in cell cytoplasm. It can take part, when efficiently compartmentalised in cell vacuoles, in turgor building and osmotic adjustments but most plant species are unable to control its uptake and/or cell compartmentalisation in high saline conditions. High soil salinity is thus a major abiotic constraint for plant growth and productivity. Several families of ion channels and transporters control the uptake by the roots and the distribution within the plant of K+ and Na+. Systems operating at the plasma membrane comprise K+ channels related to animal Shakers, acting as main cellular K+ influx or release systems, K+ or/and Na+ transporters, from two families named HAK/Kup and HKT, playing important roles in K+ shortage and salt stress conditions and without homologues in animals, and H+/K+ and H+/Na+ antiporters. In rice, a monocotyledonous plant of major agronomical interest, the two transporter families are particularly large, compared to those of the dicotyledonous model plant Arabidopsis. My talk will focus on the HKT family, which comprises 9 members in rice while a single member in Arabidopsis. I will take stock of the diversity of rice HKT transporters at the functional and expression levels and of their (possible) roles in the plant.

S-06

Guard Cell CO2 and Abscisic Acid Signal Transduction in Plants

Julian I. Schroeder, Shintaro Munemasa, Felix Hauser, Noriyuki Nishimura, Honghong Hu, Tae-Houn Kim, Benjamin Brandt, Maria Israelsson-Nordstrom, Aurelien Boisson-Dernier, Dennis Brodsky, Cawas Engineer
Division of Biological Sciences, UCSD, La Jolla, CA 92093-0116, USA

The continuing rise in atmospheric CO2 causes reduction of stomatal pore apertures. Furthermore, the rise in atmospheric CO2 down-regulates stomatal development. Together these CO2 responses affect CO2 influx into plants and transpiration. Under drought conditions, the plant hormone abscisic acid (ABA) triggers stomatal closing. Elevations in atmospheric CO2 and the ABA both trigger signaling in guard cells that converges resulting in ion channel regulation, stomatal closing and plant water loss reduction. However, the CO2 signal transduction mechanisms have remained largely unknown. We have characterized CO2-binding carbonic anhydrases, the SLAC1 anion channel and calcium signaling and phosphorylation mechanisms that are essential for triggering CO2 signal transduction (Hu et al., 2010 *Nature Cell Biol.*; Vahisalu et al., 2008 *Nature*; Negi et al., 2008 *Nature*; Xue et al., 2011 *EMBO J.*). Mechanisms that mediate CO2 and ABA control of plant gas exchange will be presented. The PYR/RCAR proteins were identified as ABA receptors through chemical genetics, protein-protein interactions and in our research through ABI1 protein complex member characterizations (Park et al., *Science* 2009; Ma et al., *Science* 2009; Nishimura et al., *Science* 2009). Using chemical genetics, we have recently identified early effector-triggered immune signaling mechanisms that rapidly down-regulate early Ca2+-dependent abscisic acid signal transduction and ion channel regulation. Recent advances in understanding guard cell signal transduction and ion channel regulation will be presented.

S-07

Excitement and movement in plants

Rainer Hedrich[1]; Felix Bemm[2]; Sönke Scherzer[1]; Elzbieta Krol[1]; Khaled Al-Rasheid[3]; Ines Kreuzer[1]; Erwin Neher[4]; Hubert Bauer[1]; Peter Ache[1];
1: Julius-von-Sachs-Intstitute, University of Wuerzburg, Germany
2: Department for Bioinformatics, University of Wuerzburg, Germany
3: Center of Excellence in Biodiversity Research, Kind Saud University, Saudi Arabia
4: Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

Carnivory is best known from the animal kingdom, but the plant kingdom has flesh eaters as well. The Venus Flytrap (Dionaea muscipula) is a carnivorous, excitable plant recognized as such by Darwin. Prey getting in contact with the touch-sensory organs protruding from the trap surface activate mechanosensitive ion channels and induce action potentials eliciting trap closure in a fraction of a second entrapping the visiting prey. Since the time of Charles Darwin, scientists have struggled to understand the sensory biology of this carnivore. Recently, we could show that prey capture by Dionaea combines plant-specific signaling pathways, involving the touch hormone OPDA and stress hormone ABA that trigger ion channels, action potentials, and Ca2+ signals. Upon recognition of the prey's nature by motion and chemical stimuli, glands on the inner surface of this "green stomach" start to secrete a lytic cocktail. Following prey digestion, the same gland-based endocrine system absorbs the animal-derived nutrients.

On the basis of an ERC Advanced Grant "Carnivorom" we are currently investigating the nature of receptors, channels, and signaling pathways behind the mechano-electric (trap) contraction-coupling and behind the senses of touch and taste. To understand the genomic basis of these remarkable features, we sequenced the Dionaea genome & transcriptome. Based on these resources we will compare the signaling elements in Dionaea glands with those operating Arabidopsis guard cells. For the latter system we have identified the entire ABA biosynthesis pathway. When the relative humidity drops, stomata located in the leaf boundary to the atmosphere are the first to face dry air. Upon perception of dry air guard cells produce their own ABA. The endogenous stress hormone is then recognized by a cytosolic ABA receptor. The underlying fast ABA signalling pathway is initiated when the cytosolic threshold of the stress hormone is passed and binding of ABA to the cytosolic receptor facilitated. The ABA-bound receptor in turn addresses guard cell anion channels. In this process the protein kinases phosphorylate and thereby activate SLAC1. Opening of anion channels depolarizes the plasma membrane and anions and K+ released drag water out of guard cells. In turn, turgor drops and stomata close.

S-08

Role in symbiosis of medicago root hair mechanosensitive msls channels and clcs anion transporters

Daniel Tran[1]; Marjorie Guichard[1]; Lydia Alloul[1]; Tiffanie Girault[1]; Sophie Filleur[2]; Jean-Marie Frachisse[1];
1: Institut des Sciences du Végétal, CNRS UPR 2355, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France
2: Institut des Sciences du Végétal, CNRS UPR 2355, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France - Université Paris-7 Denis Diderot, Paris, France

The root hair plays crucial roles in water and nutrient uptake, and thus in plant development. In legumes, it also plays a central role in the establishment of the nitrogen-fixing symbiotic interaction with rhizobia, which has a crucial importance for sustainable agriculture as it largely contributes to limitation of nitrogen fertilizers. In this study we have addressed the MSL and CLC channels families which might be involved in key processes of symbiosis in the model legume Medicago truncatula. Mechanosensitive ion channels belonging to MSL (Mechanosensitive Small-conductance Like) family represent strong candidates to participate in mechanoperception and thus in processes leading to cell signalling in Arabidopsis (Haswell et al., Current Biol., 2008). In Medicago, the bacterial infection initiated by bacterial adhesion to root hairs will lead to hair curling that entraps the bacteria.The hypothesis of the involvement of physical sensors at the surface of the root hair during early symbiotic interaction is worth considering. CLC genes encode a nitrate and chloride channel/transporter family that we contributed to identify and characterize in Arabidopsis (Barbier-Brygoo et al., Annual Rev. Plant Biol., 2011). Amongst the seven isoforms, AtClCa is involved in nitrogen storage through its ability to accumulate nitrate in the vacuole. Considering the ability of plant legume to establish symbiosis in order to assimilate atmospherical nitrogen when nitrate concentrations are low in soils, the root nitrate channel/transporter equipment of Medicago is worth analyzing. From in silico analysis of Medicago genome, we have identified 8 strong putative MSL candidates divided into two clades, and 6 CLC members distribute into two clades as observed in Arabidopsis. Semi-quantitative PCR indicates that MSL and CLC of clade II, are present in root hair and other root tissues. Preliminary results showed that transient expression of one MSL in a silenced-system provides a channel activity dependant on membrane tension. Further investigations are in progress in order to get a functional characterization of these root hair channels and to obtain an integrated view of their role in early symbiosis. Acknowledgment: This study is supported by a grant (CAROLS) of the French ANR

PLENARY LECTURE

Monday, september 16th
19:00

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John WOOD

(London, UK)

Pain pathways; modality-specific wiring and ion channels

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S-21

Peripheral Pain Pathways

John N Wood;
Molecular Nociception Group, Wolfson Institute for Biomedical Research, University College London, London, UK.

Pain afflicts a fifth of the population; there is an urgent need for new analgesic drugs with minimal side effects. Mechanistic studies in transgenic mice have transformed our understanding of peripheral pain pathways, but the translation of genetic information into useful drugs has been, so far, disappointing. Human monogenic disorders of pain perception have focused attention on voltage-gated sodium channels, particularly Nav1.7 as a potential drug target, because loss of function in this channel leads to pain-free people. However, general analgesia is not desirable for the treatment of chronic pain conditions, because of the dangers of accidental self-harm. Dissecting molecular mechanism linked to particular types of pain is a route to modality-specific pain treatments. We have provided evidence that distinct sets of sensory neurons are involved in different types of pain sensations (Abrahamsen et al. 2008). More recently, we have gained insights into some of the mechanotransducing molecules that may be involved in mechanical hyperalgesia and allodynia, where innocuous stimuli become painful (Eijkelkamp et al. 2013). Surprisingly, a role for the sympathetic nervous system not only in chronic pain, but even acute thermal pain perception has recently been demonstrated (Minett et al. 2012). By comparing a range of chronic pain conditions, (including cancer pain, the pain caused by cytostatic drugs and nerve injury pain) in mice lacking sodium channels in different sets of sensory and sympathetic neurons , we found that phenotypically identical pain conditions arise through different cellular and molecular mechanisms. These observations are important for the analysis of clinical trials data, because they suggest that the mechanistic stratification of pain patients is essential for a rational approach to treatment. Interestingly, the deletion of Nav1.7 in transgenic mice does not compromise the development of pain in a bone cancer model relevant to breast and prostate cancer metastases, or the development of pain caused by the chemotherapeutic agent oxaliplatin. Thus multiple mechanisms contribute to pain, and whilst human phenotypic stratification is an essential start, further mechanistic insights are a prerequisite for the identification of useful analgesic drugs.

We thank the Wellcome Trust, the BBSRC and MRC and ARUK for generous support.

The family of Pannexin proteins: structure and functions of a new class of channels

Organized by Carole Levenes (Paris, France)

Tuesday, september 17th

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8:45 Dale Laird (London, Canada)

The emergence of pannexins as a new class of channels"

9:15 Isabelle Couillin (Orléans, France)

Role of ATP release through connexin/pannexin channels in Nlpr3 inflammasome activation

9:45 Panagiotis Bargiotas (Lubeck, Germany)

Channel forming proteins in cerebral ischemia

10:15 Sarah Lalisse (Montpellier, France)

Organotypic hippocampal slice cultures and microglia

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S-09

The emergence of pannexins as a new class of channels

Dale Laird ; Silvia Penuela;
Department of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada

The mammalian pannexin family of channel-forming proteins consisting of Panx1, Panx2 and Panx3 has received considerable attention in the last dozen years given their newly discovered physiological roles in development and disease. Pannexins exhibit diverse subcellular profiles indicating that they may serve distinct roles in cells and tissues of different origin. These long-lived membrane proteins are typically trafficked through the classical secretory pathway before reaching the plasma membrane, although Panx2 tends to often be retained in intracellular compartments. Trafficking, stability and function of pannexins likely enlist the services of an interactome that continues to expand. We have used a variety of tools from expression constructs and custom antibodies, to knock-out mouse models to evaluate their interplay in normal differentiation and development as well as in malignant transformation and disease. The primary function of pannexins is defined by their capacity to form single-membrane channels that are regulated by post-translational modifications, channel intermixing, and sub-cellular expression profiles. Panx1 is ubiquitously expressed in many mammalian tissues, while Panx2 and Panx3 appear to be more restricted in their expression. Paracrine functions of Panx1 as an ATP release channel have been extensively studied and this channel plays a key role, among others, in the release of "find-me" signals for apoptotic cell clearance. Panx1 channel opening can also be detrimental, contributing to cell death and seizures under ischemic or epileptic conditions. In normal development, Panx2 is involved in differentiation of neurons while Panx3 plays a role in the differentiation of chondrocytes and osteoblasts. Panx1 regulates early stages of skin differentiation, and upon malignant transformation is dysregulated in skin cancer tumors, highlighting the variety of roles that pannexins exhibit in healthy development as well as disease states. This presentation will focus on pannexins as channel forming molecules and how these channels are important in health and disease. Supported by the Canadian Institute of Health Research and the Canadian Cancer Society.

S-10

Role of ATP release through connexin/pannexin channels in Nlpr3 inflammasome activation

Aurélie GOMBAULT[1]; Ludivine BARON[1]; Nicolas RITEAU[1]; Bérengère VILLERET[1]; Florence SAVIGNY[1]; Marc LE BERT[1]; Vincent LAGENTE[2]; François RASSENDREN[3]; Isabelle COUILLIN[1];
1: INEM, CNRS UMR7355/ University of Orleans, Orleans, France
2: UMR991 INSERM/ University of Rennes 1, France
3: IGF, CNRS UMR5203, INSERM U661, University of Montpellier, France

Deposition of uric acid crystals in joints causes the acute and chronic inflammatory disease known as gout and prolonged airway exposure to silica crystals leads to the development of silicosis, an irreversible fibrotic pulmonary disease. Aluminum salt (alum) crystals are frequently used as vaccine adjuvant. The mechanisms by which these crystals activate innate immunity through the Nlrp3 inflammasome are not well understood.
We show that MSU, silica and alum crystals induce maturation and secretion of IL-1beta through a process that involves extracellular release of endogenous ATP (eATP) by hemichannel opening in macrophages. Connexin/pannexin channel blockers reduced significantly both ATP and IL-1beta release indicating these hemichannels are involved in ATP release and subsequent mature IL-1beta secretion. Nevertheless, IL-1beta secretion was not impaired in macrophages from pannexine-1 (Panx-1) hemichannel deficient mice suggesting Panx-1 is dispensable and redundancy between pannexin and/or connexin hemichannels. After release through hemichannels, eATP interacts with specific purinergic receptors or is degraded via different ecto-ATPases to di- and monophosphates and then to adenosine. Those nucleotides are attracting interest as a new class of extracellular mediators released after cellular stress or activation, acting on membrane purinergic receptors (P2X, P2Y or adenosine P1 receptors). Interestingly, we observed that not only ATP but also ADP and adenosine are involved in IL-1beta production upon these Nlrp3 inflammasome activators. In conclusion, our results demonstrate connexin/pannexin hemichannels play a crucial role in ATP release. Extracellular ATP but also ADP and adenosine play a crucial role in the secretion of mature IL-1beta triggered by Nlrp3 inflammasome activators, through both P2 and P1 purinergic receptor signaling. These data provide new molecular mechanisms to explain how danger signals involved in chronic inflammatory diseases differentially activate the Nlrp3 inflammasome.

S-11

Channel forming proteins in cerebral ischemia.

Panagiotis Bargiotas;
University of Heidelberg and University of Lübeck, Germany

In addition to the connexins, the recently discovered pannexins (Px) comprise another family of channel forming proteins. There is increasing evidence that they are able to form functional channels in the membrane and in intracellular compartments, independent of gap junction formation. Although the physiological role of pannexins is still unclear, a considerable number of studies suggested an implication of pannexin-based channels in several pathological conditions. Ischemia opens large-conductance channels, with biophysical properties similar to those of pannexins, leading to neuronal death via ionic dysregulation. This channel activity was blocked by pannexin inhibitors and was completely abrogated in neurons from pannexin-deficient animals. Moreover, the genetic interference with pannexin function leads to reduced infarct size and robustly improves the functional outcome after in vivo cerebral ischemia.

How are pannexins being activated in cerebral ischemia? Several complex molecular mechanisms following cerebral ischemia lead to cellular dysfunction or even death and expansion of the brain damage. Are pannexins involved in the inflammatory cascade and in IL-1β release? How might pannexins influence the glutamate-mediated excitotoxicity, apoptosis or oxidative stress? Do they have a role in cortical spreading depression after ischemia, similar to connexins (e.g. Cx36)?

We are just beginning to uncover the interesting role that channel forming proteins may play in ischemia and the belief that targeting those proteins in a more specific way could be a promising strategy to prevent neuronal death after stroke, gains everyday more and more support.

S-12

Organotypic hippocampal slice cultures and microglia

Sarah Lalisse; Frédéric De Bock; Lauriane Ulmann; François Rassendren;
IGF, 141 rue de la cardonille 34094 Montpellier, France

Microglia plays a critical role in determining the spatial and temporal extent of inflammation in different brain pathologies including epilepsy and neuropathic pain. Purinergic receptors, particularly ATP-gated P2X channels regulate several facets of microglia activation notably through their implication in the release of pro-inflammatory mediators or trophic factors. Among the different P2X receptors, P2X4 seems to play a crucial role in activated microglia. Indeed, following peripheral nerve injury P2X4 expression is induced de novo in activated spinal microglia, where it controls the release of microglial BDNF and the subsequent increase of local network excitability. Similarly, following induction of a status epilepticus, P2X4 is up regulated in hippocampal microglia and P2X4-deficient mice display altered microglial activation and reduced CA1 pyramidal cell death after status epilepticus. In the hippocampus, P2X4 receptors are also expressed in pyramidal neurons where their activation coincides with episode of high electrical activities. The purpose of our work is to elucidate the respective roles of neuronal and microglial P2X4R in the hippocampus following induction of SE. Here, we use a mouse organotypic hippocampal slice culture (OHC) as an in vitro model of status epilepticus, which offers the possibility to manipulate microglia. Our strategy relies on the specific clodronate-mediated depletion of microglia in OHC from wild type mice and their replenishment with microglia from P2X4-deficient mice. To that aim we use the CX3CR1eGFP/+ mice, in which microglia express the green fluorescent protein, and allows for an easy analyze of microglia. Here we present preliminary data showing that an efficient microglial depletion is obtained following a 7 days treatment of OHC with clodronate. We show that microglial depletion has several side effects such as an apparent increase in cell death, activation of astrocytes, both likely due to the reduction of the phagocytic function of microglial and the accumulation of dead cells. We also provide evidence that replenishment of clodronate-depleted microglial OHC can be efficiently obtained.

