ASSOCIATION « CANAUX IONIQUES »

PROGRAM
&
ABSTRACTS

www.canaux-ioniques.fr

CONTENTS

Organizing Committee

PRESIDENTS

Thomas Grutter
Laboratoire de Conception et Application de Molécules Bioactives, UMR 7199 CNRS,
Université de Strasbourg, Illkirch, France
Tel: 33-(0)13 68 85 41 57 – Fax: 33-(0)3 68 85 43 06 - E-mail: grutter@unistra.fr

Christian Mazars
LRSV-UPS CNRS,
Université Toulouse III Paul Sabatier, Toulouse, France
Tél: 33-(0)5 34 32 38 36 - E-mail: mazars@lrsv.ups-tlse.fr

Aurelien Chatelier
Institut de Physiologie et Biologie Cellulaires, Pôle biologie Santé, Batiment B36
Université de Poitiers 1 rue George Bonnet, Poitiers - France
Tel: 33-(0)549 453 747 - Fax: 33-(0)549 454 014 - aurelien.chatelier@univ-poitiers.fr

Claude Collet
INRA, UR406 Abeilles et Environnement, Toxicologie Environnementale
Site Agroparc, Avignon - France
Tel: 33-(0)4 32 72 26 49 - Fax: 33-(0)4 32 72 26 02 - E-mail: claude.collet@avignon.inra.fr

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

Sylvain Feliciangeli
Institut de Pharmacologie Moléculaire et Cellulaire, CNRS UMR7275
Université de Sophia-Antopolis, Valbonne, France
Tel: 33-(0)4.93.95.77.32 - E-mail: feliciangeli@ipmc.cnrs.fr

Hélène Guizouarn
CNRS, Institut de biologie de Valrose UMR 7277, Bâtiment de Sciences Naturelles
Université de Nice, 28 av. Valrose, Nice - France
Tel: 33-(0)492076837 - Fax: 33-(0)492076834 - E-mail: helene.guizouarn@unice.fr

Carole Levenes
Laboratory of Neurophysique and Physiologie, CNRS UMR 8119
Université Paris Descartes, Paris, France
Tel: 33-(0)142864157 - E-mail: carole.levenes@gmail.com

David Stroebel
INSERM U1024 / CNRS UMR 8197
École Normale Supérieure – IBENS, Paris, France
Tel: 33-(0)144323892 - Mobile : 33-(0)628250129 - E-mail: David.stroebel@ens.fr

Michel Vignes
UMR5247 Institut des Biomolécules Max Mousseron
Université Montpellier, 2 Place Eugène Bataillon, Montpellier - France
Tel: 33-(0)467143699 - E-mail: michel.vignes@univ-montp2.fr

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

Cyril Sarrauste de Menthière (Web Development & Database)
IGH, CNRS UPR1142, Montpellier
Tel : 33-(0)4 34 35 99 81 - mobile : 33-(0)6 11 35 79 47 - E-mail: cyril.sarrauste@igh.cnrs.fr

Sponsors

Nous remercions pour leur soutien :
We thank for their support :

ION CHANNEL SCIENCE AND THERAPEUTICS

www.canaux-ioniques.fr/

PROGRAM Oléron 2014

Sunday, september 14th 2014

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

20:15 « Oleron Oysters » cocktail

21:15 Dinner

Monday, september 15th 2014

8:30 Meeting opening

8:45 Symposium 1. Stretch-activated channels and mechanotransduction

Organized by Thomas Ducret, Bordeaux

Frederick Sachs (USA) Mechanosensitive ion channels: Piezo from biophysics to bedside

Philippe Gailly (Belgium) Role of TRPV in osmosensation

Fabrice Duprat (France) Role of polycystins in pressure sensing

Selected speaker: Hélène Gautier (UK) Influence of the mechanical environment on neuronal electrical properties

10:30 Coffee break

11:00-12:00 Oral communication 1. Pharmacology ragtime

Talks selected by David Stroebel, Paris

Sébastien Marra (France) Activation of ASIC3 ion channel by endogeneous lipids

Zied Landoulsi (Tunisia) Subtype-selective activation of Kv7 Channels by AATXKb(2-64), a novel toxin from androctonus australis scorpion venom

Jean-François Desaphy (Italy) Pharmacological characterization of a new HNAV1-4 sodium channel mutation causing myotonia permanens : a step forward personalized medecine

Christian Legros (France) The sensitivity of GABAA receptors to the anxiolytic compound etifoxine is modulated by both α and β subunit composition

12:15 Wine tasting

13:00 Lunch

15:00-17:00 Poster session 1

17:00 Symposium 2. Chemical environmental stressors & ions channels

Organized by Claude Collet, Avignon

Isaac Pessah (USA) RyR Channel Mutations Confer Susceptibility to Environmental Triggers of Disease.

Christopher Connoly (UK) The impact of cholinergic pesticides on bee neuronal function.

Michael Hans (Germany) The ubiquitous Bisphenol A and its relationship to Ca channel.

Selected speaker: Caroline Strube (France) Remodeling of glial coverage of rats NTS (nucleus tractus solitarii) glutamatergic synapses after ozone inhalation.

19:00 Plenary lecture

Anne Feltz (France) Toward imaging cellular calcium microdomains.

20:00 Dinner

Tuesday, september 16th 2014

8:45 Symposium 3. Cardiac electrophysiology : New experimental approaches

Organized by Aurélien Chatelier, Poitier

Emilia Entcheva (USA) Towards All-Optical Electrophysiology via Optogenetics : Combining Optical Actuation and Optical Sensing.

Julia Gorelik (UK) Scanning Ion Conductance Microscopy in Cardiac Biology.

Albano Meli (France) Functional properties of hiPSC-derived cardiomyocytes and disease modeling.

Selected speaker: Jean-François Rolland (Italy) Lighting up Ion Channel Drug discovery.

10:30 Coffee break

11:00-12:00 Oral communication 2. Physiology ragtime

Talks selected by Carole Levenes, Paris

Baptiste Rode (UK) Disruption of TRPC channel ion permeation protects against weigth gain in hypercholesterolaemic mice.

Marina Balycheva (Italy) Microdomain-specific location of L-type calcium channels with increased open probability in patients with atrial fibrillation : role of channel subunits.

Amélie Borie (France) Oxytocin and Vasopressin regulate electrophysiological properties of lateral septum neurons.

Benjamin Compans (France) Dynamic and nanoscale organization of AMPA Receptors during Long Term Depression.

12:15 Lunch

15:00-17:00 Poster session 2

17:00 Symposium 4. Ion Channels and Cancer

Organized by Hélène Guizouarn, Nice

Luis Pardo (Germany) Kv10.1 in tumor biology: mechanisms and therapeutic implications.

Ildiko Szabo (Italy) Mitochondrial channels: ion fluxes and more.

Christophe Vandier (France) The calcium activated SK3 channel: from a role on metastasis development to the emergence of the Canceropole Grand Ouest ion channel network.

Selected speaker: Raphael Rapetti-Mauss (Ireland) β-catenin regulates KCNQ1 Potassium channel expression in colon cancer cells.

18:45 Annual meeting of the association Canaux ioniques.

20:15 « Menu Boyard » Special Dinner

21:30 Poster Prize

21:40 « Mort Subite » session

22:15 Evening Party

Wednesday, september 17th 2014

9:45 Symposium 5. Intracellular Calcium Homeostasis

Organized by Michel Vignes, Montpellier

Stefan Feske (USA) Regulation of CRAC channels and their role in immune function.

Dimitri Rusakov (UK) Monitoring uneven landscapes of resting Ca2+ in neurons and glia using time-resolved fluorescence imaging.

Bruno Allard (France) Sarcoplasmic reticulum Ca2+ permeation explored from the lumen side provides new insights into excitation-contraction coupling in skeletal muscles.

Selected speaker: Elizabeth Aguettaz (France) Effect of Axial strech on calcium regulation in mouse dystrophic cardiomyocytes: involvment of TRPV2 channels ?

11:30 Oral communication Prize

11:40 Meeting closure

12:00 Lunch

13:00 Shuttle departure

SYMPOSIA AND ORAL COMMUNICATION ABSTRACTS

RESUMES DES SYMPOSIA ET DES COMMUNICATIONS ORALES

Symposium 1

Stretch-activated channels and mechanotransduction

Organized by Thomas Ducret, Bordeaux

.....

Monday, september 15th 2014

8:45 Frederick Sachs (USA) Mechanosensitive ion channels: Piezo from biophysics to bedside

9:15 Philippe Gailly (Belgium) Role of TRPV in osmosensation

9:45 Fabrice Duprat (France) Role of polycystins in pressure sensing

10:15 Selected speaker: Hélène Gautier (UK) Influence of the mechanical environment on neuronal electrical properties

.....

S-01 MECHANOSENSITIVE ION CHANNELS: PIEZO FROM BIOPHYSICS TO BEDSIDE

Frederick Sachs;
SUNY, Buffalo, NY 14214

All cells have mechanosensitive ion channels (MSCs), and of the eukaryotic cells, the cation selective PIEZO and K+ selective 2P families seem to be the most easily studied. These two families have no physical relationship other than mechanosensitivity. PIEZO1 is a monomer and the 2P channels are dimers. Recently cloned, PIEZO1 is found in many cell types including red blood cells where they play a functional role since mutations can cause anemia. The human version of the channel has been cloned from HEK293 cells so all experiments on HEK cells have a potential background of PIEZO channel activity. hPIEZO1 channels are blocked by the D and L enantiomers of the peptide GsMTx4. The channels appear to exist in physical domains whose line tension can alter the internal tension affecting channel activation. Breaking the domains leads to a loss of channel inactivation by groups of channels. Xerocytotic anemias are caused by mutations in human hPIEZO1 and these mutations all slow channel inactivation and introduce latency into activation. GsMTx4 may be an appropriate therapy for Xerocytosis. Skeletal muscle also has hPIEZO1 channels whose hypersensitivity in Duchenne dystrophy may be the cause of muscle degeneration by letting excess Ca+2 leak into the muscle and activating calpains, etc. GsMTx4 may be a therapy for dystrophy since it is specific, nontoxic and also suppresses cardiac arrhythmias.