ORAL COMM. 2 ***(O05-O-08)***

ION CHANNEL MELTING POT

Organized by Fabien Vanden Abeele
(Villeneuve d'Ascq, France)

Tuesday, september 17th

11:00 Rémi Peyronnet (London, UK)

Mechanoprotection by polycystins against apoptosis is mediated through the opening of stretch-activated K2P channels.

11:15 Karima Habbout (Nice, France)

A novel skeletal muscle sodium channel mutation underlays congenital myasthenic syndrome.

11:30 Michel Ronjat (Grenoble, France)

CACNB4 subunit of voltage-gated calcium channels promotes the formation of a nuclear protein platform that controls gene expression.

11:45 Ludovic Bannwarth (Paris, France)

Gating of KIRBAC3.1 channels: structural investigation of mutants.

O-05

Mechanoprotection by polycystins against apoptosis is mediated through the opening of stretch-activated K2P channels

Rémi Peyronnet[1]; Reza Sharif-Naeini[2]; Michel Tauc[3]; Christophe Duranton[3]; Isabelle Rubera[3]; Florian Lesage[2]; Amanda Patel[2]; Eric Honoré[2]; Fabrice Duprat[2];
1: Imperial College London, National Heart and Lung Institute, Heart Science Centre, Harefield UB9 6JH, UK
2: Institut de Pharmacologie Moléculaire et Cellulaire, UMR CNRS 7275, Université de Nice Sophia Antipolis, 06560 Valbonne, France
3: CNRS-FRE 3427, Transport Ionique, Aspects Normaux et Pathologiques Université de Nice-Sophia Antipolis, 06108 Nice Cedex 2, France

How renal epithelial cells respond to increased pressure and the link with kidney disease states remain poorly understood. Pkd1 knockout or expression of a PC2 pathogenic mutant, mimicking the autosomal dominant polycystic kidney disease, dramatically enhances mechanical stress-induced tubular apoptotic cell death. We show the presence of a stretch-activated potassium channel dependent on the TREK-2 K2P subunit in mouse proximal convoluted tubule epithelial cells (isolated cells were obtained following the protocol described in Barriere et al. 20031). Our findings further demonstrate both on isolated cells and on the whole kidney in vivo, that polycystins protect renal epithelial cells against apoptosis in response to mechanical stress, and this function is mediated through the opening of stretch-activated K2P channels. Thus, to our knowledge, we establish for the first time, both in vitro and in vivo using mouse model, a functional relationship between mechanotransduction and mechanoprotection. We propose that this mechanism is at play in other important pathologies associated with apoptosis and in which pressure or flow stimulation is altered, including heart failure or atherosclerosis.

1 Barriere, H., Belfodil, R., Rubera, I., Tauc, M., Lesage, F., Poujeol, C., Guy, N., Barhanin, J., and Poujeol, P. (2003). Role of TASK2 potassium channels regarding volume regulation in primary cultures of mouse proximal tubules. J. Gen. Physiol. 122, 177–190.

O-06

A novel skeletal muscle sodium channel mutation underlays congenital myasthenic syndrome

Karima HABBOUT[1]; Damien STERNBERG[2]; François RIVIER[3]; Sophie NICOLE[4]; Saïd BENDAHHOU[1];
1: University Nice Sophia-Antipolis, LP2M-CNRS, Parc Valrose, Nice, France
2: AP-HP, GH Pitié-Salpêtrière, unité de cardiomyogénétique, Fédération de Biochimie, Paris, France
3: CHU de Montpellier, Hôpital Gui de Chauliac, Service de neuropédiatrie, Montpellier, France
4: Inserm, U975; UPMC-Paris VI, UMRS975; CNRS, UMR7225; CRICM, Paris, France

Mutations of the skeletal muscle voltage-gated sodium channel (Nav1.4) classically cause dominantly-inherited myotonia, and periodic paralysis. One severe case of congenital myasthenic syndrome (CMS) due to an apparently dominant mutation of the SCN4A gene (that encodes for the α-subunit of Nav1.4) has been described in the literature, but this relationship has never been reported in additional cases. CMS is a clinically and genetically heterogeneous group of orphan diseases characterized by muscle weakness and fatigability. CMSs result from the impairment of neuromuscular transmission resulting from mutations in 18 different genes encoding for proteins critical for the neuromuscular junction such as acetylcholine receptors (AChR), AChR-clustering proteins (rapsyn, Dok7, MusK), synaptic basal lamina proteins (ColQ, agrin, laminin β2), and choline acetyltransferase. We have identified a novel homozygous SCN4A mutation (p.R1454W) in a patient with a form of recessively-inherited CMS associated with cold sensitivity. The p.R1454W missense mutation is located on the segment S4 of domain IV of the channel. We have expressed this mutation in the human embryonic kidney 293 cells and analyzed its biophysical properties using the whole cell patch clamp technique. The p.R1454W substitution induces an important impairment of fast inactivation and shifts the activation curve towards depolarizing potentials compared to the wild type. In addition, the mutant channels have a slower inactivation kinetics than the wild type. However, cooling does not aggravate the effects induced by the mutation. A slower current decay combined with a shift in channel availability at rest potentials can ultimately lead to membrane inexcitability and muscle weakness. Altogether, these data demonstrate that this mutation may well be associated with the clinical CMS phenotype reported in the member, but its recessive pattern of inheritance remains to be explored.

O-07

CACNB4 subunit of voltage-gated calcium channels promotes the formation of a nuclear protein platform that controls gene expression.

Michel Ronjat[1]; Shigeki Kiyonaka[2]; Maud Barbado[3]; Katell Fablet[3]; Yasuo Mori[2]; Michel De Waard[1];
1: Unité Inserm U836, Grenoble Institute of Neuroscience, Site Santé, 38700 La Tronche, France. Université Joseph Fourier, Grenoble, France. LabEx Ion Channel Science and Therapeutics
2: Laboratory of Molecular Biology, Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan.
3: Unité Inserm U836, Grenoble Institute of Neuroscience, Site Santé, 38700 La Tronche, France. Université Joseph Fourier, Grenoble, France.

Calcium entering neurons through voltage-gated calcium channels (VGCC) controls expression of numerous genes. Here, we describe a novel signaling pathway in which the VGCC Cacnb4 subunit directly couples neuronal excitability to transcription. Membrane depolarization promotes the association of Cacnb4 with both the regulatory (B56d) and catalytic subunit (PP2A) of the PP2A phosphatase. This complex thus relocates within the nucleus. The human R482X CACNB4 mutation, associated to a form of juvenile myoclonic epilepsy, prevents the formation of the Cacnb4/B56d/PP2A complex and thus its nuclear targeting. Using two hybrid approach, we identified potential partners of Cacnb4 among which the transcription factor thyroid hormone receptor alpha (TRa). We show that Cacnb4 regulates the activity of TRa leading to the repression of transcription of the tyrosine hydroxylase (TH) gene. Absence of Cacnb4 in lethargic mice leads to an overexpression of several genes among which the TH gene, indicating that Cacnb4 acts as repressor of TH gene. We highlighted two levels of regulation of the TH promoter by Cacnb4: i) inhibition of TRalpha activating effect on TH promoter by Cacnb4; ii) dephosphorylation of Ser10 histone H3 by the TRa/Cacnb4/B56d/PP2A complex favoring the interaction of the heterochromatin protein 1 gamma (HP1g) with histone H3. We demonstrate that Cacnb4/HP1g interaction is controlled by B56d. Interaction of HP1g with histone H3 is a well characterized landmark of chromatin remodeling and silencing. This pathway is controlled by the interaction between the SH3 and guanylate kinase (GK) modules of Cacnb4. These findings demonstrate that an intact VGCC subunit acts as a repressor recruiting platform to control neuronal gene expression.

O-08

Gating of KIRBAC3.1 channels: structural investigation of mutants.

Ludovic Bannwarth[1]; Rita de Zorzi[2]; Catherine Vénien-Bryan[1];
1: IMPMC - UPMC - UMR7590 - 75252 Paris cedex 05
2: Harvard Medical School, Boston MA 02115

Inwardly-rectifying potassium (Kir) channels regulate membrane electrical excitability and K+ transport in many cell types where they control such diverse processes as heart rate, vascular tone, insulin secretion and salt/fluid balance. Their physiological importance is highlighted by the fact that genetically inherited defects in Kir channels are responsible for a wide-range of channelopathies. To elucidate how channel function becomes defective in the disease state requires a detailed understanding of channel structure in both the open and closed states. Here we report the structure of a KirBac potassium channel with an open bundle crossing indicating a mechanism of channel gating determined by X-ray crystallography at 3Å resolution. In this model, the rotational twist of the cytoplasmic domain is coupled to opening of the bundle-crossing gate via a network of inter- and intra-subunit interactions [1]. We have also used EM analysis of 2D crystals of the same Kir channel trapped in an open state and compared these results with the 3D structure. Intriguingly, the projection maps from the EM experiments suggest a larger opening of the pore in the 2D crystal form compared to that observed in the 3D crystal structure. The organization of these two crystal forms is different and suggests that the 2D crystals may permit stabilization of an open state structure that is not compatible with 3D crystallization
In addition, we are also currently investigating the structural effect of point mutations in Kirbac3.1 using X-ray crystallography and 2D crystallography as it was reported in the literature that point mutations could be held responsible for various pathologies (Andersen syndrome, Bartter syndrome, neonatal diabetes…). These results not only have major implications for our understanding of the open state structure of the Kir channel, but more importantly they demonstrate the general utility and importance of methods such as electron microscopy and 2D crystallography for the study of membrane protein structure.

[1] BavroVN, De Zorzi R, Schmidt MR, Muniz JR, Zubcevic L, Sansom MS, Vénien-Bryan C, Tucker SJ. Structure of a KirBac potassium channel with an open bundle crossing indicates a mechanism of channel gating Nat. Struct. Mol. Biol. 7, 158–163 (2012)

Symposium 4 (S13-S16)

Molecular and functional diversity of iGluRs

Organized by David Stroebel (Paris, France)

Tuesday, september 17th

17:00 Christophe Mulle (Bordeaux, France)

Activity-dependent plasticity of synaptic NMDA receptors

17:30 Andrew Plested (Berlin, Germany)

Glutamate receptors: fast, loose and full of surprises

18:00 Ivet Bahar (Pittsburg, USA)

Allosteric dynamics and druggability of iGluRs N-terminal Domains: Insights from Computations

18:30 Abdessattar Khlaifia (Marseille, France)

Presynaptic LTD in brainstem synapses involves anandamide

S-13

Specification of glutamate receptors at synapses

Christophe Mulle;
Interdisicplinary Institute for Neuroscience, CNRS, University of Bordeaux

Glutamatergic synapses show a large diversity of structural and functional properties. A single neuron may receive inputs from several distinct sources, and these inputs do not necessarily play the exact same role in synaptic integration. Hippocampal CA3 pyramidal cells receive three main type of excitatory afferents from the dentate gyrus through the mossy fibers (Mf), from the entorhinal cortex through the perforant path, and from CA3 pyramidal cells through the associative commissural fibers (A/C). We show that Mf-CA3 and A/C-CA3 synapses show very distinct composition in glutamate receptors, with a restricted distribution of kainate receptors at Mf-CA3 synapses, and with NMDARs composed of the GluN2D subunit but excluding GluN2B, at variance with A/C synapses. Using a molecular replacement strategy and focal glutamate uncaging, we provide evidence for the molecular mechanisms underlying the subcellular distribution of kainate receptors, which likely depends on a specific N-Cadherin complex. We also show that the subunit composition of NMDA receptors is subject to activity-dependant modifications leading to a subunit switch. Altogether the work to be presented helps undertanding the mechanisms underlying the specification of synaptic properties within a single neuron.

S-14

Glutamate Receptors: Fast, Loose and Full of Surprises

Andrew Plested;

Leibniz Institut für Molekulare Pharmakologie, Berlin 13125 Germany

The glutamate receptors of excitatory synapses are found throughout the mammalian brain, and display remarkable diversity of function. Some glutamate receptor subtypes are activated by glutamate in hundreds of microseconds, and switch off just as rapidly. Other subtypes can remain inactive for more than a minute following activation. These distinct kinetic profiles arise from molecular tuning of a common architecture. Here, I will discuss the conformational transitions that underlie channel gating by glutamate, and the molecular basis for diverse signalling roles of glutamate receptors in the brain.

S-15

Allosteric dynamics and druggability of iGluRs N-terminal Domains: Insights from Computations

Dutta A,[a] Shrivastava IH,[a] Sukumaran M,[b] Greger I[b] and Bahar I*[a]

[a] *Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States;* [b]*Neurobiology Division, MRC Laboratory of Molecular Biology, Cambridge, UK.*

Ionotropic glutamate receptors (iGuRs) encompass a family of tetrameric glutamate-gated channels that mediate the majority of excitatory neurotransmission in the vertebrate central nervous system. The iGuR subunits are modular in nature, composed each of four discrete, domains: the N-terminal domain (NTD), the ligand-binding domain, the transmembrane-domain, and the intracellular carboxyl-terminal domain. Despite accumulating structural and functional data, the mechanism of function of iGluRs, and especially, the role of NTDs in non-NMDA receptors, apart from subunit assembly, remains to be clarified. In NMDA receptors, structural changes at the NTD are known to allosterically modulate channel opening, and channel opening probability is reduced in response to binding of Zn^{2+} and other NTD ligands like ifenprodil. Ligand binding to the clefts of the NTDs of non-NMDA receptors has not been seen so far. The mechanism of function of NTDs in non-NMDA receptors has not been established, either. Utilizing a combination of structural and computational studies, we suggested recently that the NTD of AMPARs may also have an allosteric signaling potential enabled by their intrinsic intraprotomer clamshell motions and interprotomer counter-rotations.[1] A comparative analysis of the collective dynamics of AMPAR and NMDAR NTDs revealed an unexpected parallel between the two subfamilies.[2] Further investigation of druggability properties of AMPAR NTDs, using a recently developed methodology[3] reveals multiple binding sites suggesting new pharmaceutical strategies for controlling iGluRs' functional movements upon targeting AMPAR NTDs' intramolecular signaling mechanisms.[4]

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S-16

Presynaptic LTD in brainstem synapses involves anandamide

Abdessattar KHLAIFIA; Hanad FARAH; Florian GACKIERE; Fabien TELL;
CRN2M-UMR CNRS 7286 Faculte de Médecine secteur Nord 51, Boulevard Pierre Dramard-CS80011 13344 Marseille Cedex 15, France

Presynaptic long-term depression (LTD) of synapse efficacy generally requires coordinated activity between pre and postsynaptic neurons and a retrograde signal synthesized by the postsynaptic cell in an activity-dependent manner. In this study, we examined LTD in the rat nucleus tractus solitarii (NTS), a brainstem nucleus that relays homeostatic information from the internal body to the brain. We found that co-activation of N-methyl-D-aspartate receptors (NMDARs) and type 1 cannabinoid receptors (CB1Rs) induces long-term depression (LTD) at the first central excitatory synapse between visceral fibers and NTS neurons. This LTD is presynaptically expressed. However, neither postsynaptic activation of NMDARs nor postsynaptic calcium influx are required for its induction. Direct activation of NMDARs triggers cannabinoid dependent LTD. In addition, LTD is unaffected by blocking 2-arachidonyl-glycerol synthesis but its induction threshold is lowered by preventing fatty acid degradation. Altogether our data suggest that LTD in NTS neurons may be entirely expressed at the presynaptic level by local anandamide synthesis.

Symposium 5 (S17-S20)

Ion channels, from physiology to pathology

Organized by Sylvain Feliciangeli (Valbonne, France)

Wednesday, september 18th

9:45 Haijun Chen (Albany, USA)

Dynamic ion selectivity of K2P channels: physiology and pathophysiology

10:15 Daniela Pietrobon (Padova, Italy)

Neuronal calcium channel dysfunction and migraine

10:45 Stephane Hatem (Paris, France)

Up and down of potassium channels in cardiac myocytes

11:15 Anne Baron (Valbonne, France)

Mamba toxins reveal new roles for Acid-Sensing Ion channels in pain

S-17

Dynamic ion selectivity of K2P channels: physiology and pathophysiology

Haijun Chen;
Department of biological Sciences, State University of New York, Albany, USA

Abstract: Highly selective K+ channels play a key role in regulation of cellular excitability and electrolyte homeostasis. It was previously thought that ion selectivity of K+ channels, which is mainly determined by the selectivity filter, does not change in response to physiological stimuli. However, our recent studies indicate that two-pore domain K+ (K2P) channels, the newest subfamily of K+ channels, change ion selectivity and permeability in response to physiological or pathophysiological stimuli, demonstrating a new mechanism that regulates cellular function. In pathological hypokalemia that blood K+ concentrations are lower than normal levels, K2P1 (also known as TWIK-1) channels change ion selectivity, become permeable to Na+, and conduct inward leak Na+ currents, so they contribute to a well-known phenomenon in human cardiomyocytes under hypokalemia, which is called paradoxical depolarization. Similarly, K2P1, K2P3, and K2P9 channels also become permeable to Na+ in physiological external acidification. With activation of G-protein-coupled receptors, K2P2 (also known as TREK-1) channels become to conduct glutamate and mediate astrocytic glutamate release. Here we further investigate the mechanisms of why and how K2P1 channels became permeable to Na+ in hypokalemia or acidification and K2P2 channels are able to conduct glutamate. The work provides insights on the molecular basis that K2P channels regulate cellular behaviors by dynamic changes in ion selectivity and permeability.

S-18

Neuronal Calcium Channels Dysfunction and Migraine

Daniela PIETROBON;
Dept. of Biomedical Sciences and CNR Inst of Neuroscience, University of Padova, Italy

CaV2.1 (P/Q-type) calcium channels are located in presynaptic terminals and somatodendritic membranes throughout the brain and play a prominent role in initiating action potential-evoked neurotransmitter release at central nervous system synapses. Gain-of-function mutations in CACNA1A, the gene encoding the pore-forming subunit of these channels, cause familial hemiplegic migraine (FHM), a rare autosomal dominant form of migraine with aura. Cortical spreading depression (CSD) is the neurophysiological correlate of migraine aura. In animal studies, a single CSD can lead to prolonged activation of meningeal nociceptors and central trigeminovascular neurons, suggesting that CSD may also initiate the headache mechanisms. Induction and propagation of CSD are both facilitated in Cacna1a knockin mice carrying human FHM mutations. To gain insights into the primary brain mechanisms leading to increased susceptibility to CSD in FHM we studied synaptic transmission and spontaneous network activity in acute slices of the barrel cortex of wild-type and FHM knockin mice. Here I present findings that reveal roles of CaV2.1 channels and functional consequences of FHM mutations on i) unitary synaptic transmission and short-term synaptic plasticity at the main synapses of cortical microcircuits involving pyramidal cells, fast spiking interneurons and somatostatin-expressing interneurons, and ii) the balance between the total excitatory and inhibitory synaptic drive in individual cortical neurons during spontaneous cortical network activity. The insights into CSD and migraine mechanisms obtained from these studies will be discussed.

S-19

Trafficking of cardiac ion channels : from physiology to diseases

Stéphane Hatem;
UMRS 956 Faculté de médecine Pitié salpétrière Paris France

Cardiac arrhythmias are often associated with profound alterations of the myocardial electrical properties. For instance, during atrial fibrillation, there is a shortening of the action potential which is due to an unbalance between the inward and outward currents that activated during the plateau phase. Notably, potassium currents are maintained or only slightly decreased while the L-type calcium current is markedly reduced. There is an apparent paradox between the maintaining of the potassium currents and the reduced potassium channel subunits both at the transcript and proteins levels in diseased atria. This suggests the activation of post-transcriptional processes that can modulate the surface expression of ion channels. Therefore, there is a major interest to decipher the mechanisms underlying trafficking and surface expression of cardiac ion channels and to understand their involvement in the formation of the arrhythmogenic substrate. Atrial myocytes are continuously exposed to mechanical forces including shear-stress. Here, we report that shear-stress activates a large outward current which is mainly composed of the Kv1.5-encoded current in rat atrial myocyte. The activation of the shear-induced current is associated with a parallel decrease in action potential duration. The shear-induced current is caused by the recruitment of Kv1.5 from sub-membranous intracellular pools to the sarcolemma. The process requires integrin signalling through focal adhesion kinase, an intact microtubule system and relies on intracellular calcium and SNARE proteins. In a rat model of atrial hemodynamic overload and atrial fibrillation, the shear-stress machinery is already activated and could participate to the enhanced Kv1.5 functional expression at the membrane. Thus, pools of Kv1.5 may comprise an inducible reserve of channels which can participate in the tuning of cardiac electrical activity in response to mechanical stress.

S-20

Mamba toxins reveal new roles for Acid-Sensing Ion channels in pain

Anne Baron[1]; Sylvie Diochot[1]; Miguel Salinas[1]; Dominique Douguet[2]; Valérie Friend[1]; Abdelkrim Alloui[3]; Michel Lazdunski[2]; Eric Lingueglia[1];
1: IPMC, CNRS-UNS UMR7275, LabEx ICST, Valbonne, France
2: IPMC, CNRS-UNS UMR7275, Valbonne, France
3: Neuro-Dol, Inserm/Université d'Auvergne, Clermont-Ferrand, France

Peptide toxins isolated from animal venoms are invaluable tools to understand physiological and physiopathological functions of ion channels. We have identified from the deadly venom of the African snake black mamba two 57-aa isopeptides, mambalgin-1 and mambalgin-2, which define a new class of three-finger peptides with the property to specifically inhibit Acid-Sensing Ion Channels (ASICs). ASICs are excitatory Na+ channels largely expressed in the peripheral and the central nervous systems and involved in pain. Mambalgins inhibit all the ASIC channel subtypes expressed in central neurons, i.e. homomeric ASIC1a and heteromeric ASIC1a + ASIC2a and ASIC1a + ASIC2b channels, as well as ASIC1b-containing channels that are specific of sensory neurons. Mambalgins show potent analgesic effects in mice on acute pain and inflammatory hyperalgesia upon central and peripheral injections, without any apparent toxicity. In the central nervous system, the analgesic effects can be as potent as morphine but are resistant to naloxone and do not involve opioid receptors. Mambalgins also seem to produce fewer side effects than morphine. The central analgesic effects are absent in ASIC1a-knockout mice, demonstrating the specificity of the effects and the essential implication of ASIC1a-containing channels. They are also reduced by in vivo silencing of the ASIC2a subunit, which supports the involvement of heteromeric ASIC1a+ASIC2a channels in the central analgesic effects. Subcutaneous peripheral injection of mambalgins in the mice hindpaw also induces analgesic effects that are unchanged in ASIC1a-knockout mice but reduced after in vivo silencing of the ASIC1b subunit in nociceptors, supporting the involvement of ASIC1b-containing channels in these effects. These findings identify a potent role in the central and peripheral pain pathways for different ASIC channel subtypes never associated with pain before (i.e., central heteromeric ASIC1a+ASIC2a channels and peripheral ASIC1b-containing channels), and introduce at the same time new natural peptides that block them and could be of potential therapeutic value.

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POSTERS' ABSTRACTS

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P-01 LACTATE INCREASES TREK1 CHANNEL ACTIVITY

Swagata Ghatak; Aditi Banerjee; Sujit Sikdar;
MBU, IISc, Bangalore- 560012, India

Background: TREK1 is an outward rectifying leak potassium channel that is involved in neuroprotection. Global ischemia increases TREK1 activity in brain and heart (Patel and Honoré, 2001). Greater neuronal damage is observed in TREK1 deficient mice during ischemia (Heurteaux et al, 2004). During cerebral ischemia, 25-30 mM lactate has been shown to reduce neuronal lesions (Berthet et al., 2012).

Objective: Both lactate and TREK1 have been implicated in ischemia. But the effect of lactate on TREK1 channels has not been elucidated. Thus, we wanted to find out if lactate is involved in modulation of TREK1 channel activity by studying the electrophysiology of TREK1 channels both in heterologous system and in in vitro rat hippocampal slices.

Results: Bath application of lactate showed a concentration-dependent increase in the open probability of hTREK1 channel, which reached a saturation at 40 mM lactate, in both whole cell and inside-out excised patches of HEK293 cells. 30 mM lactate decreased the dwell time of single hTREK1 channel in the longer closed state by 50%, while the dwell time in other states was unaffected. Experiments on C-terminal deletion mutants (CTD Δ119, CTD Δ100, CTD Δ89) with 30 mM lactate revealed that only the CTD Δ89 mutant showed increase in activity. Site-directed mutagenesis of histidine at 313th position to alanine abolished the lactate-induced increase in hTREK1 channel activity. Further, whole cell patch clamp studies on CA1 stratum radiatum astrocytes showed 30 mM lactate significantly increased TREK channel activity by 23%.

Conclusion: The experimental observations suggest that lactate increases TREK1 channel activity intracellularly by acting on the histidine residue at 313th position in the C-terminal domain.

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P-02 ESTIMATING THE ENERGY COMPONENTS OF AGONIST INTERACTIONS WITH THE NEUROMUSCULAR ACETYLCHOLINE RECEPTOR-CHANNELS

Iva Bruhova; Anthony Auerbach;
University of Buffalo, Dept. of Physiology & Biophysics, 3435 Main street, Buffalo, USA

The neuromuscular acetylcholine receptor-channel (AChR) is involved in the electrochemical-signalling pathway leading toward muscle contraction. In AChR, αW149, αY190, and αY198, called the aromatic triad (Purohit et al., 2012), are key residues for acetylcholine (ACh) to activate a gating conformation. Ligands resembling the components of ACh, particularly tetramethylammonium (TMA) and choline (Cho), are 'partial' agonists as they cause a weaker response than ACh (by 10 and 552 times in diliganded gating). However, it is unknown how the aromatic triad interacts with Cho and TMA. We used single-channel electrophysiology (mouse α2βδε, HEK cells, -100 mV), kinetic analyses and mutations to estimate the energy for gating from the aromatic triad for Cho and TMA. The comparison of wt (Y or W), F and A mutants activated by ACh, Cho, or TMA allowed us to estimate the energy and dissect the components (OH-group and aromatic group) of the aromatic triad residues. All mutations reduced the energy for all three agonists, in the order of αY190>αW149≈αY198. The αY198F and αY190F mutations produced the same reduction in energy for ACh, TMA, and Cho. For all three agonists, the OH group of αY190 significantly reduced energy for gating by -2.0 kcal/mol, whereas the OH group of αY198 is not important for gating. Dissimilarities between agonists were observed with αW149F and Ala mutants. The mutation αW149F demonstrated that ACh is sensitive to the type of aromatic residue at position 149, whereas Cho is not. The removal of the aromatic group (W/F mutations to A) had a greater effect on ACh and TMA than Cho. We conclude that the aromatic triad provide a significant amount of energy in AChR gating activated by ACh, TMA, or Cho, but in the order of ACh≈TMA>Cho. Supported by CIHR and NIH.

P-03 PANNEXIN1 ARE EXPRESSED IN ZEBRIN POSITIVE BANDS IN THE ADULT MOUSE CEREBELLUM.

David Dubayle[1]; Visou Ady[1]; Véronique Bernard[2]; Valery I. Shestopalov[3]; Carole Levenes[1];
1: Laboratoire de Neurophysique et Physiologie - CNRS UMR 8119, Université Paris Descartes, 45 rue des Saints Pères, 75270 Paris Cedex 06, France
2: Laboratoire de Physiopathologie des maladies du SNC - INSERM U 952, CNRS UMR 7224, Université Pierre et Marie Curie, 9 quai Saint Bernard, 75252 Paris Cedex 05, France
3: Bascom Palmer Eye Institute, University of Miami, Miami, FL, USA

Pannexins, a class of membrane channels, are structural homologs of the invertebrate protein innexins and of the gap junction forming proteins connexins. However, they do not seem to form gap junction in neurons but rather to function as large pore ion channels at the surface of membranes. Their physiological role in the central nervous system is still unknown but they have been implicated in several pathological conditions such as ischemic, excitotoxic and ATP-dependent cell death, inflammasome activation. They are expressed in the cerebellum, particularly in Purkinje cells. Here, using immunochemistry staining, we show that Pannexin1 are not homogeneously distributed in the overall cerebellar Purkinje cells population. Rather, they are distributed along sagittal bands that match that of Zebrin II expression. As for Zebrin II proteins, sagittal section reveals their increasing expression from rostral to caudal lobules. Expression is rare in lobules I-II while lobules IX-X are strongly labelled all over the cerebellum. The specificity of the Pannexin 1 antibody was verified by pre-incubation with immunogenic peptide. Electron microscopy of the cerebellar cortex reveals that Pannexin1 localize to cell membranes including spines and dendrites of Purkinje cells. In some cases, Pannexin1 was located at postsynaptic sites facing climbing fiber terminals. This heterogeneous distribution of Pannexin1 among Purkinje cells makes them new members of the large family of membrane channel proteins distributed along Zebra stripes. This super

family includes ion channels, receptors, transporters and enzymes, that are possible key regulators of the cerebellar activity. Pannexin1 are good candidates to contribute to or synchronize the activity in the Zebra stripes of the cerebellar cortex.

P-04 SYNTHESIS AND CHARACTERIZATION OF A CYCLOOCTAPEPTIDE ANALOGUE OF OMEGA-AGATOXIN IVB ENHANCING THE ACTIVITY OF CAV2.1 CALCIUM CHANNELS ACTIVITY IN CULTURED HIPPOCAMPAL NEURONS

Emilie Pringos; Nadine Crouzin; Mélanie Cavalier; Janique Guiramand; Catherine Cohen-Solal; Jean Martinez; Michel Vignes; Valérie Rolland;
UMR5247 IBMM CNRS UM1 UM2 Montpellier

The structure of the toxin omega-agatoxin IVB, extracted from the venom of funnel-web spider Agelenopsis Aperta, is an important lead structure when considering the design of modulators of synaptic transmission which largely involves P/Q-type (CaV2.1) voltage gated calcium channels (VGCC) at central synapses. Focusing on the loop 2 of the omega-agatoxin IVB that seems to be the most preeminent interacting domain of the toxin with the CaV2.1 VGCC, cyclooctapeptides mimicking this loop were synthesized. While 14Trp is essential for the binding of the neurotoxin to the CaV2.1 VGCC, the substitution of the 12Cys for a glycidyl residue led to a cyclooctapeptide named EP14 able to enhance CaV2.1 VGCC-associated currents measured with patch-clamp recordings and to evoke omega-agatoxin IVA-sensitive intracellular Ca2+ increase as measured by fura-2 spectrofluoroimaging. Furthermore, this cyclooctapeptide was able to potentiate spontaneous excitatory synaptic transmission in a network of cultured hippocampal neurons, consistent with the activation of presynaptic VGCC by EP14. In addition, this peptide did not affect cell survival measured with the MTT assay. Therefore, such new cyclopeptidic structures are potential good candidates for synthesis of new agents aimed at the restoration deficient excitatory synaptic transmission.

P-05 VARIABILITY OF MINIATURE CURRENTS SUGGESTS CO-REGULATION OF AMPA AND NMDA RECEPTOR EXPRESSION IN NUCLEUS TRACTUS SOLITARII GLUTAMATERGIC SYNAPSES

Layal SALIBA; Florian GACKIERE; Virginie PENALBA; Jean-Pierre KESSLER; Caroline STRUBE;
CRN2M, CNRS - Université Aix Marseille, Bd Pierre Dramard, 13344 Marseille

Most excitatory synapses in the central nervous system co-express the two main types of ionotropic glutamatergic receptors, the AMPA receptors (AMPAR) and the NMDA receptors (NMDAR). These two famillies of receptors give rise to postsynaptic currents with very different time courses. While AMPAR activate and deactivate within a few milliseconds, NMDAR generate slow and long lasting currents. Thus, the relative abondance of each receptor type at a given synaptic contact is a key factor that determines its integrative properties. In the present study, we have recorded excitatory post-synaptic currents (EPSCs)

from neurons of the nucleus tractus solitarii, a brainstem sensory relay nucleus, in order to determine the respective contributions of AMPA and NMDA receptors.
Most of the recorded neurons displayed EPSCs with both AMPAR and NMDAR components (iAMPA and iNMDA). Miniature EPSCs recorded from a single NTS neuron had peak amplitude that varied between 5 pA and 60 pA. The amplitude of iAMPA was strongly correlated to the amplitude iNMDA, suggesting that all the synapses from a same neuron have similar proportions of NMDAR and AMPAR. Nevertheless, the iNMDA to iAMPA ratio was highly variable from one neuron to another, ranging from 0 to 0.6 depending on the neuron. Furthermore, recording performed after retrograde labeling showed that NTS neurons projecting to the caudal ventrolateral medulla had lower iNMDA to iAMPA ratios than NTS neurons projecting to the parabrachial nucleus.
These data suggest that postsynaptic factors co-regulate the expression of NMDAR and AMPAR at NTS synapses.

P-06 ALTERATIONS OF EXCITATION - CALCIUM RELEASE COUPLING IN MDX CARDIOMYOCYTES : SURFACE AND INTERNAL MEMBRANES ELEMENTS DISRUPTIONS AT REST AND UNDER STRETCHING

Charlotte LORIN; Elizabeth AGUETTAZ; Mélanie GUEFFIER; Patrick BOIS; Jean-François FAIVRE; Stéphane SEBILLE; Christian COGNARD;
Laboratoire Signalisation et Transports Ioniques Membranaires, FRE CNRS/Université de Poitiers n°3511, Pôle Biologie Santé Bât B36, BP 633, 1 rue Georges Bonnet, 86022 Poitiers, France

A dilated cardiomyopathy (DCM) is associated with Duchenne Muscular Dystrophy (DMD). The loss of dystrophin leads to membrane instability and calcium dysregulation in muscle but effects of such a loss are not elucidated at the cardiomyocytes level. In the present reported work, we studied membrane and transverse tubules damages occurring in ventricular myocytes from the mdx mouse model of DMD and how they impact the function of excitation-calcium movements coupling. At rest, Scanning Ion Conductance Microscopy (SICM) was used to characterize the integrity loss of living mdx cardiomyocytes surface. 2D Fourier transform analysis of labelled internal networks (transverse tubules, alpha-actinin, dihydropyridine receptors, ryanodine receptors) was performed to evaluate internal structure alterations. During calcium measurements, "smart microperfusions" of depolarizing solutions were applied through the SICM nanopipette, stimulating single tubules elements. These approaches revealed structural membrane surface and transverse tubules disorganization in mdx as compared to control. These disruptions were associated with functional alterations (increase of calcium signal duration and of sparks frequency). In DCM associated with DMD, myocytes display evident membrane alterations at the surface level but also in the cell depth as observed in other cases of heart failure. Similar experiments are initiated (work in progress) in cardiomyocytes stretched by means of carbon fibres in order to assess the consequences of these disturbances during mechanical constraints. In summary it can be hypothesised that these disruptions may play a role in calcium dysregulation through excitation-contraction coupling mechanism perturbation and suggest a transverse tubules stabilizing role for dystrophin.