A key to understanding MSCs is that in general we do not know the stress applied to the channels; we only know the stress we apply to the system. The coupling of stress to the channel is certainly unknown in cells and probably ill-defined even in lipids. Patches are samples of the cell cortex, not the bilayer, and the chemical composition is different from the cell it came from. Nearly all cells have cationic MSCs but most of the time they are protected ("mechanoprotection") by the cytoskeleton. Thus if you transfect with a DNA and see a cationic MSC, do not assume it is an expression of your particular gene. Expression of channels, and probably other proteins, can greatly alter the cytoskeleton and change the local stress on MSCs.

S-02 ROLE OF TRPV ION CHANNELS IN OSMOSENSATION

Philippe Gailly;
University of Louvain - Institute of Neuroscience - av. Mounier 53. Box B1.53.17, 1200 Brussels, BELGIUM

Systemic osmoregulation is a vital process whereby brain and kidney act in concert to modulate ingestive behaviour and renal function to stabilize the tonicity and volume of the extracellular fluid. Extracellular fluid hypertonicity is however transiently buffered by intracellular water coming from cells that shrink in response to hypertonicity. Due to its proportional mass in the body, skeletal muscle potentially plays an important role in whole body water balance. Its activity is also source of perturbation. Cell shrinkage (CS) induced by increased plasma osmolarity is followed by activation of a regulatory volume increase (RVI).

In skeletal muscle, osmotic CS is accompanied by transverse tubule (TT) dilatation inducing a small membrane depolarisation and a release of Ca2+ from intracellular pools (sarco-endoplasmic reticulum). We investigated the possible involvement of TRP ion channels in muscle RVI response to a hyperosmotic shock. We observed that both hyperosmotic shock-

induced Ca2+ transients and RVI were inhibited by Gd3+, by ruthenium red and by the Grammostola spatulata GsMTx4 toxin, three inhibitors of mechanosensitive channels. The response was also completely absent in muscle fibres overexpressing a dominant negative mutant of TRPV2 ion channel (TRPV2-DN), suggesting the involvement of TRPV2 channel or of a TRP isoform susceptible to heterotetramerize with TRPV2. The release of Ca2+ induced by hyperosmotic shock was increased after pretreatment with cannabidiol and decreased after pretreatment with tranilast, suggesting a role for TRPV2 channel itself.
The RVI subsequent to CS has been shown to require the sequential activation and phosphorylation of SPAK (STE20/SPS1-related proline/alanine-rich kinase) and of NKCC1, a Na+, K+ and Cl- cotransporter allowing ions entry and osmotic water driving. We observed that in fibres overexpressing TRPV2-DN as well as in fibres in which Ca2+ transients were artificially abolished by the presence of BAPTA, the level of P-SPAKSer373 in response to hyperosmotic shock was largely reduced, suggesting a modulation of SPAK phosphorylation by intracellular Ca2+. Surprisingly, muscles treatment with bumetanide, a specific inhibitor of NKCC1 also decreased P-SPAKSer373, suggesting a positive amplification loop between NKCC1, [Ca2+]i and P-SPAKSer373.
In summary, our results show that TRPV2 is involved in osmosensation in skeletal muscle fibres, acting in concert with P-SPAK-activated NKCC1.

S-03 ROLE OF POLYCYSTINS AND FILAMIN A IN CARDIOVASCULAR MECHANOSENSING

Fabrice Duprat;
duprat@ipmc.cnrs.fr

Autosomal-dominant polycystic kidney disease, the most frequent monogenic cause of kidney failure, is induced by mutations in the PKD1 or PKD2 genes, encoding polycystins TRPP1 and TRPP2, respectively. Polycystins are proposed to form a flow-sensitive ion channel complex in the primary cilium of both epithelial and endothelial cells. However, how polycystins contribute to cellular mechanosensitivity remains obscure. Our previous work shows that TRPPs are regulators of stretch-activated cationic channels (SACs) and stretch-activated potassium channels (SAKs). Deletion of TRPP1 in smooth muscle cells reduces SAC activity and the arterial myogenic tone. Inversely, depletion of TRPP2 in TRPP1-deficient arteries rescues both SAC opening and the myogenic response. We have also showed that TRPP2 interacts with filamin A and demonstrate that this actin crosslinking protein is critical for SAC regulation. We now used an inducible smMHC Cre system to specifically delete FlnA in smooth muscle cells of adult mice, and this lead to a progressive and robust inactivation of FlnA in arterial myocytes. Inactivation of FlnA in smooth muscle cells produces a mild hypotensive effect. Moreover, caudal arteries spontaneously remodel around a smaller lumen diameter upon FlnA inactivation. We also observed reduced amplitude of myogenic tone and a diminution of calcium influx through L-type calcium channels at increased intraluminal pressure. On the contrary, SAC activity induced by a step increase in pressure was significantly higher in myocytes from FlnA KO mice. In conclusion, our work shows the strong implication of FlnA in smooth muscle cells mechanosensitivity and a role in arterial remodeling.

S-04 INFLUENCE OF THE MECHANICAL ENVIRONMENT ON NEURONAL ELECTRICAL PROPERTIES

Helene Gautier[1]; Ragnhildur Karadottir[2]; Kristian Franze[1];
1: Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Anatomy Building, Cambridge CB2 3DY, UK
2: Wellcome Trust - MRC Stem Cell Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK

In mammals, spinal cord injuries lead to axonal degeneration and the formation of a glial scar. Despite its beneficial role in limiting the spread of the injury, the glial scar is thought to be a chemical and physical barrier to axonal regeneration. Thus, understanding why mammalian axons fail to regrow could lead to new therapeutic strategies. Although the biochemical environment of glial scars has been widely studied, mechanical aspects have so far largely been neglected. To understand how central nervous system (CNS) neurons respond to mechanical signals, we patch-clamped CNS neurons cultured on polyacrylamide gels of different stiffness (shear moduli of 100 Pa and 10 kPa). The development of neuronal electrical properties seems to be influenced by their mechanical environment. Our results will provide insights into how the physical properties of the environment affect neuronal function, and may ultimately lead to novel therapeutic approaches to facilitate neuronal regeneration after spinal cord injuries.

Oral communications 1

Pharmacology ragtime

Talks selected by David Stroebel, Paris

.....

Monday, september 15th 2014

11:00 Sébastien Marra (France) Activation of ASIC3 ion channel by endogeneous lipids.

11:30 Zied Landoulsi (Tunisia) Subtype-selective activation of Kv7 Channels by AATXKb(2-64), a novel toxin from androctonus australis scorpion venom.

12:00 Jean-François Desaphy (Italy) Pharmacological characterization of a new HNAV1-4 sodium channel mutation causing myotonia permanens : a step forward personalized medecine.

12:30 Christian Legros (France) The sensitivity of GABAA receptors to the anxiolytic compound etifoxine is modulated by both α and β subunit composition

.....

O-1 ACTIVATION OF ASIC3 ION CHANNEL BY ENDOGENOUS LIPIDS

Sébastien Marra; Anne Delaunay; Marine Christin; Jacques Noël; Eric Lingueglia; Emmanuel Deval;
IPMC - 660 route des Lucioles - Sophia Antipolis

Acid-sensing ion channel 3 (ASIC3) are excitatory ion channel activated by extracellular acidification. These channels are expressed in peripheral sensory neurons, where they were involved in the detection of inflammatory and post-operative pain (Deval et al, 2011; Deval et al, 2008; Ikeuchi et al, 2008; Sluka et al, 2007). A recent study shows that ASIC3 channels can be activated by compounds other than protons (Yu et al, 2010). Indeed, GMQ, a synthetic compound, and agmatine, a derivative of arginine, activate ASIC3 without any acidification of the extracellular medium, suggesting the existence of other endogenous activators, different from protons. In this study, we show that lipids (arachidonic acid and lysophospholipid), produced after hydrolysis of the plasma membrane by Phospholipase A2, are new endogenous activators of ASIC3. In heterologous expression systems, those two lipids activates reversibly ASIC3 without any changes in the extracellular pH. This activation is progressive and generates sustained currents. We also show that subcutaneous injections of these two lipids in rats lead to a characteristic pain behavior which is partially inhibited by pharmacological blockers of ASIC3 and ASIC channels. These data show the activation ASIC3s by endogenous compounds other than protons and products in case of inflammation, thus confirming the important role of this channel as a sensor of inflammatory mediators at the peripheral levels.

O-2 SUBTYPE-SELECTIVE ACTIVATION OF KV7 CHANNELS BY AATXKβ(2-64), A NOVEL TOXIN VARIANT FROM THE ANDROCTONUS AUSTRALIS SCORPION VENOM

Zied Landoulsi[1]; Francesco Miceli[2]; Angelo Palmese[3]; Angela Amoresano[3]; Gennaro Marino[3]; Mohamed El Ayeb[1]; Maurizio Taglialatela[4]; Rym Benkhalifa[1];
1: Laboratory of Venoms and Therapeutical Molecules, Institut Pasteur de Tunis, Tunis, Tunisia
2: Department of Neurociences, Division of Pharmacology, University of Naples Federico II, Naples, Italy
3: Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
4: Department of Medecine and Health Sciences, University of Molise, Campobasso, Italy.
Department of Neurociences, Division of Pharmacology, University of Naples Federico II, Naples, Italy

K+ channels encoded by the Kv7 gene family control membrane excitability in a wide variety of cells including neurons, cardiac myocytes, smooth and skeletal muscle cells, and sensory cells. Kv7 channel activators might be effective against neuropsychiatric disorders characterized by neuronal hyperexcitability, as well as hearing loss, gastro-intestinal diseases or hypertension, and skeletal muscles disorders. Scorpion venoms are known as a large source of peptides active on K+ channels. Therefore, we have optimized an experimental process alternating biochemical and electrophysiological tests to identify specific activator(s) of

Kv7.4 channels from the venom of the North African scorpion Androctonus australis (Aa). We report, the purification and functional characterization of AaTXKβ(2-64), a new variant of AaTXKβ, from a non-toxic fraction of Aa venom, which acts as the first peptide activator of Kv7.4 channel. More specifically, in Xenopus oocytes and CHO cells, AaTXKβ(2-64), and not AaTXKβ(1-64), hyperpolarized the threshold voltage of current activation and increased the amplitude of the maximal current of heterologously expressed Kv7.4 channels. This toxin effectively increased both maximal conductance and caused a leftward shift in the activation curve of Kv7.4 channels. Furthermore, AaTXKβ(2-64) activated also homomeric Kv7.3 and heteromeric Kv7.2/3 and Kv7.5/3 channels, without affecting the current amplitude and the activation threshold of Kv7.1, Kv7.2 and Kv1.1 channels. These findings are of great interest in exploring new biological roles of AaTXKβ(2-64)-senstive Kv7 channels and developing novel pharmacological tools that allow subtype-selective targeting of these channels.