P-07 CHARACTERIZATION OF IKA CURRENT IN THE NUCLEUS OF THE SOLITARY TRACT

Caroline STRUBE; Layal SALIBA; Estelle MOUBARAK; Virginie PENALBA; Marie-France MARTIN-EAUCLAIR; Fabien TELL; Nadine CLERC;
CRN2M, Faculté Médecine Nord, Bd Pierre Dramard, 13344 Marseille Cedex 15, France

Neuronal transient A-type potassium currents (IA) play a major role in the integration of neuronal information, and its amplitude variations is a conditioning factor of the fidelity of synaptic transmission. It has been shown that the amplitude of IA is highly variable in neurons from the nucleus of the tractus solitarius (NTS, a brainstem sensory relay nucleus) but the molecular identity of the channel underlying this current has never been determined. In order to identify which Kv channels underly IA in the NTS, the gating properties and the pharmacology of the Kv channels were determined using whole cell patch clamp recordings in slices. Complementary information was brought by immunohistochemistry. Activation and inactivation of IA occurred at membrane potentials around the threshold for generating action potentials, and the rate of recovery from inactivation was rapid and voltage dependent. These gating properties were typical of the Kv4 channel subfamily but did not fit with those of Kv1.4, Kv3.3, Kv3.4. Remarkably, the time course of the inactivation varied in a large range, from 3 to 655 ms. In addition, the inactivation dependence of IA displaying fast inactivation was U-shaped, as previously described for Kv4 channels expressed with Kv-channel interacting proteins (KChIPs). Pharmacologically, AmmTX3 (10-6M), a scorpion toxin of the α-KTX15 family that has been shown to block all the members of the Kv4 family (Maffie et al. 2013) blocked 80% of IA whatever was its inactivation kinetics. Finally, Kv4 expression in the NTS was confirmed by immunohistochemistry, which demonstrate that Kv4.2 is under detection level whereas Kv4.3 is differentially expressed throughout the NTS. Altogether, our data show that IA in the NTS is mainly carried by Kv4 channels, and likely by the sub-unit Kv4.3.

P-08 CALCIUM-OSCILLATIONS AND CALCIUM-DEPENDENT ACTIVATION OF RHODOPSIN GENE TRANSCRIPTION IN CULTURED RETINAL PRECURSORS FROM CHICKEN EMBRYO

Camille Dejos; Marianne Bernard; Matthieu Regnacq; Thierry Bergès; Pierre Voisin;
IPBC - FRE 3511 CNRS - Université de Poitiers Bât. B36 - Pôle Biologie Santé 1, rue Georges Bonnet - BP 633 86022 Poitiers Cedex

Introduction : A striking feature of photoreceptor differentiation in rodent and avian retinas is the 8-10 days lag between the exit from mitosis and the expression of opsin genes. In the chicken retina, photoreceptors exit mitosis around embryonic day 6 (E6) and they immediately express visinin (a photoreceptor-specific calcium-binding protein), suggesting they are rapidly committed to a photoreceptor fate. Yet, opsin gene expression cannot be detected before E14 (LWS opsin) or even E16 (rhodopsin). It is not known whether this time lag reflects the implementation of a differentiation program or the need for a signal that activates opsin gene transcription.

Methods : Chicken retinal precursors were placed in culture either at E8 or at E13. Transcriptional activation of the rhodopsin gene in the cultured cells was monitored by real-

time RT-PCR and by rhodopsin promoter-driven luciferase activity from a transiently transfected plasmid. Oscillations in Ca2+ concentration were monitored by Fluo8 fluorescence on a spinning disc laser microscope. Cell cultures were treated with drugs that modify calcium signalling and pacemaker channel activity.

Results : In retinal precursors isolated at E8, rhodopsin gene transcription was not spontaneously activated during a 4-day culture, but was efficiently stimulated by membrane depolarization (KCl or veratridine). Retinal precursors isolated at E13 spontaneously activated rhodopsin gene transcription in 4-day cultures. This spontaneous expression of the rhodopsin gene was efficiently blocked by calmodulin-dependent kinase inhibitors (KN62 and KN93) and by general and T type-selective Ca2+ channel blockers (Cd2+, Ni2+, bepridil, mibefradil, ML218). In contrast, Ca2+ channel blockers of other selectivities had no effect. The primary event in Ca2+-dependent activation of rhodopsin gene transcription could be the pacemaker activity of HCN channels because it was blocked by ZD7288. Correlatively, Ca2+ oscillations were observed in retinal precursors cultured at E13 and were inhibited by ZD7288.

Conclusions : Rhodopsin gene expression in cultured retinal precursors is activated by Ca2+ signalling. Destabilization of the membrane potential by an HCN channel may be the origin of the Ca2+ signalling cascade that activates rhodopsin gene transcription. It is not known at this point whether the entire cascade is cell-autonomous or if it requires intercellular communication.

P-09 THE NATURAL VASORALEXANT AGENT DODONEINE AND ITS ANALOGUES INHIBIT L-TYPE CALCIUM CURRENT IN THE CARDIOVASCULAR SYSTEM.

Gregoire Carre[1]; Helene Carreyre[2]; Maurice Ouedraogo[3]; Frederic Becq[1]; Patrick Bois[1]; Sebastien Thibaudeau[2]; Clarisse Vandebrouck[1]; Jocelyn Bescond[1];
1: Institut de Physiologie et de Biologie Cellulaires IPBC - FRE 3511 CNRS - Université de Poitiers
2: Synthese et Reactivite des Substances Naturelles, Université de Poitiers, CNRS/UMR 6514
3: Institut de Recherche en Sciences de la Santé, Université de Ouagadougou, Burkina Faso.

Background and objectives: Dodoneine (Ddn) is a new dihydropyranone isolated from Agelanthus dodoneifolius, an African pharmacopeia traditional medicinal plant used for the treatment of hypertension. We have previously shown that Ddn exerts hypotensive, vasorelaxant and negative inotropic effects, respectively on anesthetized rats and isolated organs. The aims of this study were (i) to characterize the molecular target on isolated cardiac ventricular myocytes and aortic smooth muscle cells, and also (ii) to investigate the effect of Ddn analogues produced with some structural modifications performed by using direct superacid catalyzed reactions.

Experimental approach: In-vitro, the effects of Ddn were characterized on freshly dissociated cardiac ventricular myocytes and on aortic smooth muscle cell line (A7r5) using the whole-cell patch-clamp configuration. Ex-vivo, vascular response to the dodoneine and its derivatives were measured on aortic rings in an organ bath apparatus.

Key results: Both in ventricular myocytes and vascular smooth muscle cells, Ddn dose-dependently reduced ICa,L density of about 30 % with an IC50 value estimated at 1 μM. Ddn did not change current voltage relation but it shifted the inactivation curve toward negative potentials and modified the half inactivation potentials. Furthermore, Ddn induced a phasic-dependent blocking on ICa,L. Among Ddn's derivatives, bicyclic-OH Ddn appears to be the most effective by inducing the vasorelaxation of precontracted aortic rings with an IC50 of 15 ± 1 μM and reducing ICa,L density of about 40 % with an IC50 value estimated at 0.5 ± 0.2 μM.

Conclusions: Our study demonstrates for the first time that Ddn is a new natural L-type Ca2+ current inhibitor. The produced analogues should permit to define more precisely the action mode of this new kind of calcium blockers.

P-10 INVOLVEMENT OF MSCs LIKE MECHANOSENSITIVE CHANNELS IN PLANT SIGNALING

Tiffanie Girault; Daniel Tran; Jean Marie Frachisse;
Institut des Sciences du Végétal, CNRS UPR 2355, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

Plants are constantly subjected to mechanical stimulations from their surrounding environment. These solicitations may vary in length, frequency and intensity depending on their origin. They are relayed in natural condition by a large scale of mechanical stimuli from the slight touch produced by an insect to damages produced by a storm. In all these situations, forces applied to the plant will produce deformations of the tissues. These deformations are transduced into internal signals and thus will induce an adaptive growth response called thigmomorphogenesis. In plant, the molecular nature of protein mediating mechanoperception is still elusive. Mechanosensitive ion channels belonging to MSL (Mechanosensitive Small-conductance Like) family represent strong candidates to be involved in mechanoperception and cell signalling. MSL proteins have been recently characterized in Arabidopsis in our laboratory [1] [2]. These proteins provide a mechanosensitive channels activity, anion selective, elicited by plasma membrane stretching. Using the GUS reporter gene, we have shown that MSL4, MSL5, MSL6, MSL9 and MSL10 have a wide range of expression from root to rosette leaves and interestingly, for two members, the expression is enhanced by wounding. Based on approaches combining q-PCR, patch clamp and extracellular potential recording, we have evaluated the putative role of MSL in local and transmitted response to mechanical stimulation.
Data concerning the function of MSL channels and new insight associated to biophysical properties of these channels will be presented and discussed.
[1] Haswell, E. et al. (2008). Curr. Biol., 18: 730-734
[2] Peyronnet, R., et al. (2008). Plant Signaling & Behavior, 3: 726-729.
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P-11 TWO TYPES OF VACUOLAR CATION-SELECTIVE CHANNELS IN THE MOSS PHYSCOMITRELLA PATENS

Mateusz Koselski; Kazimierz Trębacz; Halina Dziubińska;

Department of Biophysics, Institute of Biology and Biochemistry, Maria Curie-Skłodowska University, Akademicka 19, Lublin, 20-033, Poland

The patch-clamp technique was used to investigate vacuolar ion channels in the moss Physcomitrella patens. Measurements carried out in the whole vacuole configuration in the symmetrical (in the bath and in the pipette) concentration of 100 mM KCl and 2 mM CaCl2, allowed recording currents with different kinetics. Slow activated currents were observed at positive voltages, and fast currents at negative and partially at positive voltages. Both the currents were carried by K+, because the reversal potential obtained in the KCl gradient was close to the reversal potential to K+ (EK). The fast activated currents were inhibited after replacement of K+ with Na+. Such results indicate that the fast currents are the effect of the activity of K+-selective VK channels (Vacuolar K+-selctive channels), which have been so far recorded only in vacuoles of higher plants. Unlike the fast activated currents, slow currents did not vanish in the presence of Na+ instead of K+. In the symmetrical concentration of Na+, only positive slow activated currents were recorded, indicating their outward rectifying abilities. The above features of the whole-vacuole currents are attributed to SV channels (Slowly activating Vacuolar channels) – the best examined plant vacuolar channels permeable to many cations including Na+ and K+. Recordings of single channel activity in the cytoplasm-out configuration carried out at different concentrations of cytoplasmic Ca2+ [Ca]cyt, revealed another difference between VK and SV channels in Physcomitrella. The results proved stronger [Ca]cyt dependence of SV channels than VK channels. Ca2+ dependence of VK channel activity was checked by removal of Ca2+ from both sides of the tonoplast. Such conditions caused a decrease in the open probability of VK channels, but did not completely inhibit their activity.

P-12 FUNCTIONAL PROPERTIES OF TWO GAIN-OF-FUNCTION MUTANTS OF egl-19, WHICH ENCODES THE α1 SUBUNIT OF THE L-TYPE Ca2+ CHANNEL IN C. ELEGANS

Viviane Lainé; Jean Rony Segor; Maëlle Jospin;
CGPhiMC, CNRS UMR5534, 16 rue Raphael Dubois, 69622 Villeurbanne, FRANCE

L-type voltage-gated calcium channels play fundamental role in excitation-contraction coupling of striated muscle cells. Even small perturbations in the channel function may dramatically alter the development and the cell homeostasis: mutations in the genes encoding these channels cause congenital pathologies, like hypokalemic periodic paralysis or Timothy syndrome. The effects of the mutations are not well understood, partially because of the difficulty to express these channels in heterologous systems. The use of Caenorhabditis elegans could be an alternative approach to study in vivo the consequences of mutations on muscle function, since i) a functional L-type voltage-gated calcium channel is expressed in striated muscles, and ii) more than 150 mutants of the α1 subunit of the channel, encoded by egl-19, are available. Among these mutations, two have been so far described as gain-of-function, and one is concerning a residue modified in patients affected by the Timothy syndrome. By coupling phenotypic analyses and in situ electrophysiological recordings, we characterized in vivo the effects of these gain-of-function (gf) mutations within egl-19. We show that egl-19(gain-of-function) have defects in locomotion linked to a high muscle tone,

which is due to a shift of the voltage-dependence towards negative potentials, associated with slowed-down inactivation of calcium currents.

P-13 EFFECT OF HYPOXIA ON TRPV1 AND TRPV4 CHANNELS IN INTRAPULMONARY ARTERIES

Thibaud Parpaite; Guillaume Cardouat; Marthe Mauroux; Jennifer Gillibert-Duplantier; Roger Marthan; Jean-Pierre Savineau; Thomas Ducret;
Centre de Recherche Cardio-Thoracique de Bordeaux, INSERM U1045, Université Bordeaux Segalen, Bordeaux, France

Pulmonary hypertension (PH) is often defined as a small pulmonary artery disease characterized by increased pulmonary vascular resistance, leading to right ventricular heart failure and ultimately death. PH subtypes of group 3 are, in part, related to a prolonged hypoxia exposure. During hypoxia, hypoxic pulmonary vasoconstriction allows the efficiency of gas exchanges by increasing the intraluminal pressure. This leads to stretch smooth muscle cell membranes inside the vascular wall and thus can activate stretch-activated channels (SAC) among them transient receptor potential vanilloid channels (TRPV). It has been previously shown that TRPV1 and TRPV4 are implicated in proliferation and migration of pulmonary vascular cells. Furthermore, these two TRPV isoforms are overexpressed in case of PH. Whether these features are directly caused by hypoxia alone or are a consequence of stretch is a matter of debate. In the present study, we thus investigated the role of hypoxia independently to the stretch effect, conditioning rat pulmonary arteries smooth muscle cells (PASMC) with 1% O2 during 48h. Using microspectrofluorimetry (indo-1), we observed that hypoxia (1% O2 during 48h) was sufficient to significantly increase the calcium response to 5 μM 4α-phorbol-12,13-didecanoate (4α-PDD), but not to 10 μM capsaicin, selective agonists of TRPV4 and TRPV1, respectively. Using immunofluorescence experiments, we showed that TRPV1 and TRPV4 activation triggered the Ca2+/calcineurin/NFATc4 pathway. Furthermore, hypoxia induced relocalization of TRPV1 from intracellular stores to the plasma membrane, and remodelling of the cytoskeleton. Finally, measurement of PASMC migration (wound-healing migration assay), proliferation (BrdU) and cell viability (Wst-1) showed a direct effect of hypoxia on TRPV1- and TRPV4-induced migration. In conclusion, these data point out, for the first time, the direct effect of hypoxia on TRPV1 and TRPV4 channels in rat PASMC.

P-14 CHARACTERIZATION OF A NOVEL MOUSE MODEL TO STUDY THE ZINC MODULATION OF CAV3.2 T-TYPE CALCIUM CHANNELS

Tiphaine Voisin; Emmanuel Bourinet; Philippe Lory;
IGF, Montpellier

T-type Cav3.2 channels are voltage-gated calcium channels that play an important role in controlling neuronal excitability, particularly in dorsal root ganglia (DRG) neurons where they are involved in pain signaling. Cav3.2 channels are also expressed in thalamic and hippocampal neurons and likely participate to epileptic seizures. How Cav3.2 channels are regulated in neurons is therefore important to understand. Interestingly, studies on recombinant Cav3 channels have revealed that Cav3.2 channels are preferentially inhibited by low concentrations of zinc (IC50 ≈ 0,8 μM), and that the mutation of a single histidine residue

at position 191 severely attenuated zinc sensitivity of Cav3.2. In order to study the zinc action on Cav3.2 channels in vivo, which is hypothesized to impact the activity of DRG neurons, we have generated a knock-in mouse carrying this mutation (H191Q). Electrophysiological studies were done on a specific type of DRG neurons, D-hair cells. We found that these neurons from the H191Q-Cav3.2 mice have lost their sensitivity to zinc. To investigate further the role of this modulation in vivo, we conducted behavioral studies. These H191Q-Cav3.2 mice showed no alteration of their locomotor phenotype. Experiments to assess the pain phenotype on this animal model are ongoing. Of interest, the KI mice showed an increased reaction to inflammatory pain (formalin test). Additional experiments to characterize further the pain phenotype of the H191Q-Cav3.2 mice will be presented.