O-3 PHARMACOLOGICAL CHARACTERIZATION OF A NEW HNAV1.4 SODIUM CHANNEL MUTATION CAUSING MYOTONIA PERMANENS: A STEP FORWARD PERSONALIZED MEDICINE

Jean-François Desaphy[1]; Roberta Carbonara[1]; Marilena Dinardo[1]; Anna Modoni[2]; Adele D'Amico[3]; Serena Pagliarani[4]; Mauro Lo Monaco[2]; Diana Conte Camerino[1];
1: University of Bari, Italy
2: Catholic University, Rome, Italy
3: Bambino Gesù Hospital, Rome, Italy
4: University of Milan, Italy

Mutations in the skeletal muscle (Nav1.4) sodium channel are responsible for paramyotonia congenita or sodium channel myotonia, two muscle diseases characterized by impaired muscle relaxation after voluntary contraction. The Nav blocker mexiletine has received orphan drug designation in nondystrophic myotonia. We identified a new Nav1.4 mutation (p.P1158L) in a young Algerian girl showing a severe myotonia permanens phenotype. She obtained some improvement with mexiletine but experienced side effects. Treatment was shifted to flecainide, another Nav blocker previously shown to exert antimyotonic activity in patients resistant to mexiletine (Desaphy et al., Eur J Clin Pharmacol 2013). The patient claimed great improvement of stiffness with flecainide. Recombinant P1158L hNav1.4 mutant was expressed in tsA201 cells for patch-clamp studies. Compared to wild-type, the mutant channel shows a slower rate and a ~6-mV positive shift in voltage dependence of fast inactivation. Voltage dependences of activation and slow inactivation is not altered. The P1158L currents are less sensitive to mexiletine compared to WT; The half-maximum inhibitory concentration for tonic block (holding potential of -120 mV, stimulation frequency of 0.1 Hz) increases from 295 in WT to 479 μM in P1158L. In a myotonic–like condition (-90 mV, 50 Hz), inhibition of P1158L currents by 10 and 30 μM mexiletine is significantly reduced compared to WT. Conversely, the sensitivity to flecainide is not altered by P1158L. In conclusion, the impairment of sodium channel fast inactivation caused by the P1158L mutation likely accounts for the symptoms. The reduced sensitivity of P1158L to mexiletine may have contributed to the unsatisfactory response of patient. Supported by Telethon-Italy and AFM-France.

O-4 THE SENSITIVITY OF GABAA RECEPTORS TO THE ANXIOLYTIC COMPOUND ETIFOXINE IS MODULATED BY BOTH ALPHA AND BETA SUBUNIT COMPOSITION

Cesar MATTEI[1]; Antoine TALY[2]; Marc VERLEYE[3]; Daniel HENRION[1]; Nathalie C GUERINEAU[1]; Christian LEGROS[1];

1: 1Department of Integrated Neurovascular and Mitochondrial Biology, CNRS UMR6214, INSERM U1083, University of Angers, 49045 Angers cédex, France.

2: Laboratoire de Biochimie Théorique - UPR 9080 Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.

3: Biocodex, Service de Pharmacologie, ZAC de Mercières, 60200 Compiègne, France.

The anxiolytic compound Etifoxine (EFX, Stresam®) potentiates GABAA receptor function through both a direct binding and a stimulation of neuroactive steroids synthesis. The GABAA receptor β subunit is crucial for EFX effects. The GABA-evoked current potentiation induced by EFX is more important at receptors containing β2 or β3-subunit than at β1-receptors. Here, we examined the potentiating effect of EFX on both synaptic GABAA receptors, containing α3, α4 or α6 associated with β3γ2 and extrasynaptic GABAA receptors, containing α4 or α6 with β3γ2 or α5β3δ2. All combinations were expressed in Xenopus oocytes for electrophysiological recordings of GABA-evoked currents. α3β3γ2 GABAA receptors were 2.5-fold more sensitive to EFX than α5 or α6 containing GABAA receptors. In comparison, receptors with α4 subunit were resistant to EFX. Our data are in agreement with the lack of secondary effects of EFX, including sedation, amnesia and tolerance. Altogether, our data showed that EFX-mediated potentiation of GABA-evoked currents relies on the α subunit, but not on γ2 or δ subunits. We next built a model of interaction between EFX and GABAA receptors. It predicts a binding site of EFX between α and β subunits in the extra-cellular domain. In conclusion, the anxiolytic EFX preferentially potentiates GABA-evoked currents elicited by synaptic GABAA receptors containing α2-3/β2-3 subunits, which are involved in the anxiolytic-like effects mediated by positive modulation at GABAA receptors. Thus, this non-benzodiazepine compound displays a pharmacological profile appropriate for the long-term treatment of anxiety with minimal side effects. Further investigation of the structure-function relationship will allow deciphering the molecular basis of the discriminative effects of EFX.

Symposium 2

Chemical environmental stressors and ions channels

Organized by Claude Collet, Avignon

.....

Monday, september 15th 2014

17:00 Isaac Pessah (USA) RyR channel mutations confer susceptibility to environmental triggers of disease.

17:30 Christopher Connolly (UK) The impact of cholinergic pesticides on bee neuronal function.

18:00 Michael Hans (Germany) The ubiquitous Bisphenol A and its relationship to Ca channel.

18:30 Selected speaker: Caroline Strube (France) Remodeling of glial coverage of rats NTS (nucleus tractus solitarii) glutamatergic synapses after ozone inhalation.

.....

S-05 NON-DIOXIN LIKE POLYCHLORINATED BIPHENYLS AND DIPHENYLETHERS- FROM RYR1 CHANNELS TO MUSCLE DYSFUNCTION

Isaac Pessah;
Department of Molecular Biosciences, UC Davis School of Veterinary Medicine

Non-dioxin like (NDL) such as polychlorinated biphenyls (PCBs) and diphenylethers (e.g., triclosan; TCS) are both persistent legacy environmental contaminants and have significant contemporary sources. NDL-PCBs interact with ryanodine receptors (RyRs), Ca2+ channels of sarcoplasmic/endoplasmic reticulum (SR/ER) that regulate excitation-contraction coupling (ECC) and Ca2+-dependent cell signaling in muscle and nerve cells. Activities of 4 chiral PCB congeners and their respective 4- and 5-hydroxy (-OH) derivatives toward skeletal muscle ryanodine receptor (RyR1) were compared to the pervasive chlorinated diphenylether TCS using [3H]ryanodine binding, single channel voltage clamp, and SR Ca2+ flux analyses. Parent PCB congeners exhibit a stringent structure-activity relationship toward enhancing RyR1 activity across all three measures, including pronounced stereoselectivity that extends to the cardiac RyR2 channel. Although 5-OH metabolites have comparable activity to their respective parent structures in all three assays, 4-OH derivatives were unable to trigger Ca2+ release from SR microsomes in the presence of Ca2+-ATPase activity. PCB 95 its OH-metabolites and TCS were investigated further using primary murine myotubes and FDB fibers for their influences on excitation-contraction coupling (ECC). Ca2+ imaging of muscle cells loaded with Fluo-4 showed that acute exposure to PCB 95 (5μM) potentiates ECC and partially depletes SR Ca2+ stores. Exposure to 5-OH-PCB 95 or TCS (5 μM) transiently increases cytoplasmic Ca2+, leading to rapid ECC failure. By contrast, 4-OH-PCB 95 neither increases baseline Ca2+ nor causes ECC failure but rather depletes SR Ca2+ and depresses ECC. Subacute exposure of muscle cells to 10-300 nM of each compound impaired myotube ECC by primarily depleting SR Ca2+ stores (PCB 95 and 4-OH PCB 95) or uncoupling ECC (5-OH-PCB 95 and TCS). Certain NDL persistent organic pollutants directly target RyR channels and impair ECC at concentrations relevant to human health, especially in muscle with expressed RyR mutations that confer heightened susceptibility.

S-06 THE IMPACT OF NEONICOTINOIDS ON THE BEE BRAIN

Christopher Moffat[1]; Mary Palmer[1]; Christopher Connolly[2];
1: Division of Neuroscience Medical Research Institute Ninewells Medical School, Mail box 6 University of Dundee Dundee DD1 9SY
2: Division of Neuroscience Medical Research Institute Ninewells Medical School (level 6, Rm 093) Mail box 6 University of Dundee Dundee DD1 9SY

Pesticides that target cholinergic neurotransmission are highly effective, but their use has been implicated in insect pollinator population decline. Honeybees are exposed to two widely used classes of cholinergic pesticide: neonicotinoids (nicotinic receptor agonists) and organophosphate miticides (acetylcholinesterase inhibitors). Although sublethal levels of neonicotinoids are known to disrupt honeybee learning and behaviour, the neurophysiological basis of these effects has not been shown. Using recordings from mushroom body Kenyon cells in acutely isolated honeybee brain, we show that the neonicotinoids imidacloprid and clothianidin, and the organophosphate miticide coumaphos oxon, cause a depolarization block of neuronal firing and inhibit nicotinic responses. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with

combined application. Bumblebees appear more sensitive to neonicotinoids and at environmentally relevant levels they cause neuronal vulnerability to innocuous insults.