P-15 ROLE OF CAV3.2 T-TYPE CALCIUM CHANNEL AND ITS REGULATORY SUBUNITS IN PROSTATE CELL PROLIFERATION

Marine Warnier; Morad Roudbaraki; Natalia Prevarskaya; Pascal Mariot;
Laboratoire physiologie cellulaire INSERM U1003

Prostate cancer, the third cause of death by cancer in men, depends on androgens in its early stages. However, after few years of anti-androgen therapy, the disease resumes its progression towards an androgen-refractory and aggressive stage. In addition, we have shown that the progression of prostate cancer is accompanied by the appearance of cancer cells overexpressing the Cav3.2 subunit of voltage-activated T-type calcium channels (T-channels). We have established that the overexpression of Cav3.2 channels favored prostatic acid phosphatase secretion and other mitogenic factors responsible for the proliferation of neighboring epithelial cells. Although it has been shown previously that T-channels in the prostate consist of a Cav3.2 pore subunit, T-channels multimeric structure still remains unknown. This channel could be constituted by a pore subunit (α1H) associated with accessories subunits ($\alpha 2\delta$, β, γ) as described for other high voltage-activated calcium channels.
We carried out in this study a screening of potential regulatory subunits for voltage-activated calcium channels in prostate tissues and cancer cell lines. We demonstrate the expression of a $\alpha 2\delta 2$, $\beta 4$ and $\gamma 4$ regulatory subunit in prostate tissue and various prostate cancer cell lines. We show that the $\alpha 2\delta 2$ subunit is more frequently expressed in cancerous tissue than in non-cancer tissues. Using immunofluorescence, immunoprecipitation and patch-clamp techniques, we show for the first time that Cav3.2 and $\alpha 2\delta 2$ subunits belong to the same protein complexes, thereby forming functional T-channels in prostate cells, and that $\alpha 2\delta 2$ modulates the activity of this channel leading to an increased cytosolic calcium concentration. In addition, using overexpression and siRNA approaches, we demonstrate the stimulatory role of these $\alpha 2\delta 2$ and Cav3.2 subunits in proliferation of prostate cancer cell lines. Thus, our study leads to a better understanding of the involvement of T-type calcium channels in prostate cancer. It remains to clarify the mechanisms by which these proteins activate proliferation and particularly to determine whether the association of Cav3.2 channel with other subunits is required for cell proliferation regulation

P-16 STUDY OF THE ANTICONVULSANT AND ANTIEPILEPTIC ACTIVITIES OF MEDICINAL PLANT EXTRACTS IN A ZEBRAFISH-SEIZURE MODEL AND THEIR EFFECT ON T-TYPE CALCIUM CHANNEL ACTIVITY

Chaymae El Alaoui[1]; Adèle Faucherre[1]; Christian Jopling[1]; Taoufiq Fechtali[2]; Philippe Lory[1];
1: Institut de Génomique Fonctionnelle ; CNRS UMR 5203, INSERM, U661; Université de Montpellier, Montpellier, France
2: Laboratoire de Neurosciences, Physiopathologies intégrées et substances Naturelles; Faculté des Sciences et Techniques Mohammedia, Université Hassan II Mohammedia-Casablanca, Maroc

Epilepsy is a widespread neurological disorder that affects nearly 300.000 people in Morocco, 500.000 people in France and up to 65 million people worldwide. It is characterized by the occurrence of excessive and hypersynchronous electrical discharges (seizures) of neurons in the brain. T-type calcium channels, a subfamily of voltage-gated calcium channels, appear to contribute to seizure genesis. Drug side effects and interactions, drug resistance, and narrow therapeutic index are all major problems that are facing epilepsy treatment. Therefore, the identification of novel active substances would be of great asset for people with epilepsy. Medicinal plants represent a significant reservoir of unexplored substances for early-stage drug discovery. Here, we have investigated the effects of methanolic extracts of Mediterranean plants and of their corresponding purified active compounds (e.g. harmaline from Peganum harmala). We describe substances that inhibit voltage-gated T-type calcium channels in a concentration-dependent manner, using the whole-cell configuration of the patch-clamp technique. Using zebrafish larvae, we report that valproic acid, an AED clinically in use, as well as TTA-A2, a selective blocker of T-type calcium channels, could suppress convulsant drug-mediated larval movements. The anticonvulsant / antiepileptic activity of the Mediterranean plant substances is currently evaluated using this paradigm in zebrafish larvae.

P-17 QUINIDINE, RANOLAZINE AND TTX MODULATE NOREPINEPHRINE-INDUCED AUTOMATIC ACTIVITY IN RAT PULMONARY VEIN CARDIOMYOCYTES: ROLE OF NA CHANNELS

Claire O. MALECOT; Pierre BREDELOUX; Ian FINDLAY; Véronique MAUPOIL;
CNRS FRE 3511, Institut de Physiologie et de Biologie Cellulaires, Physiologie des Cellules Cardiaques et Vasculaires, Faculté des Sciences, Parc de Grandmont, F-37200 TOURS

Ectopic activity arising from pulmonary vein (PV) cardiomyocytes plays a prominent role in the onset of atrial fibrillation (AF) in humans. In rat PV cardiac muscle, the superfusion of norepinephrine (NE) induces an automatic activity occurring in bursts (catecholaminergic automatic activity, CAA). Because the interburst membrane potential varies within the limits of the Na window current, we assessed the effects of Na channel blocking drugs (quinidine - QUIN -, ranolazine - RAN - and Tetrodotoxin - TTX) upon (i) CAA in isolated rings and strips of rat PVs (contractility and intracellular microelectrode recordings) and (ii) whole-cell INa in isolated rat PV cardiomyocytes.
QUIN and RAN (1-10μM) decreased CAA incidence in a concentration-dependent manner. Total arrhythmia duration over a 10min period was reduced due to an increase in the interval between bursts and also a decrease in burst duration for RAN. TTX (10μM) reduced burst duration and increased interval. Only RAN could entirely abolish CAA, leaving a quiescent membrane potential of ~-50mV. To explain these differences, we investigated the effects of these three compounds on INa. At 1Hz, QUIN, RAN and TTX (10μM each) inhibited peak INa at -20mV by 29, 28 and 98%, respectively. There was no evidence for a TTX, QUIN or

RAN-sensitive late INa under our experimental conditions. Na conductance (gNa) was best described by a double Boltzmann function. QUIN decreased both gNa components and RAN decreased only the gNa component activating at negative potentials. Both QUIN and RAN induced a negative voltage shift (2.8 and 2mV, respectively) of INa steady-state availability curve. In conclusion, Na channels modulate CAA in rat PV myocardium. Reduction of the Na window current appears more important than the amount of peak current block. RAN also has an anti α1-adrenergic effect.

P-18 HUMAN INDUCED PLURIPOTENT STEM CELLS FROM ANDERSEN'S SYNDROME PATIENTS: IMPLICATION OF A POTASSIUM CHANNEL IN BONE MORPHOGENESIS

Jonathan PINI[1]; Matthieu ROULEAU[1]; Sabrina SACCONI[2]; Claude DESNUELLE[2]; Saïd BENDAHHOU[1];
1: University Nice Sophia-Antipolis, LP2M-CNRS, Parc Valrose, Nice, France
2: Centre de référence maladies neuromusculaires, CHU de Nice et UMR CNRS 7277 / IBV Faculté de Médecine de Nice

Andersen's syndrome is a rare disorder characterized by a triad of symptoms: periodic paralysis, cardiac arrhythmia and dysmorphic features. Patients with Andersen syndrome have bone developmental defect as craniofacial features, dental and skeletal anomalies. Most of the patients have a mutation in the KCNJ2 gene that encodes for the inward rectifier potassium channel Kir2.1. This mutation leads to a loss of function with a dominant-negative effect. Kir 2.1 channels play a major role in maintaining the rest membrane potential in excitable cells such as cardiac and skeletal muscle cells. The electrical characteristics of mutations in Kir2.1 are well known but the implication of this channel in the bone development is actually unknown. KCNJ2 knockout mice are born with a cleft palate and die few hours after birth preventing further investigations. To investigate the molecular determinants that require Kir2.1 channels in bone morphogenesis, we use human myoblasts to generate induced pluripotent stem (iPS) cells. The iPS cells are adult cells, which are reprogrammed to an embryonic stem cells state. These cells could proliferate without differentiation and give all cell types from the three germ layers. We have generated iPS cells from healthy as well as Andersen's syndrome patient muscular biopsies (taken from Vastus lateralis). We infected adult patient myoblasts with lentivirus, which contain the 4 genes required reprogramming (Oct4, Sox2, Klf4 and cMyc). The cells obtained after infection have the characteristics of classical iPS cells and embryonic stem cells. They express pluripotent, genetics, as well as surface markers. These cells could also differentiate into the three germ layers by the embryoid bodies formation and express specific markers of ectoderm, endoderm and mesoderm. To go further in the bone development, we differentiated patient and control iPS cells into the mesodermal pathway, and mesenchymal stromal cells obtained from both iPS cells exhibit the same characteristics. Altogether, our results show that Kir2.1 channels are not important for the reprogramming process and for the differentiation into mesenchymal stromal cells. The iPS cells from Andersen patients may provide a good tool to study the implication of the Kir2.1 potassium channels in bone development.

P-19 FUNCTIONAL CHARACTERIZATION OF A RICE SHAKER K+ CHANNEL IN XENOPUS OOCYTES

Limin Wang; Meriem Daly; Hervé Sentenac; Anne-Aliénor Véry ;
B&PMP, UMR 5004 CNRS/386 INRA/SupAgro-M/UM2, Campus SupAgro-INRA, Montpellier, France

Plant Shaker channels have been shown to play major roles in inwardly or outwardly directed transports of K+ through the plasma membrane. AKT1, an inwardly rectifying Shaker channel from the model plant Arabidopsis is in particular strongly active in root peripheral cell layers where it plays a main role in potassium uptake from the soil, thus contributing importantly to plant growth. Here, we were interested in the rice homologue of AKT1, OsAKT1, with the aim to further analyze, in this essential food crop, the channel-mediated mechanism of plant K+ uptake. Performing heterologous expression of OsAKT1 in Xenopus oocytes, we were able to unravel the rice Shaker channel electrophysiological properties. The results showed that OsAKT1 is, as expected, a voltage-gated inwardly-rectifying K+ channel, transporting K+ with low affinity (half maximal conductance for a K+ concentration close to 30 mM). It is not regulated by external Ca2+, slightly sensitive to external pH (50% of conductance decrease upon pH change from 5 to 7) and strongly blocked by Cs+. OsAKT1 is, like other Shaker channels, K+-selective, but provides also a significant pathway to NH4+, which may have physiological relevance in this crop species growing in NH4+-rich environment.

P-20 INTER-SUBUNIT INTERACTIONS IN THE ECTODOMAIN ARE IMPORTANT FOR ACID-SENSING ION CHANNEL ACTIVITY

Karolina Gwiazda; Stephan Kellenberger;
Department of Pharmacology and Toxicology, rue Bugnon 27, 1005 Lausanne, Switzerland

Acid-sensing ion channels (ASICs) are voltage-independent, proton-activated channels. They are implicated in the expression of fear, pain perception and diseases associated with a tissue acidification. ASIC subunits are composed of two transmembrane domains, intracellular N- and C-termini and a large ectodomain with the sub-domains palm, thumb, finger, knuckle and β-ball. Three subunits are required to form a functional channel and interactions between subunits are important for the assembly and stability of the channel. Here, we focused on inter-subunit interactions in the ectodomain and their importance for channel function. To this aim we identified inter-subunit residue pairs in the crystal structure that were within a distance of 5 Å and mutated them to Cys in human ASIC1a. Oxidation/reduction treatments were applied and functional consequences of knuckle-β-ball and palm-thumb interactions of adjacent subunits were investigated. Oxidation-induced, inter-subunit disulfide bond formation between the palm and the thumb domains affected ASIC1a function. Oxidation led to a significant decrease in current amplitude, in part due to an acidic shift of ASIC1a activation. This permanent effect was only reversed by the treatment with a reducing agent. Thus, our data indicate that disulfide bond formation between palm domain of one subunit and the thumb of another favors the closed/inactivated state of ASIC1a. Another relevant inter-subunit interaction was identified between residues of the knuckle and the β-ball of adjacent subunits. The corresponding ASIC1a double Cys mutant presented a leak current (~10% of maximal pH-activated current amplitude), unlike WT ASIC1a-expressing cells, suggesting that a disulfide bond was formed during the expression phase and locked a

proportion of the channels in an open conformation. This leak current was prevented by incubation in a medium containing a reducing agent and was not observed in the single Cys mutants. In summary, this study identifies functionally relevant inter-subunit interactions between the knuckle and β-ball as well as the palm and the thumb domains of ASIC1a.

P-21 A CROSSLINKING STUDY OF ASIC1 SHOWING A MYSTERIOUS TETRAMERIC CHANNEL COMPLEX

Delphine Huser; Ivan Gautschi; Laurent Schild;
Department of Pharmacology & Toxicology, University of Lausanne, Switzerland

Acid-sensing ion channels (ASICs) are proton-gated cation channels that belong to the ENaC/Deg family of non-voltage-gated cation channels. ASICs are expressed in the central and peripheral nervous system and play a role in learning, fear-related behavior and various pathological situations such as brain ischemia or inflammation associated pain. ASIC1 has been crystalized as a homotrimer; each subunit is composed of a large extracellular domain between 2 transmembrane α helices arranged pseudosymmetrically around a collapsed non-conducting pore, presumably in a desensitized state. The subunit oligomerization of the functional ASIC1 at the cell surface has not been specifically addressed. We have crosslinked functional ASIC1 channels directly at the cell surface of Xenopus oocytes using a bismaleimide crosslinker with a 8 Å spacer arm to stabilize the multimeric channel, and assessed the ASIC1 oligomeric state on SDS-PAGE under reducing conditions After the irreversible, DTT-resistant, crosslinking of cysteines in the TM2 and TM1, the ASIC1a protein runs as a ladder of 4 distinct bands on SDS-PAGE, which by mass correspond to multiples of a ASIC1a monomer (84 ± 1, 172 ± 2, 230 ± 3, 305 ± 5 kDa, n=4) consistent with 4 reproducible oligomeric states. No complexes were observed at molecular weight higher than 305 kDa. We could exclude crosslinking of ASIC1 with other surface proteins. Additional crosslinking between C-terminal cysteines stabilized at the cell surface a predominant ASIC1a complex of 305 kDa which by mass corresponds to a homotetramer. Our experiments provide direct evidence that the functional ASIC1a at the cell surface of Xenopus oocytes does not correspond by mass to a trimer but likely to a tetramer.

P-22 ALIVE AND KICKING: A NON-FUNCTIONAL FHM SCN1A SODIUM CHANNEL MUTANT TRANSFORMED INTO GAIN OF FUNCTION BY PARTIAL RESCUE OF FOLDING DEFECTS

Sandrine Cestèle[1]; Emanuele Schiavon[1]; Raffaella Rusconi[1]; Silvana Franceschetti[2]; Massimo Mantegazza[1];
1: IPMC, CNRS UMR7275 and University of Nice-Sophia Antipolis, 06560 Valbonne, France
2: Department of Neurophysiopathology, Carlo Besta Foundation Neurological Institute, 20133, Milan, Italy

Familial hemiplegic migraine (FHM) is a rare subtype of migraine with aura. Mutations causing FHM (type 3) have been identified in SCN1A, the gene encoding Nav1.1 Na+ channel, which is also a major target of epileptogenic mutations and is particularly important

for the excitability of GABAergic neurons. However, functional studies of NaV1.1 FHM mutations have generated controversial results. In particular, it has been shown that the NaV1.1-L1649Q mutant is non-functional when expressed in a human cell line because of impaired plasma membrane expression, similarly to NaV1.1 mutants that cause severe epilepsy. Notably, we have observed gain of function effects for other NaV1.1 FHM mutants. Here we show that NaV1.1-L1649Q is non-functional because of folding defects that are rescuable by incubation at lower temperature or co-expression of interacting proteins, and that a partial rescue is sufficient for inducing an overall gain of function because of the modifications in gating properties. Strikingly, when expressed in neurons the mutant was partially rescued and was a constitutive gain of function. A computational model showed that 30% rescue can be sufficient for inducing gain of function. Interestingly, previously described folding defective epileptogenic NaV1.1 mutants show loss of function also when rescued. Our results are consistent with gain of function as the functional effect of NaV1.1 FHM mutations and hyperexcitability of GABAergic neurons as the pathomechanism of FHM-type 3.

P-23 ORAI1 CALCIUM CHANNEL IS INVOLVED IN SKIN AGING AND HOMEOSTASIS

Maylis Raphaël[1]; Vyacheslav Lehenkyi[1]; Matthieu Vandenberghe[1]; Dmitri Gordienko[1]; Thierry Oddos[2]; Roman Skryma[1]; Natalia Prevarskaya[1];
1: Laboratory of Cell Physiology; INSERM U1003, Lille 1, Villeneuve d'ascq, France
2: Johnson & Johnson Santé Beauté France, Val-de-Reuil, France

Because the number of persons aged 60 and older is rising since several decades and is still expecting to rise in the next ones, aging-related diseases become major health problems in developed countries. Cellular senescence is thought to be largely involved in most, if not all, the major aging-related diseases. Epidermis integrity is crucial for the skin to maintain its principal functions. During skin ageing epidermis morphology is progressively modified leading to numerous alterations such as an impaired skin barrier function. To achieve and maintain this epidermal barrier, keratinocytes must intricately balance growth, differentiation and polarized motility, which are all known to be governed by calcium. These functions are largely altered during aging and cellular senescence is already recognized as a response participating to these functions in skin. It is already well known that the ion environment especially calcium plays a crucial role for the skin homeostasis. However, the role of calcium, the molecular nature of calcium transporting proteins, and mechanisms involved in cellular senescence remain poorly studied. Here we have discovered a critical and hitherto unappreciated role of Orai1 protein in skin homeostasis and keratinocytes senescence. A combination of electrophysiology, calcium imaging, cellular and molecular biology approaches was applied to both commercially available human primary keratinocytes subjected to replicative senescence in vitro, and skin samples isolated from healthy subjects 18 to 85 years old. In conclusion, we have shown the modified expression of Orai1 calcium channel during keratinocyte senescence in vitro and during skin aging in vivo. In addition, we have demonstrated the Orai1 involvement in human primary keratinocyte proliferation, differentiation, and migration. Further studies will be conducted in order to clear out the role of Orai1 calcium channel in replication-induced keratinocytes senescence.

P-24 IDENTIFICATION OF ION CHANNELS EXPRESSED IN Medicago truncatula ROOT HAIRS

Alice DRAIN; Isabelle GAILLARD; Cécile FIZAMES; Anne Aliénor VERY; Hervé SENTENAC;
B&PMP, UMR 5004 CNRS/386 INRA/SupAgro-M/UM2, Campus SupAgro-INRA, Montpellier, France

The legume-rhizobium symbiosis is of major importance in terrestrial ecosystems by allowing the host plant to assimilate atmospheric nitrogen. Mechanisms that permit plant root hairs to perceive the presence of their symbiotic bacteria partners during establishment of the interaction are complex and their analysis is a major objective for plant biologists. The earliest detected events, immediately triggered by root hair perception of the NOD signaling factors secreted by the bacteria, are rapid fluxes of anions (Cl-, NO3- ...) and cations (Ca2 +, K+, H+) across the cell membrane. Cell membrane depolarization is likely to result from activation of anion channels, allowing anion effluxes. The depolarization could induce calcium channel activation, giving rise to Ca2+ entry (and Ca2+ signaling), and resulting in further depolarization of the cell membrane. In response to this depolarization, potassium channels would be activated, resulting in repolarization of the membrane.
Our objective is to identify and characterize ion channels involved in these signaling events using Medicago truncatula root hair as model. Candidate ion channel families have been selected based on knowledge available in Arabidopsis thaliana: the Shaker family for K+ channels, the SLAC family for anion channels and the CNGC and GLR families for Ca2+ influx. A bioinformatics approach has been developed to collect the channel sequences belonging to these families in M. truncatula, to analyze phylogenetic connections between these two model plants and then to compare the expression data available on these two species. According to these results we selected 10 candidate channels, and made cDNA libraries from isolated M. truncatula root hairs to amplify and clone cDNA of candidate channels. Then, as proof of concept, we focused on a Shaker channel, MtSKOR, which is a homologue of the SKOR channel in A. thaliana and seems to be the unique outward K+ Shaker channel in M. truncatula. Electrophysiological analyses in Xenopus oocytes indicates that MtSKOR is a voltage-sensitive K+ channel, allowing K+ efflux, activated by membrane depolarization. Because of its root hair localization and its electrophysiological signature, this channel could take part to the initial events in the transduction of NOD factor signaling.

P-25 IDENTIFICATION AND ANALYSIS OF THE PROTEIN SIGNALING COMPLEX ASSOCIATED TO P2X4 AND P2X7 IN A NATIVE SYSTEM

Elvira Garcia-de-Paco[1]; Friedrich Koch-Nolte[2]; François Rassendren[1];
1: Institut de Génomique Fonctionnelle, Équipe Nouvelles familles de canaux ioniques, 141 rue de la Cardonille, 34094 Montpellier cedex 05, France
2: Universitätsklinikum Hamburg-Eppendorf, AG Molekulare Immunologie, Martinistraße 52, 20246 Hamburg, Germany

Extracellular ATP, released upon microbial infection, cell damage or inflammation, acts as alert signal towards immune cells by activating purinergic receptors. The purinergic receptors P2X4 and P2X7 are ATP-gated ion channels that are involved, among others, in secretion of

pro-inflammatory cytokines and recruitment of inflammatory macrophages. These receptors present similar functions and are strongly expressed in immune cells. Both can be co-immunoprecipitated, suggesting they belong to the same signalling complex in which P2X4 with its high affinity for ATP would represent the ATP sensor, whereas P2X7 with its low affinity for ATP but its long intracellular C-terminal region would serve as scaffolding unit. This hypothesis can be tested by determining the protein complexes associated to P2X4 and P2X7. This approach should also help to get a better understanding of the mechanisms linking purinergic receptors to inflammatory processes as well as to identify intracellular proteins involved in the regulation of these receptors. However, to obtain pertinent information such a study needs to be performed in a native environment. To reach these goals, we developed an experimental approach based on the immunopurification using primary specific monoclonal antibodies of P2X4 and P2X7 receptors associated protein complexes from Bone Marrow Derived Macrophages in primary culture, and their identification by mass spectrometry. Our study reveals that P2X7 is associated to proteins involved in cytoskeletal rearrangement, phagocytosis, motility and apoptosis, all of them essential features for macrophages to modulate inflammation. Furthermore, P2X4 was found to interact with proteins belonging to the SNARE complex, suggesting a role in vesicle fusion, as well as with a subset of proteins involved in phagocytosis, cell motility and adhesion. Moreover, we found that the apolipoprotein E (ApoE) is part of P2X4 signalling complex. This result was further confirmed by coimmunoprecipitation. A growing number of studies point the involvement of ApoE in many immunological processes as well as in Alzheimer's disease and cognition. This study provides new data on the proteomic environment of P2X7 and P2X4 in primary macrophages and opens new doors to understand the mechanisms linking purinergic signalling to inflammation as well as to the identification of new therapeutic targets.

P-26 DIFFERENT PATHWAYS IN HYPEROSMOSIS-INDUCED PCD IN TOBACCO BY-2 CELLS

Emanuela Monetti [1]; Takashi Kadono [2]; Daniel Tran[3]; Elisa Azzarello [4]; Bernadette Biligi[3]; Joël Briand[3]; Tomonori Kawano[5]; Stefano Mancuso[4]; François Bouteau[6];
1: Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain, 75013 Paris, France LINV, Department of Plant, Soil, and Environmental Science, University of Florence, 50019 Sesto Fiorentino, Italy
2: Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain, 75013 Paris, France Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
3: Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain, 75013 Paris, France
4: LINV, Department of Plant, Soil, and Environmental Science, University of Florence, 50019 Sesto Fiorentino, Italy
5: Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
6: Université Paris Diderot, Sorbonne Paris Cité, Institut des Energies de Demain, 75013 Paris, France, LINV, Department of Plant, Soil, and Environmental Science, University of Florence, 50019 Sesto Fiorentino, Italy

Plants are frequently exposed to a variety of external conditions able to affect their growth, development and productivity. Among them large hyperosmotic changes induce complex

network of signaling events leading to oxidative burst, mitochondria dysfunction, DNA damages and cell death. The aim of this study was to examine the early events induced by saline and non saline hyperosmotic changes (NaCl and sorbitol) and their putative role in programmed cell death process (PCD). By using BY2 tobacco cell we showed that sorbitol-induced PCD seemed dependent on O2 •- generation by NADPH-oxidase. In the case of NaCl, an early influx of sodium through non-selective cation channels (NSCCs) participates to the development of a PCD through mitochondrial dysfunction and also NADPH-oxidase dependent O2 •- generation. This supports the hypothesis of different pathways in NaCl- and sorbitol induced cell death. Surprisingly, other related early responses such as [Ca2+]cyt increase and 1O2 production, do not seemed to be involved in PCD.

P-27 THE ANION EXCHANGER 1 : A TRANSPORTER WITH CHANNEL LIKE BEHAVIOUR.

Damien Barneaud-Rocca[1]; Franck Borgese[1]; Catherine Etchebest[2]; Hélène Guizouarn[3]; *1: iBV, Univ. Nice Sophia Antipolis, UMR7277 CNRS, Inserm U1091, 28 av. Valrose, 06100 Nice - 2: Dynamique des structures et des interactions des macromolécules biologiques Inserm U665, Univ. Paris Diderot, UMR S665, INTS, 6 rue A. Cabanel, 75015 Paris - 3: iBV, Univ. Nice Sophia Antipolis, UMR7277 CNRS, Inserm U1091, 28 av. Valrose, 06100 Nice*

The anion exchanger 1 (AE1) is the mot abundant protein in red cell membrane. It participates to CO2 transport by exchanging Cl and bicarbonate with a one for one stoichiometry. However, studying the AE1 of trout red cells it was shown that this transporter could behave like an anion channel permeable to many different solutes such as amino-acids (taurine), organic and inorganic cations (choline, Na+, K+), urea. This behaviour is an adaptive response to changes in intracellular ionic strength in case of cell swelling. Few years ago, a similarly unexpected behaviour for an electroneutral anion exchanger was also observed in human AE1. Some specific point mutations, identified in patient with hereditary haemolytic anaemia, converted the electroneutral Cl/bicarbonate exchange into a cation conductance1,2. It was observed that human AE1 could adopt three different functional states : electroneutral anion exchanger, cation conductance or anion exchanger with cation leak. In an attempt to understand the structure and functioning of AE1 transport site, homology modelling was combined with site-directed mutagenesis and cysteine scanning mutagenesis. A 3D structure of the anion exchanger transmembrane domain has been proposed for the first time and experimentally validated. Unexpectedly, this model is based on the Uracil transporter, a cation symporter. There is a unique transport site accessible to anions and cations. Changes in the position of positive charges in the access funnel are proposed to open or close a cation conductance in this transport site which should normally exchange anions.
To some extend AE1 shows transport similarities with the glutamate transporter an anion channel with glutamate, Na and K permeability which structure relates to Na-coupled transporters.

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P-28 LOCALIZATION AND ANALYSIS OF K+ TRANSPORT SYSTEMS OF THE ECTOMYCORRHIZAL MODEL FUNGUS HEBELOMA CYLINDROSPORUM

Kevin Garcia; Amandine Delteil; Hervé Sentenac; Sabine Zimmermann;
Institut de Biologie Intégrative des Plantes IBIP Biochimie et Physiologie Moléculaire des Plantes BPMP UMR 5004 AgroM-CNRS-INRA-UM2 Campus AgroM-INRA Place Viala, Bât. 7 34060 Montpellier cedex 1, FRANCE

Ectomycorrhizal symbiosis established between woody plants and soil fungi is widespread in forest ecosystems. This mutual interaction is crucial for the vegetal partner to uptake "hidden nutrients" efficiently and for the fungus to get access to carbon sources. We are analyzing potassium (K+) transport systems of the ectomycorrhizal fungus Hebeloma cylindrosporum aiming in understanding the functional differentiation of the fungal plasma membrane in specific sites for absorption and secretion. K+ is used as model because of its importance as essential element for all organisms. Furthermore, K+ transport systems are well characterized in plants and animals. Amelioration of plant K+ nutrition by ectomycorrhization has been reported. Taking advantage from an EST library of H. cylindrosporum (1) and, more recently, from the sequenced genome for in silico identification of candidate genes, K+ transport systems comprising Shaker-like and TOK-type channels as well as transporters from the Trk- and KUP/HAK-type have been identified. Functional properties of these systems are studied by heterologous expression in yeast and Xenopus oocytes (2). In parallel, we are developing cellular biology approaches (in situ hybridization, transcriptional and translational fusions with a GFP marker) for fine localization of these transport systems within fungal tissues (3). Finally, over-expression and RNAi strategies are used to understand the exact role of these candidate proteins in vivo, in pure fungal culture and ultimately in the mycorrhizal context. A model comprising the different putative fungal actors for K+ uptake from the soil and its secretion towards the host plant Pinus pinaster will be proposed.

(1) Lambilliotte et al., New Phytol. 2004, 164:505–513 (2) Corratgé et al., J. Biol. Chem. 2007, 282:26057–26066 (3) Garcia et al., Fungal Genet & Biol., 2013, doi: 10.1016/j.fgb.2013.06.007

P-29 ULTRASTRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF MITOCHONDRIA FROM THE HONEYBEE FLIGHT MUSCLES AFTER EXPOSURE TO AN INSECTICIDE

Mercedes Charreton[1]; Isabelle Bornard[2]; Axel Decourtye[3]; Loic Teulier[4]; Caroline Romestaing[4]; Damien Roussel[4]; Romain Juge[5]; Jerome Kucharczak[5]; Abdel Aouacheria[5]; Claude Collet[1];
1: INRA, UR406 AE, Toxicologie Environnementale, Avignon - 2: INRA, UR407 PV, Avignon - 3: UMT PrADE, Avignon - 4: CNRS, UMR 5023 LEHNA, Lyon - 5: CNRS, UMR 5239 LBMC, Lyon

Honeybees are commonly exposed to a number of pesticides dedicated to protect crops from insects considered as pests in agriculture. Not only sublethal effects of insecticides are difficult to assess in honeybees, but available assays often consist in limited investigations dedicated to identify neurotoxic-induced deleterious modifications. New functional

approaches and robust biomarkers are urgently needed to explore unpredicted effects of pesticides on bees, in particular in the domain of energy metabolism. Using confocal and electron microscopy, we have characterized mitochondrial contents in the extremely energy-demanding flight muscles and mitochondrial functional integrity in both fibrillar flight muscles and tubular leg muscles. Muscle mitochondrial oxygen consumption was also explored in these muscles. Mitochondria can be directly or indirectly modified by pesticides. We tentatively explored the consequences of pollen nutrition on these functional and ultrastructural parameters

P-30 BKCa CHANNELS: A NEW THERAPEUTIC TARGET IN THE TREATMENT AGAINST FRAGILE X SYNDROME

Arnaud Menuet;
UMR7355-CNRS INEM, "neurogenetic" team, 3b rue de la Férollerie, 45100 Orléans

The most common cause of inherited mental deficiency (MD) associated with autistic features, Fragile X Syndrome (FXS), results from the FMR1 gene silencing and loss of Fragile X Mental Retardation Protein (FMRP). Among synaptic proteins deregulated by the absence of Fmrp in fmr1 knock-out (KO) mice (murine model for FXS), Kcnma1 (α-subunit of large conductance Ca2+-activated potassium channels, BKCa) seems to be the most significant deregulated one. Since several papers showed an involvement of BKCa channels in MD, this channel could be a new therapeutic target for FXS. The objectives of this study were: 1) to characterize the molecular BKCa channel anomaly in fmr1 KO mouse model and 2) to evaluate the therapeutic effect of a BKCa channel opener molecule (BCOM), BMS-204352, on dendritic spines maturation of fmr1 KO neurons and on social behavior of fmr1 KO mice. In fmr1 KO mice, Kcnma1 protein quantity was lower in cerebral structures and neurons, and associated with a reduced BKCa whole cell current. In vitro addition of BMS-204352 induced dendrite spines maturation of fmr1 KO neurons. In vivo, using a behavioral test of direct social interaction, a single injection of BMS-204352 rescued affiliative behaviors to wild-type level. In conclusion, we demonstrated 1) a Kcnma1 protein anomaly in fmr1 KO mice inducing a decrease in BKCa whole cell current, and 2) that a BCOM, BMS-204352, restored a regular phenotype of dendrite spines and social interaction in fmr1 KO mice. Therefore, BKCa channel could be a new therapeutic target in the treatment against FXS.

P-31 Tandem pore domain halothane-inhibited K+ (THIK) channels, THIK1 and THIK2, form functional homomeric and heteromeric channels

Delphine Bichet; Franck Chatelain; Sylvain Feliciangeli; Marie-Madeleine Larroque; Florian Lesage;
IPMC, CNRS-UNS UMR7275, LabEx ICST, 06560 Valbonne, France

Tandem pore domain halothane-inhibited K+ channel 1 (THIK1) produces background K+ currents. Despite 62 % amino acid identity with THIK1, THIK2 is not active upon heterologous expression. Here, we show that this apparent lack of activity is due to a unique combination of retention in endoplasmic reticulum and low intrinsic channel activity at the plasma membrane. A THIK2 mutant containing a proline residue (THIK2-A155P) in its

second inner helix (M2) produces K+-selective currents with properties similar to THIK1, including inhibition by halothane and insensitivity to extracellular pH variations. Another mutation in the M2 helix (I158D) further increases channel activity and affects current kinetics. We also show that the cytoplasmic amino-terminal region of THIK2 (Nt-THIK2) contains an arginine-rich motif (RRSRRR) that acts as a retrieval/retention signal. Mutation of this motif in THIK2 induces a relocation of the channel to the plasma membrane resulting in measurable currents, even in the absence of mutations in the M2 helix. Cell surface delivery of a Nt-THIK2-CD161 chimera is increased by mutating the arginines of the retention motif (RRSRRR), but also by converting serine embedded in this motif to aspartate suggesting a phosphorylation-dependent regulation of THIK2 trafficking. We also show that THIK1 and THIK2 form functional homomeric and heteromeric channels, further expanding the known repertoire of K+ channels.

P-32 PREYNATPIC L-TYPE VOLTAGE-GATED CALCIUM CHANNELS IN CEREBELLAR INTERNEURONS

Stéphanie Rey and Thibault Collin

CNRS-UMR8118, Laboratoire de Physiologie Cérébrale, Université des Saints-Pères, 75270 Paris.