S-07 THE UBIQUITOUS BISPHENOL A AND ITS RELATIONSHIP TO CALCIUM CHANNEL

Michael Hans;
Institute of Physiology, University of Bonn, Bonn, Germany

Bisphenol A (BPA) is a high volume chemical compound used in the production of many consumer products, including plastics, PVC, food packaging, medical tubing, flame retardants, dental sealants and thermal paper. Humans are widely exposed to BPA through their diet, respiration and dermal contact and BPA's adverse effects on human health and the ecosphere are being increasingly recognized. BPA is a known endocrine disruptor. It binds to multiple hormone receptors and influences multiple endocrine pathways. BPA interacts with physiological systems including the immune system, the endocrine pancreas, and the developmental central nervous system by acting on biologically active proteins such as enzymes and ion channels. In this study we present evidence that BPA interacts with voltage-activated Ca2+ channels as inhibitory ligand in various tissues, including neuroendocrine cells, neurons and cardiac cells. Pharmacological experiments with specific organic blockers (nifedepine and w-conotoxins) for the different Ca2+ channel types showed that BPA affects all subtypes studied here (L-, P/Q-, R-type) to the same extent. Detailed analysis of biophysical properties on human R-type Ca2+ channels strongly suggests that BPA exerts its action by binding to channels in their resting state to a site, located at the extracellular part of the pore forming subunit. Structure-function analysis of BPA and related derivatives revealed requirements necessary for BPA´s effectiveness. The structural motive a double-methylated or double-trifluoromethylated sp3-hybridized carbon atom flanked by two phenol moieties in angulated orientation strongly suggests a specific binding site at the various Ca2+ channels. This knowledge could provide a key in finding molecules which could substitute for BPA in large scale plastic production.

S-08 REMODELING OF GLIAL COVERAGE OF RAT NTS GLUTAMATERGIC SYNAPSES AFTER OZONE INHALATION.

Keodavanh Chounlamountry; Bénédicte Boyer; Anne -Marie François-Bellan; Olivier Bosler; Jean-Pierre Kessler; Caroline Strube;
CRN2M, Faculté Médecine Nord, Bd Pierre Dramard, 13344 Marseille Cedex 15, France

Ozone, a major component of air pollution, has considerable impact on public health. Besides its well described inflammatory and dysfunction effects on the respiratory tract, there is accumulating evidence indicating that ozone exposure also affects brain functions. However, the mechanisms through which ozone exerts toxic effects on the CNS remain poorly understood. We previously showed that in addition to lung inflammation, ozone exposure caused a neuronal activation in the dorsolateral regions of the rat nucleus tractus solitarius (NTS, a sensory nucleus involved in visceral information processing) overlapping terminal fields of lung primary afferent running in the vagus nerves (Gackière et al. 2011). Knowing that neuronal activity can induce glial remodeling in different brain structures (Reichenbach et al. 2010) and taking into account the high level of expression of plasticity marker PSA-

NCAM in the NTS (Bouzioukh at al. 2001), we hypothesized that ozone induced neuronal activation could lead to astroglial remodeling.

To investigate this hypothesis, we used electron microscopy and immunoblot techniques. In ozone-exposed animals, the astrocytic coverage of NTS glutamatergic synapses is increased while the astrocyte volume fraction and the astrocyte membrane densities are not significantly increased. Moreover, the expression of specific astrocyte markers such as GFAP, GLT1 and Glutamine Synthase are only slightly increased after 24 hours of ozone exposure. Altogether, our results indicate that ozone inhalation induces glial plasticity, which is restricted to the peri-synaptic coverage.

Plenary lecture

Monday, september 15th 2014
19:00

……..

Anne Feltz

(Paris, France)

……..

Toward imaging cellular calcium microdomains.

Anne FELTZ[1]; Alsu ZAMALEEVA[1]; Mayeul COLLOT[2]; Eloi BAHEMBERA[3]; Céline TISSEYRE[3]; Philippe ROSTAING[1]; Aleksey YAKOVLEV[1]; Martin OHEIM[4]; Michel DE WAARD[3]; Jean-Maurice MALLET[2];

1: IBENS, 46 rue d'Ulm, F-75005 Paris

2: Laboratoire de Biomolécules, ENS, Université Pierre et Marie Curie, F-75005 Paris

3: Grenoble Neurosciences Institute, Université Joseph Fourier, F-38042 Grenoble

4: Laboratoire de Physiologie cérébrale, Université Paris-Descartes, F-75006 Paris

Small-molecule chemical calcium (Ca2+) indicators are invaluable tools for studying intracellular signaling pathways but have severe shortco- mings for detecting local Ca2+entry. Nanobiosensors incorporating functionalized quantum dots (QDs) have emerged as promising alternatives, but their intracellular use remains a major challenge. We designed cell-penetrating FRET-based Ca2+nanobiosensors for the detection of local Ca2+ concentration transients, usingCANdot®565QD as a donor and CaRuby, a custom red-emitting Ca2+ indicator, as an acceptor. With Ca2+-binding afinities covering the range of 3 to 20 μM, our CaRubies allow building sensors with a scalable afinity for detecting intracellular Ca2+transients at various concentrations. To facilitate their cytoplasmic delivery, QDs were further functionalized with a small cell-penetrating peptide (CPP) derived from hadrucalcin (Had : UF1-11 H11), a ryanodine receptor-directed scorpion toxin identified within the venom of Hadrurus gertschi. Efficient internalization of QDs doubly functionalized with PEG5-CaRuby and H11 (in a molar ratio of 1:10:10, respectively) is demonstrated. In BHK cells expressing a N-methyl-D-aspar tate receptor (NMDAR) construct, these nanobiosensors report rapid intracellular near-membrane Ca2+ transients following agonist application when imaged by TIRF microscopy. Our work presents the elaboration of cell-penetrating FRET-based nanobiosensors and validates their function for detection of intracellular Ca2+ transients.

Symposium 3

Cardiac electrophysiology : New experimental approaches

Organized by Aurélien Chatelier, Poitier

.....

Tuesday, september 16th 2014

8:45 Emilia Entcheva (USA) Towards All-Optical Electrophysiology via Optogenetics: Combining Optical Actuation and Optical Sensing.

9:15 Julia Gorelik (UK) Scanning Ion Conductance Microscopy in Cardiac Biology.

9:45 Albano Meli (France) Functional properties of hiPSC-derived cardiomyocytes and disease modeling.

10:15 Selected speaker: Jean-François Rolland (Italy) Lighting up Ion Channel Drug discovery.

.....

S-09 Towards All-Optical Cardiac Electrophysiology via Optogenetics

Emilia Entcheva;
Department of Biomedical Engineering, Stony Brook University, Stony Brook, NY 11790, USA

Optogenetics, in the broader sense, refers to the use of genetically-encoded molecules serving as optical actuators or optical sensors for the active interrogation and imaging of biological processes and systems with high specificity and high spatiotemporal resolution. The recently developed optogenetic actuators center around microbial opsins (light-gated ion channels and pumps) with depolarizing/excitatory, e.g. Channelrhodopsin2, or hyperpolarizing/inhibitory action, e.g. Halorhodopsin or Archaerhodopsin. While optogenetics has already made an impact in neuroscience, only recently, these new tools have been extended to cardiac applications. In this talk, I will present new results from my lab dealing with viral and non-viral approaches to cardiac optogenetics, computational insights into the utility of opsins in cardiac tissue, the practical integration of optical actuation and optical sensing, and demonstration of new ways towards biological pacemakers, cardioversion and control of cardiac function by light.

S-10 DEVELOPING SCANNING ION CONDUCTANCE MICROSCOPIC TECHNIQUE FOR CARDIOVASCULAR BIOLOGY

Julia Gorelik ;
Imperial College London, Du Cane Rd, London, UK

Conventional physiological techniques for cardiac cells have attained important achievements during past decades. However, few of them resolve physiological processes at the nanoscale level in living cells. Scanning ion conductance microscopy (SICM) is a unique imaging technique that uses similar principles to the atomic force microscope, but with a pipette for the probe. The scanning technique enables simultaneous recording of high-resolution topography of cell surfaces, and cell surface fluorescence. The hybrid instrument also functions as a vastly improved patch clamp system (the "smart patch"). The method allows scanning of the surface of living cells noninvasively and enables measurement of cellular activities under more physiological conditions than is possible with other techniques.

Since the activity of various receptors and ion channels is highly organized in space and time, it is essential to correlate intracellur signalling with cell structures and subcellular compartments. We describe and validate scanning ion conductance microscopy combined with conventional methods (FRET, patch-clamp, intercellular recording and optical mapping of impulse propagation) as a new technique for cardiac cell physiology . Such hybrid technologies revealed i) functional localization of beta –adrenergic receptors; ii) location of ionic currents and membrane potential and iii) dynamics of intercellular impulse propagation in cardiac cells. We used scanning ion conductance microscopy and conventional cell-attached patch-clamp with a software modification that allows controlled increase of pipette tip diameter. The sharp nanopipette used for topography scan was modified into a larger patch pipette which can be positioned with a nanoscale resolution to a specific site of interest (crest, groove or T-tubules of cardiomyocytes), and sealed to the membrane for cell-attached recording of ion channels. Using this method, we significantly increased the probability of detecting activity of L-type calcium channels in the T-tubules of ventricular cardiomyocytes.Our new method substantially increases the throughput of recording location-

specific functional ion channels on the cardiomyocyte sarcolemma, thus allowing characterization of ion channels in relation to the microdomain in which they reside.

S-11 MODELING OF CATECHOLAMINERGIC POYMORPHIC VENTRICULAR TACHYCARDIA WITH HUMAN INDUCED PLURIPOTENT STEM CELLS

Ivana Acimovic[1]; Aleksandra Vilotic[1]; Valerie Scheuermann[2]; Petr Dvorak[1]; Vladimir Rotrekl[1]; Alain Lacampagne[2]; Albano Meli[2];

1: Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

2: INSERM U1046, University of Montpellier I and II, Montpellier, France

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal inherited arrhythmogenic disorder occurring in the absence of a structural heart disease. Predominantly it is caused by mutations in cardiac ryanodine receptor gene (RYR2). Human induced pluripotent stem cells (hiPSCs) offer a unique opportunity for disease modeling.
Aims: The goals are to derive functional cardiomyocytes (CMs) from CPVT patient via hiPSCs and test whether the novel CPVT mutation is associated with abnormal intracellular Ca2+ handling properties in CMs.
Methods: hiPSCs are reprogrammed from dermal fibroblasts from a 31 years-old female CPVT patient carrying a novel heterozygous single point mutation RyR2-D3638A. Following molecular characterization, WT- and CPVT-hiPSCs are differentiated into CMs. Using confocal microscopy, their intracellular Ca2+ handling properties are studied in resting and stress conditions.
Results: WT- and CPVT-hiPSCs express pluripotency markers (OCT4, NANOG, SSEA4) and exhibit normal karyotype. Both CMs exhibit typical cardiac markers such as cardiac troponin T and I, and α-actinin. At rest, there is no significant difference in the amplitude and frequency of spontaneous Ca2+ transients between WT- and CPVT-CMs. Significant differences in maximal amplitude and frequency of Ca2+ transients are observed when paced (0.5, 1, and 2 Hz), and under adrenergic stimulation. At rest and upon adrenergic stimulation, no difference is observed in the caffeine-induced Ca2+ release between CPVT- and WT-CMs.
Conclusions: Our results indicate that hiPSC-CMs can provide a suitable platform for CPVT disease modeling. In stress conditions, the novel heterozygous single point mutation RyR2-D3638A may cause SR Ca2+ leak.

S-12 LIGHTING UP ION CHANNEL DRUG DISCOVERY

Jean-Francois Rolland; Alberto Di Silvio; Viviana Agus; Katharina Montag; Loredana Redaelli; Lia Scarabottolo; Stefan Lohmer;

Axxam SpA, via Meucci 3 - 20091 Bresso/Milan - Italy

Channelrhodopsin-2 (ChR2) is a light-activated microbial cation channel which can be used to depolarize neurons through the incidence of blue light. This tool has opened new and interesting perspectives in the neuroscience field, but can we use it for the characterization of voltage-gated ion channels and the search for modulators in a large scale? Can we perform High Throughput Screening using Optogenetic?

Proof of principle studies have been performed to answer these questions. An HEK-293 cell line stably co-expressing the hCav1.3 and the inward rectifier hKir2.3 channels, was over-transfected with a construct for ChR2 carrying a single amino acid mutation. This mutation results in both a prolonged lifetime of the conducting state of ChR2, and an increased light' sensitivity. The aim was to achieve a full activation of ChR2 with the light emitted by the LED banks of the FLIPR. A protocol was created to perform light stimulation of ChR2, hence plasma membrane depolarization and opening of the Cav1.3 channels leading to an intracellular calcium rise recorded with a calcium-sensitive fluorescent dye. Thanks to the Kir2.3 channels, the plasma membrane potential was set close to reversal potential for potassium to keep the Cav1.3 channels in the closed state. Partial Cav1.3 inactivation was induced by illuminating the cells with two blue light pulses separated by various interval. The percentage of inactivation is taken as the relative calcium increase induced by the second pulse normalized by the first. Experiments with known state and non-state dependent Cav1.3 blockers, tested in the resting and the partial inactivated conditions, confirmed their pharmacological profile. Data obtained for the ChR2-hCav1.3-hKir2.3 cell line by light stimulation, as opposed to the "old" potassium protocol, and data produced by automated patch-clamp are consistent. The cell line was fully characterized in terms of signal reproducibility and stability over time, proving its suitability for HTS.
Our study illustrates the first use of the ChR2 tool on the FLIPR instrument, for the purpose of activating voltage-gated ion channels in physiological extracellular potassium concentration. Our innovative Ion ChannelFLASH technology is suitable for HTS application.

Oral communication 2

Physiology ragtime

Talks selected by Carole Levenes, Paris

.....

Tuesday, september 16th 2014

11:00 Baptiste Rode (UK) Disruption of TRPC channel ion permeation protects against weigth gain in hypercholesterolaemic mice.

11:30 Marina Balycheva (Italy) Microdomain-specific location of L-type calcium channels with increased open probability in patients with atrial fibrillation : role of channel subunits.

12:00 Amélie Borie (France) Oxytocin and Vasopressin regulate electrophysiological properties of lateral septum neurons.

12:30 Benjamin Compans (France) Dynamic and nanoscale organization of AMPA Receptors during Long Term Depression.

.....

O-5 DISRUPTION OF TRPC CHANNEL ION PERMEATION PROTECTS AGAINST WEIGHT GAIN IN HYPERCHOLESTEROLAEMIC MICE

Baptiste Rode; Nadira Y. Yuldasheva; Stephen B. Wheatcroft; Justin F.X. Ainscough; David J. Beech ;
School of Medicine, University of Leeds, Leeds, LS2 9JT, UK

We previously showed that Transient Receptor Potential Canonical (TRPC) channels are expressed by adipocytes and that short-term inhibition of these channels increased the concentration of circulating adiponectin, suggesting a functional role of TRPC channels in adipocyte biology in vivo (Sukumar et al, Circ Res. 2012;111:191-200). The aim of this study was to investigate if there are implications of TRPC channels for the control of body weight. Mice with conditional global expression of dominant-negative ion pore mutant TRPC5 were used to suppress ion permeation in TRPC channels in vivo in adults without deleting TRPC proteins. Mice were fed a western-style high fat diet for 12 weeks and male litter-mates were compared with and without suppression of TRPC ion permeation. Mice expressing mutant TRPC5 showed significantly lower body weight as compared to controls. No obvious adverse effects on health were evident. Analysis of adipose tissue showed that adiponectin mRNA was more abundant in the mice expressing mutant TRPC5, suggesting regulation of gene expression in adipocytes. The results suggest that ion flux through TRPC channels is a driver for weight gain and that ion pore blockers of TRPC channels could be an approach for protecting against obesity.

O-6 Microdomain-specific location of L-type calcium channels with increased open probability in patients with atrial fibrillation: role of channel subunits

Marina Balycheva[1]; Alexey Glukhov[2]; Sophie Schobesberger[2]; Jose L. Sanchez-Alonso[2]; Francisca Schultz[2]; Prakash Punjabi[2]; Giuseppe Faggian[1]; Julia Gorelik[2];
1: Cardiosurgery Department, University of Verona, P.le A. Stefani, 1 - 37126 Verona, Italy
2: National Heart and Lung Institute, Imperial Centre for Translational and Experimental Medicine Hammersmith Campus, Du Cane Road London W12 0NN, UK

Background: Atrial fibrillation (AF) is the most common arrhythmia and is associated with an increased single L-type calcium channels (LTCCs) open probability. AF is also characterized by structural changes in myocytes which could be substrate for electrical remodeling. However, it remains unknown how structural changes modulate LTCC activity.
Objective: To determine spatial distribution and biophysical characteristic of functional LTCCs and their structural composition in myocytes collected from human right atrium.
Methods and results: Scanning ion conductance and confocal microscopy were used to characterize cell surface topography and t-tubules (TTs) system in myocytes collected from 22 patients with sinus rhythm (AF-) and 28 patients with AF (AF+). Surface and TTs structures were disrupted in AF+ cells in comparison to AF- with Z-groove index 0.39± 0.04 vs 0.56 ± 0.02, $P<0.01$ and with TTs density 2.48±1.11 vs 5.4±3.41, ns, respectively. Distribution of functional LTCCs in different subcellular microdomains was revealed by super-resolution scanning patch-clamp. LTCCs clustered in the TTs demonstrated difference in amplitude (0.5± 0.06 pA vs 0.77±0.06 pA, $P<0.01$) and open probability (0.25±0.05 vs 0.06±0.02, $P<0.01$) of single channel in AF+ and AF- myocytes, respectively.

The qPCR was used to determine mRNA levels of LTCCs forming subunits in human tissue.Transcription of all LTCC subunits (α1C, β1, β2, β3 and β4) was downregulated in AF+ samples.
Conclusion:
We found significant surface and t-tubules structural damaging in atrial cells in AF. Also we demonstrated in AF+ cells microdomain-specific localisation of functional LTCCs with 2.6-fold higher open probability clustered in TTs in comparison with AF- cells.
Transcriptional downregulation of channel subunits in samples collected from patients with AF could underlie a decrease of single LTCC amplitude and their arrhythmogenic phenotype.

O-7 OXYTOCIN AND VASOPRESSIN REGULATE ELECTROPHYSIOLOGICAL PROPERTIES OF LATERAL SEPTUM NEURONS

Amélie M Borie[1]; Françoise Muscatelli[2]; Gilles Guillon[1]; Michel G Desarménien[1];
1: CNRS, UMR-5203, INSERM, U661, Universités de Montpellier 1 & 2. Institut de Génomique Fonctionnelle, Montpellier, F-34094, France
2: Institut de Neurobiologie de la Méditerranée, INMED UMR U901, INSERM, Parc Scientifique de Luminy, 13273 Marseille, France.

Oxytocin (OT) and vasopressin (VP) regulate social behaviors and they respectively have natural pro and anti-social effects both in human and rodent but their mode of action is unknown.
The lateral septum is a brain structure involved in social behavior and expressing OT and VP receptors. Strikingly, in the lateral septum, these neuropeptides seem to both have a positive effect on social behavior. Nevertheless, properties of lateral septum neurons are mostly unknown and electrophysiological effects of OT and VP on these neurons have not been precisely described so far.
To decipher the mode of action of OT and VP in this structure, we performed loose-patch and whole cell recording in acute slices of wild-type mice brain and described electrophysiological characteristics of lateral septum neurons and their modulation by OT and VP. During the recording, neurons were labeled with AlexaFluor594-cadaverin to study their morphology.
We show that 60% of lateral septum neurons have a rhythmic activity and that the majority of synaptic inputs received in this structure are GABAergic. We also demonstrate that the majority of neurons answer to OT and/or VP application by a modification of their spontaneous electrical activity (activation or inhibition) and/or of their synaptic inputs (activation). We now classify lateral septum neurons in function of their response to OT and VP, electrophysiological properties and morphology.
This study should help to decipher the organization of oxytocin and vasopressin-sensitive neurons in the lateral septum paving the way to understand the role of this structure in the regulation of social behavior.