Fast release of neurotransmitter is elicited by depolarization of the presynaptic compartment and Ca2+ entry into the terminal through voltage dependent Ca2+ channels (VDCCs). In cerebellar molecular interneurons (MLIs), only P/Q and N –types high threshold VDCCs have been shown to play a pivotal role in the release of GABA (Forti et al., 2000; Stephens et al., 2001). Although pharmacologically identified at the membrane of these neurons, L-types VDCCs have not been extensively studied. Two types of L- types VDCCs, Cav1.2 and Cav1.3 are expressed in the central nervous system. They are currently thought to mediate Ca2+-induced Ca2+ release (CICR) processes at MLIs somata through a conformational association with ryanodine receptors (Chavas et al., 2004) and involvement in neurotransmitter release has been ruled out either by electrophysiology and Ca2+ imaging (Forti et al., 2000; Stephens et al., 2001). Our electrophysiological data confirm that P/Q (Cav2.1) , N (Cav2.2) and L-type (Cav1.2/3) VDCCs are functionally expressed in MLIs. Moreover, mRNA encoding Cav2.1, Cav2.2, Cav1.2 and Cav1.3 have been detected in MLI by single-cell RT-PCR. The use of various concentrations of Isradipine (Dihydropyridine (DHP) antagonist) suggests the presence of both L-type VDCCs Cav1.2 and Cav1.3 which are also positively modulated in the presence of BayK8644 (DHP agonist). Interestingly, BayK8644 (1-10 μM) dramatically increases mIPSCs frequency indicating that L-type VDCCs could be present inpresynaptic sites although this has been ruled out by previous studies. Action potential-evoked Ca2+ transients recorded in axonal varicosities exhibit a larger amplitude and slower decay kinetics in the presence of BayK8644. Using a GABAergic presynapticmarker (VGAT), we show a colocalisation between Cav1.2, Cav1.3 and VGAT. Moreover, some electrophysiological experiments show a potential coupling between L-type VDCCs and ryanodine receptors in MLI by a Calcium-Induced-Calcium-Release (CICR) or/and a physical coupling. Taken together, our data indicate demonstrate that L-type VDCCs are functionally expressed in the presynaptic compartment and are potentially involved in the release of GABA.

LIST OF AUTHORS

ATTENDEES : contact info

Bahar Ivet
University of Pittsburgh
Systems Biology School of Medicine,
University of Pittsburgh, 3064 BST3,
3501 Fifth Ave
15213 Pittsburgh - United States
Tel: 4126483332 - Fax: 4126483163
bahar@pitt.edu

Bahar Israel
University of Pittsburgh
1814 Willow Oak Drive
15090 Wexford - United States
Tel: 7249400170 - Fax: 4126483163
bahar@pitt.edu

Bannwarth Ludovic
Institut de Minéralogie et Physique des Milieux Condensés
Case courrier 115 4 place Jussieu
75005 Paris - france
Tel: 0144275072 - Fax: 0144275152
ludovic.bannwarth@impmc.upmc.fr

Bargiotas Panagiotis
University
Perdika 3a, Sikies
56625 Thessaloniki - Greece
Tel: 00306971505247 - Fax:
pabargio@yahoo.gr

Baron Anne
IPMC, CNRS-UNS, UMR 7275
660 route des Lucioles Sophia Antipolis
06560 Valbonne - France
Tel: 0493953418 - Fax:
anne.baron@ipmc.cnrs.fr

Bendahhou Saïd
LP2M CNRS
Université de Nice Sophia Antipolis Parc Valrose
06108 Nice - France
Tel: 0624608412 - Fax: 0492076850
said.bendahhou@unice.fr

Benndorf Klaus
Institute of Physiology II
Kollegiengasse 9
07743 Jena - Germany
Tel: +49 3641 934350 - Fax: +49 3641 933202
Klaus.Benndorf@med.uni-jena.de

Berrou Mikael
World Precision Instruments
1 Hunting Gate
SG4 0TJ Hitchin - United Kingdom
Tel: +33659102138 - Fax: +441462424701
mberrou@wpi-europe.com

Bescond Jocelyn
IPBC FRE3511 CNRS-Université de Poitiers
Pôle Biologie Sante Bâtiment B36 1 rue Georges Bonnet
86022 POITIERS - France
Tel: +33549453528 - Fax: +33549454014
jocelyn.bescond@univ-poitiers.fr

Bichet Delphine
IMPC
660, route des Lucioles
06560 Valbonne - France
Tel: 04 93 95 77 32 - Fax: 04 93 95 77 08
bichet@ipmc.cnrs.fr

Blesneac Iulia
Institut de Genomique Fonctionnelle (CNRS)
141, rue de la Cardonille
34094 Montpellier - FRANCE
Tel: (33) 4.34.35.92.50 - Fax:
iulia.blesneac@igf.cnrs.fr

Blin Sandy
Institut de Pharmacologie Moléculaire et Cellulaire
660 route des Dolines - Sophia Antipolis
06560 Valbonne - France
Tel: 0493957777 - Fax: 0493957708
blin@ipmc.cnrs.fr

Bois Patrick
Université de Poitiers
Equipe Physiopathologie et Pharmacologie des Canaux Ioniques
Institut de Physiologie et Biologie Cellulaires FRE CNRS/Université de Poitiers n°3511 Pôle Biologie Santé Bât B36 BP 633 1 rue Georges Bonnet 86022 POITIERS
86022 Poitiers - France
Tel: 0549000961 - Fax: 0549454014
patrick.bois@univ-poitiers.fr

Bossu jean louis
cnrs
INCI 5 rue blaise pascal
67084 strasbourg - france
Tel: 0614296610 - Fax:
jlbossu@inci-cnrs.unistra.fr

Boué-Grabot Eric
IMN CNRS UMR 5293
Université Bordeaux Segalen 146 rue Léo saignat
33600 Bordeaux - France
Tel: 0557575763 - Fax:
eric.boue-grabot@u-bordeaux2.fr

Boulin Thomas
Université Lyon 1 - CNRS
Centre de Génétique et de Physiologie Moléculaire et Cellulaire UMR CNRS 5534 - Université Claude Bernard Lyon 1
Bât. Dubois, 2ème étage 43 bd du 11 Novembre 1918
69622 Villeurbanne - France
Tel: 06 45 92 12 46 - Fax:
thomas.boulin@gmail.com

Bourinet Emmanuel
IGF CNRS UMR5203 Montpellier
141 Rue de la cardonille
34094 Montpellier - France
Tel: 04 34 35 92 48 - Fax: 04 67 54 24 32
emmanuel.bourinet@igf.cnrs.fr

Bruhova Iva
University of Buffalo
Department of Physiology and Biophysics 3435 Main street
14214 Buffalo - USA
Tel: 716-829-5482 - Fax: 716-829-2569
ibruhova@buffalo.edu

Caro Lydia
University of Toronto
914 Yonge Street, Unit #1103
M4W3C8 Toronto - Canada
Tel: 416-807-8464 - Fax:
lydia.caro@utoronto.ca

Carré Grégoire
Signalisation et Transports Ioniques Membranaires
Institut de Physiologie et de Biologie Cellulaires IPBC - FRE 3511 CNRS - Université de Poitiers Bât. B36 - Pôle Biologie Santé 1, rue Georges Bonnet - BP 633 86022 Poitiers Cedex
86022 Poitiers - France
Tel: 05 49 45 63 42 - Fax: 05 49 45 40 14
gregoire.carre@univ-poitiers.fr

Charnet Pierre
CRBM, CNRS umr5237
1919 Route de Mende
34293 Montpellier - France
Tel: 0434359537 - Fax:
pierre.charnet@crbm.cnrs.fr

Chataigneau Thierry
Université e Strasbourg, Faculté de Pharmacie
Laboratoire de Conception et Application de Molécules Bioactives
Equipe de Chimie et Neurobiologie Moléculaire UMR 7199 CNRS/ Université de Strasbourg
67400 Illkirch - France
Tel: 03 68 85 41 33 - Fax: 03 68 85 43 06
thierry.chataigneau@unistra.fr

Chatelain Franck
IPMC
660, route des Lucioles
06560 Valbonne - France
Tel: 04 93 95 77 32 - Fax: 04 93 95 77 08
chatelain@ipmc.cnrs.fr

Chatelier Aurelien
Institut de Physiologie et Biologie Cellulaires, Université de Poitiers
Pôle biologie Santé, Batiment B36 1 rue George Bonnet
86022 Poitiers - France
Tel: 33 0549 453 747 - Fax: 33 0549 454 014
aurelien.chatelier@univ-poitiers.fr

Chen Haijun
State Universirty of New York-Albany
1400 Washington Avenue
12222 Albany - USA
Tel: 1-518-591-8854 - Fax: 1-518-442-4767
hchen01@albany.edu

Cognard Christian
Lab. STIM (Signalisation et Transports Ioniques Membranaire)s
CNRS FRE 3511 Pôle Biologie Santé 1 rue Georges Bonnet
86022 Poitiers - France
Tel: 0549454064 - Fax: 0549454014
christian.cognard@univ-poitiers.fr

Collet Claude
INRA, UR406 Abeilles et Environnement, Toxicologie Environnementale
Site Agroparc
84914 Avignon - France
Tel: 04 32 72 26 49 - Fax: 04 32 72 26 02
claude.collet@avignon.inra.fr

Couillin Isabelle
CNRS, Orleans University
UMR-INEM 7355 Experimental and Molecular Immunology and Neurogenetics 3B rue de la Ferollerie
45071 ORLEANS - FRANCE
Tel: 33238255443 - Fax: 33238257979
couillin@cnrs-orleans.fr

Dejos Camille
Université de Poitiers-FRE-CNRS-3511
IPBC-FRECNRS3511 Bat B36-Pole Biologie Santé 1, rue Georges Bonnet
86022 POITIERS - FRANCE
Tel: 05 49 45 37 99 - Fax: 05 49 45 40 14
camille.dejos@univ-poitiers.fr

Deval Emmanuel
IPMC, UMR 7275 CNRS/UNS
Institut de Pharmacologie Moléculaire et Cellulaire (IPMC) 660, route des lucioles Sophia Antipolis
06560 Valbonne - France
Tel: 0493953418 - Fax:
deval@ipmc.cnrs.fr

Dougherty Dennis
California Institute of Technology
164-30
91125 Pasadena, CA - USA
Tel: 626 395 6089 - Fax: 626 564 9297
dadougherty@caltech.edu

Drain Alice
BPMP
Campus INRA / SupAgro - IBIP - UMR BPMP - Bât. 7 - 2 place Pierre Viala
34060 Montpellier - FRANCE
Tel: 0499612057 - Fax:
alice.drain@supagro.inra.fr

Dubayle David
Université Paris Descartes - CNRS UMR 8119
45, rue des Saints-Pères
75006 Paris - France
Tel: 0142862282 - Fax:
david.dubayle@parisdescartes.fr

Dubois Charlotte
INSERMU1003
Laboratoire de Physiologie cellulaire
Bâtiment SN3 2ème étage
59650 Villeneuve d'ascq - FRANCE
Tel: 0320337078 - Fax:
charlottedubois2@aol.com

Ducret Thomas
Université Bordeaux Segalen
Centre de Recherche Cardio-Thoracique de Bordeaux INSERM U1045 Université Bordeaux Segalen 146, rue Léo-Saignat (case 13)
33076 Bordeaux - France
Tel: +33.(0)5.57.57.56.58 - Fax:
+33.(0)5.57.57.16.95
thomas.ducret@u-bordeaux2.fr

El Alaoui Chaymae
Institut de génomique fonctionnelle, CNRS
141, rue de la cardonille
34094 Montpellier - France
Tel: +33753041933 - Fax:
+33434359252
chaymae.el-alaoui@igf.cnrs.fr

Fagni Laurent
CNRS
IGF, 141 Rue de la Cardonille
34000 Montpellier - France
Tel: 06 31 13 87 06 - Fax:
laurent.fagni@igf.cnrs.fr

Feliciangeli Sylvain
CNRS UMR7275
Institut de Pharmacologie Moléculaire et Cellulaire 660 route des lucioles Sophia Antipolis
06560 Valbonne - France
Tel: (33).4.93.95.77.32 - Fax:
(33).4.93.95.77.08
feliciangeli@ipmc.cnrs.fr

Florian Lesage
IPMC CNRS
600 route des lucioles
06560 Valbonne - France
Tel: 0493957727 - Fax: 0493957704
lesage@ipmc.cnrs.fr

Frachisse Jean Marie
CNRS
Institut des Sciences Végétales CNRS bat 22, Av de la terrasse
91198 Gif sur Yvettre - France
Tel: 01 69 82 36 87 - Fax:
frachisse@isv.cnrs-gif.fr

Garcia de Paco Elvira
IGF (Institut de Génomique Fonctionnelle)
141 rue de la Cardonille
34094 Montpellier - France
Tel: 04 34 35 93 04 - Fax: 04 67 54 24 32
Elvira.Garcia-de-Paco@igf.cnrs.fr

Ghatak Swagata
Indian Institute of Science
Molecular Biophysics Unit, Indian Institute of Science.
560012 Bangalore - India
Tel: 080-22933220 - Fax:
swagata@mbu.iisc.ernet.in

Groc Laurent
CNRS - Universite Bordeaux
Cnrs umr 5297 Universite bordeaux Segalen 146 rue Leo Saignat
33077 Bordeaux - France
Tel: 0557575746 - Fax:
laurent.groc@u-bordeaux2.fr

Grutter Thomas
CNRS - University of Strasbourg
Faculty of Pharmacy 74 route du Rhin
67400 Illkirch - France
Tel: 03 68 85 41 57 - Fax: 03 68 85 43 06
grutter@unistra.fr

Guizouarn Hélène
CNRS
Institut de biologie de Valrose,
Université de Nice 28 av. Valrose
Bâtiment de Sicences Naturelles
06100 Nice - France
Tel: 0492076837 - Fax: 0492076834
helene.guizouarn@unice.fr

Gwiazda Karolina
University of Lausanne
Department of Pharmacology & Toxicology Bugnon 27
1005 Lausanne - Switzerland
Tel: 0041216925366 - Fax:
Karolina.Gwiazda@unil.ch

Habbout Karima
Laboratoire PhysioMédecine Moléculaire
Bâtiment des Sciences Naturelles, 6ème niveau Parc Valrose
06108 Nice - France
Tel: 0492076852 - Fax: 0492076850
karimahabbout@yahoo.fr

Habermacher Chloé
Equipe de Chimie et Neurobiologie Moléculaire UMR 7199 CNRS/ Université de Strasbourg Faculté de Pharmacie
74 route du Rhin
67400 Illkirch - France
Tel: (+33) 03 68 85 41 64 - Fax: (+33) 03 68 85 43 06
habermacher@unistra.fr

Haijun Chen
State University of New York-Albany
1400 Washington Ave.
12222 Albany - USA
Tel: 1-518-591-8854 - Fax: 1-518-442-4767
hchen01@albany.edu

Hatem Stéphane
Inserm/UPMC
UMRS956 91 boulevard de l'hôpital
75013 Paris - France
Tel: 0140779584 - Fax:
stephane.hatem@upmc.fr

HEBERT Betty
INEM - UMR7355 CNRS
3b rue de la férollerie
45071 ORLEANS - FRANCE
Tel: 02 38 25 70 40 - Fax: 02 38 25 79 79
bhebert@cnrs-orleans.fr

Hedrich Rainer
Julius-von-Sachs-Institute for Biosciences
Julius-von-Sachs-Platz 2
97082 Wuerzburg - Germany
Tel: +49 931 31-86100 - Fax: +49 931 31-86157
hedrich@botanik.uni-wuerzburg.de

Hirbec Hélène
IGF
141 rue de la cardonille
34094 Montpellier - France
Tel: 0647129278 - Fax:
helene.hirbec@igf.cnrs.fr

Huser Delphine
University of Lausanne
Department of Pharmacology & Toxicology Bugnon 27
1005 Lausanne - Suisse
Tel: 0041797791808 - Fax:
delphine.huser.2@unil.ch

Jospin Maelle
UMR 5534
Bâtiment Dubois, Rue Raphaël Dubois, Université Lyon 1 43 bd du 11 novembre 1918
69622 Villeurbanne - France
Tel: 33472432939 - Fax:
jospin@univ-lyon1.fr

Karanauskaite Jovita
Molecular Devices (UK) Ltd.
660-665 Eskdale Road Winnersh Triangle
RG41 5TS Wokingham - UK
Tel: +441189444642 - Fax: +441189448001
jovita.karanauskaite@moldev.com

Khlaifia Abdessattar
CRN2M - CNRS UMR 72 86 MARSEILLE CRN2M - CNRS UMR 7286 Faculté de Médecine - Secteur Nord CS80011 Bd Pierre Dramard 13344 Marseille Cedex 15, FRANCE
13344 MARSEILLE - FRANCE
Tel: 04 91 69 89 73 - Fax:
abdessattar.khlaifia@etu.univ-amu.fr

Koselski Mateusz
Department of Biophysics
Institute of Biology Maria Curie-Sklodowska University
20-033 Lublin - Poland
Tel: 815375931 - Fax: 815375902
Mateusz.koselski@poczta.umcs.lublin.pl

Lainé Viviane
CGPhiMC
Batiment Dubois, 43 bd du 11 novembre
69622 Villeurbanne - France
Tel: +33472431031 - Fax:
viviane.laine@univ-lyon1.fr

Laird Dale
University of Western Ontario
Dental Science Building London, Ontario
N6A 5C1 London - Canada
Tel: 519 661-2111 x86827 - Fax:
dale.laird@schulich.uwo.ca

Lalisse Sarah
Institut de génomique fonctionnelle
141 rue de la cardonille
34094 Montpellier - France
Tel: 04 34 35 92 85 - Fax: 04 67 54 24 32
sarah.lalisse@igf.cnrs.fr

Lemoine Damien
UMR7199 Unistra
74 route du Rhin
67401 ILLKIRCH - FRANCE
Tel: 0368854116 - Fax:
damien.lemoine@etu.unistra.fr