O-8 Dynamic and nanoscale organization of AMPA Receptors during Long Term Depression

Benjamin Compans; Estelle Toulme; Daniel Choquet; Eric Hosy;
IINS, CNRS UMR5297, 146 rue Leo Saignat 33077 Bordeaux, France

AMPA-type glutamate receptors (AMPAR) are responsible for fast synaptic transmission. The spatiotemporal organization of these receptors in post-synaptic membranes is a fundamental determinant to understand synaptic transmission and information processing by the brain. With the recent developments in Super-Resolution microscopy, we showed that in basal condition, AMPARs are organized in few nanodomains of ~70nm which are mostly stable for up to 1 hour at synapses. This observation could have important consequences on our understanding of excitatory neurotransmission.
Here, we report the effect of synaptic long term depression (LTD) on nanoscale AMPAR dynamic organization. We used NMDA treatment to induce a chemical LTD in hippocampal cell culture, and Single particle tracking microscopy (UPAINT and sptPALM) and dSTORM are applied to decipher the variation of AMPAR mobility and organization in nanodomains. We coupled this work with electrophysiological recordings to correlate synaptic AMPAR clusterization and miniature currents.

Symposium 4

Ion Channels and Cancer

Organized by Hélène Guizouarn, Nice

.....

Tuesday, september 16th 2014

17:00 Luis Pardo (Germany) Kv10.1 in tumor biology: mechanisms and therapeutic implications.

17:30 Ildiko Szabo (Italy) Mitochondrial channels: ion fluxes and more.

18:00 Christophe Vandier (France) The calcium activated SK3 channel: from a role on metastasis development to the emergence of the Canceropole Grand Ouest ion channel network.

18:30 Selected speaker: Raphael Rapetti-Mauss (Ireland) β-catenin regulates KCNQ1 Potassium channel expression in colon cancer cells.

.....

S-13 Kv10.1 IN TUMOR BIOLOGY: MECHANISMS AND THERAPEUTIC IMPLICATIONS

Diana Urrego; Araceli Sánchez; Farrah Zahed; Franziska Hartung; Luis A. Pardo;
Max-Planck Institute of Experimental Medicine

The crucial role of voltage-gated channels in the pathophysiology of cancer has become a generally accepted concept. The molecular nature of this implication is however obscure, and appears to be specific for each channel. KV10.1 is a brain-specific voltage-gated potassium channel ectopically expressed in a majority of extra-cranial solid tumors and a significant fraction of acute myeloid leukemia. Kv10.1 is not detected in normal tissues. This tumor specificity relies, rather than on an absolute lack of expression, on a time-limited transcription along the cell cycle. Expression of the channel is tightly controlled by E2F1, p53 and miR34a; alterations on any of these factors lead to aberrant expression, as it is the case after papilloma infection, which activates E2F1 and thereby Kv10.1. Once expressed, KV10.1 confers a selective advantage to the cells, at least partly mediated by increased angiogenesis (through higher HIF1 activity and VEGF secretion), but it also directly modifies cell cycle through two independent mechanisms, direct activation of cyclin-dependent kinases and altered coordination of ciliogenesis. Its specificity for tumor tissue makes KV10.1 an attractive target for tumor diagnosis and therapy. Channel expression correlates with bad prognosis in several tumor types, and pharmacological or genetic inhibition of KV10.1 is able to reduce tumor progression. Channel inhibition alone does not induce regression in immunocompromised mice, possibly because the role of KV10.1 in tumor cells is partly independent of ion permeation. Immuno-targeted tools taking advantage of the tumor specificity of channel expression (a single-chain antibody against KV10.1 fused to a mutated version of the human soluble TNF-related apoptosis-inducing ligand (TRAIL) induce apoptosis in KV10.1 positive cancer cells, but not in non-tumor or KV10.1-negative tumor cells; in vivo, this results in efficacious tumor reduction when applied in combination with doxorubicin, and allows reducing dosage of the cytotoxic drug.

S-14 INHIBITION OF A MITOCHONDRIAL POTASSIUM CHANNEL AS A STRATEGY AGAINST PANCREATIC DUCTAL ADENOCARCINOMA

Antonella Managò[1]; Angela Zaccagnino[2]; Luigi Leanza[1]; Bernhard Linder[2]; Holger Kalthoff[2]; Ildiko Szabo[1];
1: Department of Biology, University of Padova, Italy
2: Institut für Experimentelle Tumorforschung, University of Kiel, Germany

Mitochondrial ion channels are emerging as oncological targets (Leanza et al, Oncogene 2014). Among the potassium channels found in the inner mitochondrial membrane we identified mtKv1.3 as a possible target. By preventing the function of this channel using specific membrane permeant inhibitors (Psora-4, PAP-1 and clofazimine), cancer cells of different origin expressing Kv1.3 undergo cell death. In particular, inhibition of the channel causes increased mitochondrial ROS production and cytochrome c release and triggers

intrinsic apoptosis. The cells become resistant to these Kv1.3 modulators when expression of the channel is downregulated by siRNA, indicating specificity. We have previously shown that in vivo treatment of melanoma in an orthotopic mouse model with clofazimine caused 90% reduction of the tumor volume without affecting healthy tissues (Leanza et al, EMBO Mol. Med. 2012). Furthermore, these inhibitors efficiently killed primary human tumor Chronic lymphocytic leukemia (B-CLL) cells expressing Kv1.3 by intrinsic apoptosis, while sparing residual normal T-lymphocytes even of the same patient and B cells from healthy subjects. Since Kv1.3 inhibitors kill B-CLL by direct interference with mitochondrial functions, they act on these malignant cells independent of classic prognostic factors, Bcl-2 overexpression and Bax/Bak deficiency (Leanza et al, Leukemia, 2013). Here we report the expression profile of Kv1.3 in five different pancreatic ductal adenocarcinoma lines under different conditions as well as in primary tissues. The effect of Kv1.3 inhibitors and of their improved derivatives in vitro was investigated. In the case of some of these drugs, even staurosporine-resistant lines died upon treatment. All cell lines analyzed displayed mutation in p53 and have been tested previously to be largely resistant to standard chemotherapeutics. Clofazimine has been tested also in vivo in a SCID mouse model for PDAC, characterized by a local recurrent tumor and liver metastases closely resembling the clinical challenge.

S-15 THE SK3 CHANNEL: FROM A ROLE ON METASTASIS DEVELOPMENT TO THE EMERGENCE OF THE CANCEROPOLE GRAND OUEST ION CHANNEL NETWORK

Maxime Guéguinou[1]; Aurélie Chantome[1]; Romain Felix[1]; Yan Fourbon[1]; Thierry Lecomte[2]; Philippe Bougnoux[1]; Gaëlle Fromont[1]; Marie Potier-Cartereau[1]; Christophe Vandier[1];
1: University of Tours / Inserm UMR 1069
2: University of Tours / UMR CNRS 7292

If in a physiological context, the SK3 channel (that belongs the Ca2+-activated K+ channel family) regulates neuronal and smooth muscle excitabilities this is not that is observed in a tumour context where the physiological function of SK3 is hijacks by cancer cell to drive essential biological functions for tumour development such as cell migration. We have demonstrated a pivotal role of SK3 channel as a mediator of cancer cell migration and bone metastasis. When express by cancer cell, this channel form a complex with Orai1 channel, a voltage-independent Ca2+ channel, and the SK3-Orai1 complex associate within lipid rafts where it controls a constitutive Ca2+ entry. The co-localization of SK3 and Orai1 in primary human tumors and bone metastases further emphasized the clinical relevance of our observations. Interestingly, we established that the alkyl-lipid Ohmline moved the SK3-Orai1 complex outside of lipid rafts and impaired SK3-dependent Ca2+ entry, cell migration and bone metastases. This is the first report documenting that the deregulation of an ion channel complex by a synthetic lipid could influence metastases.
Regarding the unique expertise in France, of several teams of the “Grand Ouest” region in the field of KCa/ClCa (Ca2+-activated K+/Cl- channels) and voltage-independent Ca2+channels involved in cancer, we join our efforts and expertise within a network named IonChannel-CanceropoleGrandOuest (IC-CGO). The IC-CGO network is composed of 18 partners with transversal, complementary and multidisciplinary approaches. We believe that this network that will combine basic and clinical research through collaborations between clinicians, chemists and biologists should allow us to propose these channels as relevant drug targets and prognostic indicators for cancer.

S-16 B-CATENIN REGULATES KCNQ1 POTASSIUM CHANNEL EXPRESSION IN COLON CANCER CELLS

Raphael Rapetti-Mauss; Aine Nolan; Natalia Lajczak; Warren Thomas; Brian J. Harvey;
Department of molecular Medicine, Education and Research center, Royal College of Surgeons in Ireland, Beaumont Hospital PO Box 9063; Dublin 9 Republic of Ireland

Ion channels are key players in cell and tissue homeostasis, disturbance of their primary functions contribute to genesis and progression of several diseases, including cancers. In particular, potassium channels have been involved in tumorigenesis in different types of cancers. Recently, the potassium channel KCNQ1 has been identified as a partner of the TCF-4 co-activator, β-catenin which is a key player in the Wnt/TCF-4 signaling pathway. Moreover KCNQ1 has been identified as a tumor suppressor protein in human and mouse models of intestinal cancer. However the cellular functions of KCNQ1 and the molecular mechanisms that support its effects in cancer remain unclear. In the present study we addressed the question whether KCNQ1 is associated to the β-catenin/TCF-4 pathway and contributes to the phenotype of tumor cells.

We first uncovered that KCNQ1 expression is lost in poorly differentiated colon cell lines, in which a nuclear and cytosolic expression of β-catenin is observed. From this observation we reasoned that β-catenin activation could control KCNQ1 expression. Using a pharmacological approach, we inhibited the Glycogen Synthase Kinase-3β to increase β-catenin activity and translocation in the nucleus. Thus, KCNQ1 protein expression was inhibited by approximately 50%. To investigate the role of TCF-4 in the control of KCNQ1 expression, DLD-1 cells were transfected with a plasmid carrying a dominant negative for TCF-4 (DNTCF-4). This experiment reveals that the expression of KCNQ1 was restored in presence of DNTCF-4 suggesting that the transcription factor induces the repression of the channel in cancer cells. We also observed that the localization of β-catenin was more diffuse and nuclear in cell transfected with SIRNA against KCNQ1, suggesting that the absence of KCNQ1 impaired the stability of the β-catenin at the plasma membrane. Finally, the pharmacological and molecular inhibition of KCNQ1 decreased wound healing rate after injury, establishment of the trans-epithelial electrical resistance (TEER), and expression of marker of cell differentiation, suggesting that KCNQ1 participates in cell layer integrity.

We propose that KCNQ1 expression is repressed by the activation of the TCF-4/β-catenin pathway leading to the disruption of the epithelial integrity. This mechanism could be involved in the process of tumorigenesis in CRC.

Symposium 5

Intracellular Calcium Homeostasis

Organized by Michel Vignes, Montpellier

.....

Wednesday, september 17th 2014

9:45 Stefan Feske (USA) Regulation of CRAC channels and their role in immune function

10:15 Dimitri Rusakov (UK) Monitoring uneven landscapes of resting Ca2+ in neurons and glia using time-resolved fluorescence imaging

10:45 Bruno Allard (France) Sarcoplasmic reticulum Ca2+ permeation explored from the lumen side provides new insights into excitation-contraction coupling in skeletal muscles

11:15 Selected speaker: Elizabeth Aguettaz (France) Effect of Axial strech on calcium regulation in mouse dystrophic cardiomyocytes: involvment of TRPV2 channels ?

.....

S-17 CRAC CHANNELS: MOLECULAR REGULATION AND ROLE IN IMMUNITY

Stefan Feske;
New York University, Dept Pathology, New York, NY 10016, USA

Store-operated Ca2+ entry (SOCE) is a universal Ca2+ influx pathway present in almost all cell types. SOCE is activated by the emptying of endoplasmic reticulum (ER) Ca2+ stores following ligation of cell surface receptors such as G protein coupled receptors and antigen receptors on immune cells. The subsequent activation of phospholipase C (PLC) and production of inositol 1,4,5-trisphosphate (IP3) cause the release of Ca2+ from ER stores through IP3 receptor (IP3R) channels. The resulting reduction in the ER Ca2+ concentration [Ca2+]ER triggers the opening of SOC channels in the plasma membrane (PM) and Ca2+ influx from the extracellular space, hence the name SOCE. The prototypical SOC channel is the Ca2+ release-activated Ca2+ (CRAC) channel, which is present in many cell types including those of the immune system. CRAC channels are highly Ca2+ selective channels that are formed by multimers of ORAI proteins, which constitute the permeation pore for Ca2+ entry into the cell. Of the three ORAI homologues present in human, mouse and other vertebrates, ORAI1 is the best characterized and predominant channel in immune cells. The opening of ORAI1 channels is mediated by two single pass transmembrane proteins located in the ER membrane, stromal interaction molecules (STIM) 1 and STIM2 following depletion of ER Ca2+ stores.
The importance of ORAI and STIM-mediated Ca2+ influx for immunity is emphasized by mutations in human patients that abolish SOCE and cause severe combined immunodeficiency (SCID). Similarly, mice with T cell specific deletion of Stim1, Stim2 or Orai1 genes have impaired SOCE and T cell function. These mice develop autoimmunity due to impaired development and function of regulatory T cells (Tregs), but are protected from T cell mediated autoimmune diseases such as multiple sclerosis or inflammatory bowel disease due to impaired effector T cell functions. Importantly, we recently showed that STIM1 and STIM2 expression in murine T cells is required for long-lasting memory CD8 T cell responses to viral infection and antitumor immunity. In the absence of both molecules, mice fail to control viral infection and show uncontrolled tumor growth compared to wild-type littermates. Our data demonstrate an important role of SOCE in CD4 and CD8 T cell-mediated immune responses to infection, tumors and the regulation of immune tolerance to self.

S-18 MONITORING UNEVEN LANDSCAPES OF RESTING CA2+ IN NEURONS AND GLIA USING TIME-RESOLVED FLUORESCENCE IMAGING

Dimitri Rusakov; Lucie Bard; Tom Jensen; Kaiyu Zheng;
UCL Instiute of Neurology, University College London, WC1N 3BG London, U.K.

Signal transfer in the brain relies on rapid diffusion of small ions and transmitter molecules in the extracellular space. How fast these molecules move between cells is however poorly understood. A recently developed photonics approach, time-resolved fluorescence anisotropy imaging (TR-FAIM), gauges the speed of molecular rotation on the nanosecond scale. We find that TR-FAIM faithfully reports translational diffusion of small molecules in aqueous solutions of varying viscosities and adapt this methodology to evaluate extracellular molecular mobility in organised brain tissue. Two-photon excitation TR-FAIM of a small cell-impermeable fluorophore reveals that instantaneous extracellular diffusion in ex vivo

hippocampal slices is 25-30% slower than in a free physiological medium and is further retarded inside synaptic clefts of large synapses. These findings provide novel constraints for diffusion-dependent reactions that shape molecular signalling in the brain. We also find that the fluorescence lifetime (nanosecond scale) of the common Ca2+ indicator OGB-1 is highly sensitive to concentrations of free Ca2+ in the nanomolar range. Mapping the OGB lifetime in live neurons and astroglia reveals a previously unrecognised complexity of the basal Ca2+ landscape and its activity-dependent changes in organised brain tissue. These methodologies open new horizons in our understanding of the cellular machinery that controls neural circuits of the brain.

S-19 SARCOPLASMIC RETICULUM CA2+ PERMEATION EXPLORED FROM THE LUMEN SIDE PROVIDES NEW INSIGHTS INTO EXCITATION-CONTRACTION COUPLING IN SKELETAL MUSCLE

Bruno ALLARD; Gaëlle ROBIN;
Université Lyon 1, UMR 5534 CNRS, Villeurbanne, France

In active skeletal muscle fibers, the propagation of an action potential along the plasma and the tubular membrane induces a massive release of Ca2+ from the sarcoplasmic reticulum (SR) into the cytosol that activates contraction. This process of excitation-contraction (e-c) coupling engages two key proteins, the dihydropyridine receptor (DHPR) and the ryanodine receptor (RyR), embedded in the tubular membrane and in the junctional membrane of the SR respectively. RyRs operate as SR Ca2+ release channels whose gating is controlled in a time- and voltage-dependent manner by DHPRs which act as voltage sensors of the e-c coupling but also as voltage-gated Ca2+ channels. To date, experiments performed in the field have mainly consisted in measuring Ca2+ changes in the cytosol under voltage control of the cell. We recently set up the measurement of Ca2+ changes in the SR lumen of adult mouse muscle fibers under voltage control. These experimental conditions proved to provide very high resolution to explore SR Ca2+ fluxes and allowed us to resolve two key issues in e-c coupling. First, by showing that SR Ca2+ efflux is controlled by voltage and DHPR conformation within an unexpected very negative voltage range of membrane potentials, we demonstrate that DHPRs exert an active repressive control on RyRs gating in resting muscle. Secondly, in active muscle, SR Ca2+ depletion and voltage-dependent inactivation of Ca2+ release channels are assumed to both contribute to the decay of contractile performance during prolonged depolarization. However, the relative contribution of these processes is not clearly established. By showing that the voltage-dependence of SR Ca2+ depletion is 40 mV more negative than the one of inactivation, we demonstrate that SR Ca2+ depletion mainly contributes to the decline of Ca2+ release during long-lasting depolarization. This result challenges the previous studies which, by using steady-state inactivation protocols to investigate voltage-dependence of Ca2+ release inactivation, in fact probed the voltage dependence of SR Ca2+ depletion. Overall, we demonstrate that during prolonged trains of action potentials, inactivation did not occur, giving evidence that SR Ca2+ depletion is the only process that leads to Ca2+ release decline in these conditions.

S-20 EFFECT OF AXIAL STRETCH ON CALCIUM REGULATION IN MOUSE DYSTROPHIC CARDIOMYOCYTES: INVOLVEMENT OF TRPV2 CHANNELS?

Elizabeth AGUETTAZ[1]; José Javier LOPEZ BARBA[2]; Amandine KRZESIAK[1]; Bruno CONSTANTIN[2]; Christian COGNARD[1]; Stéphane SEBILLE[1];
1: Equipe Transferts Ioniques et Rythmicité Cardiac (TIRC) Lab. Signalisation et Transports Ioniques Membranaires (STIM) ERL CNRS/Université de Poitiers n°7368 Pôle Biologie Santé Bât B36/B37 1 rue Georges Bonnet TSA 51106 86073 POITIERS CEDEX 9
2: Equipe Calcium et Microenvironnement des Cellules Souches (CMCS) Lab. Signalisation et Transports Ioniques Membranaires (STIM) ERL CNRS/Université de Poitiers n°7368 Pôle Biologie Santé Bât B36/B37 1 rue Georges Bonnet TSA 51106 86073 POITIERS CEDEX

In Duchenne muscular dystrophy (DMD), deficiency of the cytoskeletal protein dystrophin leads to well-described defects in skeletal muscle, but also to dilated cardiomyopathy (DCM). Mechanisms leading to cardiomyocyte cell death and DCM are not well understood. The main goal of this study is to explore effects of a mechanical stress on cardiac cells in the pathological context of DCM associated with DMD.
To do so, we used a direct mechanical and homogeneous axial stretch method that is similar to stimuli in a physiological context, allowing to investigate the true stretch-activated damages in dystrophy. We also started to investigate TRPV2 channels, molecular candidates for stretch-activated channels (SACs) and for which sarcolemma accumulation has pathological impact on DCM progression.
All experiments have been performed on 12 months mdx cardiomyocytes, the murine model of DCM associated with DMD.
The SICM (Scanning Ion Conductance Microscopy) technique was used to characterize the topography of the surface membrane of cardiomyocytes at rest and during an axial stretch. No modification of the topography was observed during stretch even if a tendency to increase of the pattern step length is detectable in mdx cardiomyocytes.
Localization of TRPV2 channels was performed by immunostaining and sarcolemma accumulation of TRPV2 was demonstrated in mdx whereas, it seemed to be more localized in intracellular compartment in control cardiomyocytes.
By the use of the quenching technique with Fura-2 probe, a constitutive entry of calcium was observed in resting mdx cardiomyocytes. This constitutive influx was decreased with the use of tranilast, an inhibitor of TRPV2 channels. A constitutive increase in intracellular calcium was also observed in mdx, but not in control, when extracellular calcium was increased. When stretched, a similar increase was observed in control cardiomyocytes and this increase was blocked with tranilast incubation.
These findings suggest that TRPV2 channels may play a role in stretched-evoked calcium entry with a differential channel expression between control and mdx cardiomyocytes.