Levenes Carole
CNRS - Université Paris Descartes
Laboratoire de Neurophysique et Physiologie 45 rue des Saints Pères
75006 Paris - France
Tel: 33 1 42864157 - Fax:
carole.levenes@gmail.com

Lingueglia Eric
IPMC-CNRS/Université de Nice-Sophia Antipolis UMR7275
660 Route des Lucioles, Sophia Antipolis,
06560 Valbonne - France
Tel: 04 93 95 34 23 - Fax: 04 93 95 77 08
lingueglia@ipmc.cnrs.fr

Magaud Christophe
Laboratoire STIM (exIPBC) FRE-CNRS 3511
1,rue Georges Bonnet Bâtiment B36 Pôle Biologie Santé BP633
86022 Poitiers - France
Tel: 0549453891 - Fax: 0549454014
christophe.magaud@univ-poitiers.fr

Malecot Claire O.
CNRS FRE 3511, Institut de Physiologie et de Biologie Cellulaires
Physiologie des Cellules Cardiaques et Vasculaires, Faculté des Sciences, Parc de Grandmont
37200 TOURS - FRANCE
Tel: 02-47-36-70-09 - Fax: 02-47-36-71-12
malecot@univ-tours.fr

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

Martinez Audrey
IMN- CNRS 5293
Universite Bordeaux2 146 rue Leo Saignat
33076 Bordeaux - France
Tel: 0557575765 - Fax: 0557575760
audrey.martinez@u-bordeaux2.fr

Martz Adeline
Equipe de Chimie et Neurobiologie Moléculaire UMR 7199 CNRS/ Université de Strasbourg Faculté de Pharmacie
74 route du Rhin
67400 Illkirch - France
Tel: 03 68 85 41 16 - Fax: (+33) 03 68 85 43 06
adeline.martz@unistra.Fr

Mazars Christian
CNRS
Laboratoire de Recherche en Sciences Végétales (LRSV) UMR 5546 UPS/CNRS Pôle de Biotechnologies Végétales 24, chemin de Borde Rouge B.P. 42617 Auzeville
31326 Castanet-Tolosan - France
Tel: (33)534323836 - Fax: (33)534323802
mazars@lrsv.ups-tlse.fr

Mellen Nicholas
University of Louisville
Kosair Children's Hospital Research Institute Baxter Building 1 Suite 321F
570 S Preston Street
40202 Lousville - USA
Tel: 502 852 2652 - Fax:
nicholas.mellen@louisville.edu

Menuet Arnaud
UMR7355 INEM
3b rue de la Férollerie
45100 Orléans - France
Tel: 0238257940 - Fax: 0238257979
menuet@cnrs-orleans.fr

Meunier Claude
CNRS - Université Paris Descartes
Laboratoire de Neurophysique et Physiologie 45 rue des Saints Pères
75006 Paris - France
Tel: 33 1 42862141 - Fax:
claude.meunier@parisdescartes.fr

Mohamed Chahine
Laval University
Centre de recherche Institut universitaire en santé mentale de Québec
G1J 2G3 Québec - Canada
Tel: 418 663 5747 #4723 - Fax: 418 663 8756
mohamed.chahine@phc.ulaval.ca

Monetti Emanuela
Università di Firenze Université Paris 11
Polo scientifico Viale delle Idee 30 Sesto F.no
50019 Firenze - Italy
Tel: +39 055 4574093 - Fax: +39 055 4574017
emanuela.monetti@unifi.it

Monteil Arnaud
Institut de Génomique Fonctionnelle - CNRS UMR5203
141, rue de la Cardonille
34094 Montpellier Cedex 5 - France
Tel: (33) 4.34.35.92.50 - Fax:
arnaud.monteil@igf.cnrs.fr

Moreau Bertrand
INRA
BPMP INRA-Supagro Place Viala
34060 Montpellier - France
Tel: 0499613169 - Fax: 0467525737
moreaub@supagro.inra.fr

Moubarak Estelle
Inserm UMR 1072
Faculté de Médecine secteur Nord
Boulevard Pierre Dramard
13015 MARSEILLE - FRANCE
Tel: 0491698710 - Fax: 0491090506
estelle.moubarak@gmail.com

Mulle Christophe
CNRS
University of Bordeaux - Interdisciplinary Institute for Neuroscience
33076 Bordeaux - France
Tel: 0557574086 - Fax: 0557574086
mulle@u-bordeaux2.fr

Noël Jacques
Université de Nice Sophia Antipolis
IPMC, UMR 7275 CNRS/UNS 660 Route des Lucioles
06560 Valbonne - France
Tel: -33 49395 3421 - Fax: -33 49395 7708
jnoel@unice.fr

Parpaite Thibaud
Université Bordeaux Segalen
Centre de Recherche Cardio-Thoracique de Bordeaux INSERM U1045 Université Bordeaux Segalen 146, rue Léo-Saignat (case 13)
33076 Bordeaux - France
Tel: +33.(0)5.57.57.16.94 - Fax: +33.(0)5.57.57.16.95
thibaud.parpaite@etud.u-bordeaux2.fr

Perroy Julie
IGF / CNRS
141 rue de la cardonille
34094 Montpellier - france
Tel: 0434359210 - Fax:
julie.perroy@igf.cnrs.fr

Peyronnet Rémi
National Heart & Lung Institute
Imperial College, room RG81 Heart Science Centre Hill End Road
UB9 6JH Harefield - United Kingdom
Tel: 441895453805 - Fax:
r.peyronnet@imperial.ac.uk

Pietrobon Daniela
University of Padua
University of Padua Viale Giuseppe Colombo, 3
35121 Padua - Italy
Tel: 049 8276052 - Fax: 049 8276049
daniela.pietrobon@unipd.it

Pini Jonathan
Laboratoire de PhysioMédecine Moléculaire LP2M
LP2M - CNRS FRE 3472 Université Nice Sophia-Antipolis Laboratoire de Physio-Médecine Moléculaire Bâtiment des Sciences Naturelles, 6ème Niveau Parc Valrose
06108 Nice Cedex 2 - France
Tel: 0492076852 - Fax: 0492076850
jonathan.pini@unice.fr

Plested Andrew
FMP Berlin
Robert-Rössle-Str 10
13125 Berlin - Germany
Tel: +493094063071 - Fax:
plested@fmp-berlin.de

Pumain René
Inserm
177 ave du Mal Foch
92220 Bagneux - France
Tel: 0146631337 - Fax:
rene.pumain@inserm.fr

Raphael Maylis
Laboratory of Cell Physiology INSERM U1003
Bât. SN3 2ètage, porte 223 USTL
59650 Villeneuve d'Ascq - France
Tel: +33320337078 - Fax:
maylis.raphael@inserm.fr

Rettinger Jürgen
Multi Channel Systems
Aspenhaustr. 21
72770 Reutlingen - Germany
Tel: +497121909250 - Fax:
+4971219092511
rettinger@multichannelsystems.com

Rey Stephanie
CNRS
45, rue des St Pères
75006 Paris - France
Tel: 0142863829 - Fax:
stephanie.rey@parisdescartes.fr

Riou Morgane
Institut de Biologie de l'Ecole Normale Supérieure (IBENS)
46 rue d'Ulm
75005 PARIS - FRANCE
Tel: 0673522550 - Fax:
morgane.riou@ens.fr

Ronjat Michel
Grenoble Institut des Neurosciences
Inserm U836, équipe 3 LabEx Ion Channel Science and Therapeutics Bât. Edmond J. Safra Université Joseph Fourier Site Santé de la Tronche BP 170
38042 Grenoble - France
Tel: 04 56 52 05 65 - Fax: 04 56 52 05 72
michel.ronjat@ujf-grenoble.fr

Roy Jérôme
U1046
INSERM - U1046 - 371 Avenue du Doyen G. Giraud, CHU Arnaud de Villeneuve, Bâtiment INSERM Crastes de Paulet, 34295 Montpellier Cedex 5 France
Laboratoire : PHYSIOLOGIE & MÉDECINE EXPÉRIMENTALE du Cœur et des Muscles
34295 Montpellier - France
Tel: Tel : 04 67 41 52 40 - Fax:
jerome.roy@inserm.fr

Salinas Miguel
IPMC UMR7275 CNRS
660, route des Lucioles Sophia Antipolis
06560 Valbonne - FRANCE
Tel: 04 93 95 34 22 - Fax: 04 93 95 77 04
salinas@ipmc.cnrs.fr

Sandoz Guillaume
iBV - CNRS
CNRS UMR7277 - Inserm U1099 - UNS Faculté des Sciences - Université de Nice Sophia Antipolis Bât. Sciences Nat., 3ème Etage, 4ème niveau 28 Av. de Valrose
06108 Nice - France
Tel: +33 492076804 - Fax:
sandoz@unice.fr

Sarrauste de Menthière Cyril
IGH - CNRS UPR1142
141, rue de la Cardonille
34396 Montpellier - France
Tel: +33(0)4 34 35 99 81 - Fax:
cyril.sarrauste@igh.cnrs.fr

Schroeder Julian
UC San Diego, Division of Biological Sciences
9500 Gilman Drive, MC-0116
92093 La Jolla, California - USA
Tel: 858-534-7759 - Fax: 858-534-7108
jischroeder@ucsd.edu

Séguéla Philippe
McGill University
Montreal Neurological Institute 3801 University, Suite 778
H3A 2B4 MONTREAL - Canada
Tel: 5143985029 - Fax: 5143988106
philippe.seguela@mcgill.ca

Sentenac Hervé
INRA Montpellier
b&pmp, Campus SupAgro/inra
34060 Montpellier - France
Tel: 0499612605 - Fax: 0467525797
sentenac@supagro.inra.fr

Stroebel David
IBENS/CNRS
46, rue d'Ulm
75005 Paris - France
Tel: 0144323892 - Fax:
david.stroebel@ens.fr

Strube Caroline
CNRS - Aix Marseille Université
Faculté de Médecine Nord Bd Pierre Dramard
13344 Marseille - France
Tel: 33-4 91 69 89 83 - Fax: 33-4 91 69 89 77
caroline.strube@univ-amu.fr

Szponarski Wojciech
INRA
BPMP INRA-SupAgro Place Viala
34060 Montpellier - France
Tel: 0499612706 - Fax: 0467525737
szponarski@supagro.inra.fr

Tell Fabien
aix-marseille université
Centre de recherche en Neurobiologie-Neurophysiologie de Marseille UMR CNRS 7286 Faculté de Médecine - Secteur Nord - CS80011
13344 Marseille - FRANCE
Tel: 04 91 69 89 83 - Fax:
fabien.tell@univ-amu.fr

Temporal Simone
UMR 1072 (anciennement UMR 641) Unité de Neurobiologie des canaux Ioniques et de la Synapse (UNIS) Faculté de Médecine - Secteur Nord Université Aix Marseille
51, Bd Pierre Dramard
13015 Marseille - France
Tel: 0491698710 - Fax: 0491090506
simone.temporal@univ-amu.fr

Thibaud Jean-Baptiste
Biochimie et Physiologie Moléculaire des Plantes
Institut de Biologie Intégrative des Plantes Claude Grignon Campus INRA-SupAgro
34060 Montpellier cedex 2 - France
Tel: +33 (0)499 612 609 - Fax: +33(0)467 525 737
thibaud@supagro.inra.fr

Tompkinson Suzanne
Scientifica Ltd
Unit 1A Kingfisher Court, Brambleside, Bellbrook Industrial Estate
TN22 1QQ East Sussex - UK
Tel: 01825 749933 - Fax: 01825 749934
colette.lewis@scientifica.uk.com

Toulme Estelle
IMN-CNRS5293
Universite Bordeaux2 146 rue Leo Saignat
33076 Bordeaux - France
Tel: 0557575763 - Fax: 0557575760
estelle.toulme@u-bordeaux2.fr

Tran Daniel
CNRS
Institut des Sciences du Végétal Avenue de la Terrasse Bâtiment 22
91198 Gif-sur-Yvette - France
Tel: +33169823870 - Fax:
daniel.tran@isv.cnrs-gif.fr

Trauner Dirk
University of Munich | Dept. o. Chemistry
Butenandtstr. 5-13
81377 Munich - Germany
Tel: 004989218077800 - Fax: 004989218077972
Dirk.Trauner@lmu.de

Trebacz Kazimierz
Department of Biophysics
Institute of Biology Maria Curie-Sklodowska University
20-033 Lublin - Poland
Tel: 815375931 - Fax: 815375902
kazimierz.trebacz@poczta.umcs.lublin.pl

Uber, Dr. Andreas
HEKA Elektronik Dr. Schulze GmbH
Wiesenstrasse 71
67466 Lambrecht - Germany
Tel: +49-6325-95530 - Fax: +49-6325-955350
sales@heka.com

Vanden Abeele Fabien
INSERMU1003
Laboratoire de physiologie cellulaire
Batiment SN3 2ème étage
59650 Villeneuve d'ascq - FRANCE
Tel: 0671031555 - Fax:
fabien.vanden-abeele@inserm.fr

Very Anne-Aliénor
CNRS
Laboratoire B&PMP UMR 5004
CNRS/386 INRA/ Supagro Montpellier/ UM2 Campus SupAgro-INRA
34060 Montpellier - France
Tel: 04-99-61-25-74 - Fax: 04-67-52-57-37
very@supagro.inra.fr

Vignes Michel
UMR5247 Institut des Biomolécules Max Mousseron
Université Montpellier 2 Place Eugène Bataillon
34095 Montpellier - France
Tel: 0467143699 - Fax:
michel.vignes@univ-montp2.fr

Voisin Tiphaine
IGF
141 rue de la Cardonille
34094 Montpellier - France
Tel: 0434359250 - Fax:
tiphaine.voisin@igf.cnrs.fr

Wang Limin
CNRS
Laboratoire B&PMP UMR 5004
CNRS/386 INRA/ SupAgro M/UM2
Campus SupAgro-INRA
34060 Montpellier - France
Tel: 04-99-61-20-57 - Fax: 04-67-52-57-37
limin.wang@supagro.inra.fr

Warnier Marine
Laboratoire physiologie cellulaire
INSERM U1003
Université Lille 1 UFR Biologie Rue Paul Langevin Bât SN3 2e étage
59655 Villeneuve d'Ascq - France
Tel: 0320336423 - Fax: 0320434066
marine.warnier@etudiant.univ-lille1.fr

Wong Lik-Wei
University of Melbourne
Department of Pharmacology and Therapeutics Level 8, Medical Building
Corner of Grattan street and Royal Parade
3010 Victoria - Australia
Tel: +61430095762 - Fax:
l.wong2@student.unimelb.edu.au

Wood John
UCL
Gower Street London
WC1E 6BT London - UK
Tel: 44-20767967954 - Fax:
j.wood@ucl.ac.uk

Xiong Tou Cheu
Biochimie et Physiologie Moléculaire des Plantes
Institut de Biologie Intégrative des Plantes Claude Grignon Campus INRA-SupAgro
34060 Montpellier cedex 2 - France
Tel: +33 (0)499 612 711 - Fax:
+33(0)467 525 737
xiong@supagro.inra.fr

Ye-lehmann Shixin
Institut de biologie, École Normale Supérieure
46 rue d'Ulm
75005 Paris - France
Tel: 01.44.32.38.92 - Fax:
yelehman@biologie.ens.fr

Zimmermann Sabine
CNRS
Institut de Biologie Intégrative des Plantes IBIP Biochimie et Physiologie Moléculaire des Plantes BPMP UMR 5004 AgroM-CNRS-INRA-UM2 Campus AgroM-INRA Place Viala, Bât. 7
34060 Montpellier cedex1 - France
Tel: + 33 4 99 61 27 18 - Fax: + 33 4 67 52 57 37
sabine.zimmermann@supagro.inra.fr

DUTSCHER s.a.
Jocelyne CACHAT
30, rue de l'industrie
BP 62
67172 BRUMATH
FRANCE

MOLECULAR DEVICES (UK) LTD
Carlene Whitehead, BSc (Hons
European Marketing Communications Specialist
660-665 Eskdale Road | Winnersh Triangle
Wokingham | Berkshire RG41 5TS

MULTI CHANNEL SYSTEMS
Karla Burgert
Aspenhaustrasse 21
72770 Reutlingen
Germany

QIAGEN - SAMPLE & ASSAY TECHNOLOGIES
ISABELLE BITAULD
Senior Executive Assistant
Communication & Marketing Coordinator
QIAGEN France SAS
3 Avenue du Canada
LP 809
91974 Courtaboeuf cedex
France

SCIENTIFICA LTD.
Catherine Arnold
Kingfisher Court
Brambleside
Bellbrook Industrial Estate
Uckfield, East Sussex
TN22 1QQ
United Kingdom

SMARTOX BIOTECHNOLOGY
AURELIEN CLAEYSSEN
570 rue de la chimie Bâtiment Nanobio Campus
38 400 Saitn Martin d'Hères France

THERMO FISHER SCIENTIFIC
Ruben Lonneville
Tradeshow Manager
Biosciences Division and Laboratory Product Group
Industriezone III
Industrielaan 27
B-9320 Erembodegem

WORLD PRECISION INSTRUMENTS
Mikael Berrou | Market Manager Be,Fr,Es
1 Hunting Gate, Hitchin, Hertfordshire.
SG4 0TJ
Industrielaan 27
B-9320 Erembodegem

HEKA Elektronik
Dr. Schulze GmbH
Wiesenstrasse 71
D-67466 Lambrecht/Pfalz
Germany

ALOMONE
Avi Wener
Har Hotzvim Hi-Tech Park,
P.O.Box 4287 Jerusalem 9104201,
Israel

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