POSTERS LIST

LISTE DES COMMUNICATIONS AFFICHEES

……..

Session I : Monday, september 15th , 15:00-17:00
Posters with Odd numbers / Posters numéros Impairs

Session II : Tuesday, september 16th , 15:00-17:00
Posters with Even numbers / Posters numéros Pairs

……..

P-01 PIEZO1-DEPENDENT STRETCH-ACTIVATED CHANNELS ARE INHIBITED BY POLYCYSTIN-2 IN KIDNEY
Rémi Peyronnet[1]; Joana Martins[2]; Sophie Demolombe[2]; Malika Arhatte[2]; Martine Jodar[2]; Michel Tauc[3]; Christophe Duranton[3]; Marc Paulais[4]; Eric Honore[2]; Amanda Patel[2];

P-03 THE ERYTHROID ANION EXCHANGER (AE1) FUNCTION IS MODULATED BY STOMATIN
Youcef Chouali[1]; Sandrine Genetet[1]; Yves Colin-Aronovicz[1]; Corinne Armari-Alla[2]; Patricia Aguilar-Martinez[3]; Pierre Ripoche[1]; Claude Lopez[1]; Isabelle Mouro-Chanteloup[1];

P-04 REGULATION OF TRPC AND ORAI1 CHANNELS BY CARDIAC MINERALOCORTICOID RECEPTOR: GENOMIC EFFECT OF ALDOSTERONE
Jessica Sabourin; Ana-Maria Gomez; Jean-Pierre Benitah;

P-05 IDENTIFICATION OF TWO NATIVE NAV1.5 PHOSPHORYLATION SITES AS POTENTIAL DETERMINANTS OF THE INCREASED LATE SODIUM CURRENT IN HEART FAILURE
Fabien Coyan[1]; Sophie Burel[1]; Matthew Meyer[2]; Cheryl Lichti[3]; Joan Brown[4]; Flavien Charpentier[1]; Jeanne Nerbonne[5]; Reid Townsend[6]; Lars Maier[7]; Céline Marionneau[1];

P-06 AGGRAVATION OF CARDIAC MYOFIBROBLAST ARRHYTHMOGENEICITY BY MECHANICAL STRESS
Teddy Grand[1]; Nicolò Salvarani[2]; Florian Jousset[2]; Stephan Rohr[2];

P-07 CARDIOMYOCYTES DIFFERENTIATED FROM URINE-DERIVED PLURIPOTENT STEM CELLS CAN BE USED AS MODELS TO RECAPITULATE ELECTROPHYSIOLOGICAL CHARACTERISTICS OF CARDIAC ARRHYTHMIAS
Mariam Jouni; Karim Si-Tayeb; Zeineb Es-Salah-Lamoureux; Xénia Martin Latypova; Anaïs Rungoat; Flavien Charpentier; Gildas Loussouarn; Patricia Lemarchand; Nathalie Gaborit;

P-08 TRAFFICKING DEFECTIVE MUTATIONS MODULATE NAV1.5 N GLYCOSYLATION STATES.
Aurélie Mercier; Romain Clément ; Patrick Bois; Aurélien Chatelier;

P-09 THE SKELETAL MUSCLE ClC-1 CHANNEL ACCOUNTING FOR MACROSCOPIC RESTING CHLORIDE CONDUCTANCE IS A TARGET OF ANGIOTENSIN-II VIA AT-1 RECEPTOR AND NADPH OXIDASE
Anna Cozzoli; Antonella Liantonio; Elena Conte; Maria Cannone; Sabata Pierno; Giulia Maria Camerino; Annamaria De Luca;

P-11 MODIFICATION OF CHLORIDE CONDUCTANCE AND CLC-1 CHLORIDE CHANNEL EXPRESSION DUE TO STATIN TREATMENT IS WORSENED BY AGING IN RAT SKELETAL MUSCLE
Sabata Pierno; Giulia Maria Camerino; Michela De Bellis; Maria Cannone; Antonella Liantonio; Elena Conte; Kejla Musaraj; Adriano Fonzino; Diana Conte Camerino;

P-12 KCNK3 CHANNEL PARTICIPATES TO THE DEVELOPMENT OF PULMONARY ARTERIAL HYPERTENSION.
Fabrice Antigny; Philippe Jourdon; Marc Humbert; Frederic Perros;

P-13 CAVEOLAE ARE INVOLVED IN STRETCH-INDUCED CA2+ SIGNALING IN PULMONARY HYPERTENSION
Guillaume Gilbert; Thomas Ducret; Jean-Pierre Savineau; Roger Marthan; Jean-François Quignard;

P-14 INFLUENCE OF CELLULAR ENVIRONMENT ON PULMONARY ARTERIAL SMOOTH MUSCLE CELLS.
Thibaud Parpaite; Guillaume Cardouat; Marthe Mauroux; Eva Delbrel; Jennifer Gillibert-Duplantier; Jean-François Quignard; Arnaud Courtois; Roger Marthan; Jean-Pierre Savineau; Thomas Ducret;

P-15 TIAGABINE IMPROVES HIPPOCAMPAL LONG-TERM DEPRESSION DEFICITS INDUCED BY MATERNAL IMMUNE STRESS IN RATS
Aline Rideau Batista Novais; Nadine Crouzin; Mélanie Cavalier; Mathilde Boubal; Janique Guiramand; Catherine Cohen-Solal; Marie-Céleste de Jésus Ferreira; Gilles Cambonie; Michel Vignes; Gérard Barbanel;

P-16 IMPLICATION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID-1 AND -4 CHANNELS IN INTRACELLULAR CALCIUM SIGNALING TRIGGERED BY ENGINEERED NANOPARTICLES
Ninon Hontans[1]; Thibaud Parpaite[1]; Thomas Ducret[1]; Jean-François Quignard[1]; Isabelle Baudrimont[1]; Stéphane Mornet[2]; Roger Marthan[1]; Bernard Muller[1]; Jean-Pierre Savineau[1]; Arnaud Courtois[1];

P-17 CHARACTERIZATION OF APIS MELLIFERA VOLTAGE-GATED CA2+ CHANNELS IN APIS NEURONS AND AFTER EXPRESSION IN XENOPUS OOCYTES
Thierry Cens[1]; Matthieu Rousset[1]; Claude Collet[2]; Mohamed Chahine[3]; Jean-Christophe Sandoz[4]; Pierre Charnet[1];

P-18 PYRETHROIDS DIFFERENTIALLY ALTER VOLTAGE-GATED SODIUM CHANNELS FROM THE HONEYBEE CENTRAL OLFACTORY NEURONS
Aklesso Kadala[1]; Mercédès Charreton[1]; Ingrid Jakob[1]; Thierry Cens[2]; Matthieu Rousset[2]; Mohamed Chahine[3]; Yves Le Conte[1]; Pierre Charnet[2]; Claude Collet[1];

P-19 INVOLVEMENT OF POTASSIUM CHANNELS IN CIRCUMNUTATION OF THE INFLORESCENCE STEM IN ARABIDOPSIS THALIANA
Maria STOLARZ[1]; Halina DZIUBINSKA[1]; Jean-Baptiste THIBAUD[2]; Kazimierz TREBACZ[1];

P-20 BINDING SITE AND INHIBITORY MECHANISM OF THE MAMBALGIN-2 PAIN-RELIEVING PEPTIDE ON ACID-SENSING ION CHANNEL 1A
Salinas Miguel; Besson Thomas; Delettre Quentin; Diochot Sylvie; Boulakirba Sonia; Douguet Dominique; Lingueglia Eric;

P-21 UNDERSTANDING THE FUNCTIONAL COUPLING BETWEEN SUR AND KIR6.2 SUBUNITS IN THE ATP-SENSITIVE POTASSIUM CHANNEL
Maria Antonietta Principalli; Jean Revilloud; Julien Dupuis; Michel Vivaudou;

P-22 THIK2 ASSOCIATES WITH THIK1 TO FORM FUNCTIONNAL HETERODIMERS
Sandy Blin[1]; Dawon Kang[2]; Franck Chatelain[1]; Sylvain Feliciangeli[1]; Maud Larroque[1]; Donghee Kim[2]; Florian Lesage[1]; Bichet Delphine[1];

P-23 GENETICALLY ENCODED PHOTO-CROSS-LINKER TO STUDY INTERFACES FOR POSITIVE ALLOSTERIC REGULATION OF GLUTAMATE-GATED ION CHANNELS
Meilin TIAN[1]; Pierre PAOLETTI[2]; Shixin YE-LEHMANN[2];

P-24 FUNCTIONAL ASSESSMENT OF CRYSTALLIZATION-OPTIMIZED G PROTEIN-COUPLED RECEPTORS USING ION CHANNEL-COUPLED RECEPTORS
Katarzyna Niescierowicz[1]; Lydia Caro[2]; Vadim Cherezov[3]; Michel Vivaudou[1]; Christophe Moreau[1];

P-25 AUTOMATED MICROFLUIDIC TECHNOLOGY FOR SCREENING OF ACTIVITY OF NANOPORES AND ION CHANNELS USING DROPLET INTERFACE BILAYERS
Tomasz Kaminski[1]; Magdalena Czekalska[1]; Slawomir Jakiela[1]; Tanuj Sapra[2]; Hagan Bayley[2]; Piotr Garstecki[1];

P-26 THE VOLTAGE-GATED SODIUM CHANNEL IN THE PACIFIC OYSTER CRASSOSTREA GIGAS : ITS SENSITIVITY TO PARALYTIC SHELLFISH TOXINS (PST) PRODUCED BY ALEXANDRIUM MINUTUM.
Floriane Boullot[1]; Evelyne Benoit[2]; Justine Castrec[1]; Maëva Leitwen[1]; Antoine Beuzit[1]; Hélène Hégaret[1]; Pierre Boudry[1]; Philippe Soudant[1]; Caroline Fabioux[1];

P-01 PIEZO1-DEPENDENT STRETCH-ACTIVATED CHANNELS ARE INHIBITED BY POLYCYSTIN-2 IN KIDNEY

Rémi Peyronnet[1]; Joana Martins[2]; Sophie Demolombe[2]; Malika Arhatte[2]; Martine Jodar[2]; Michel Tauc[3]; Christophe Duranton[3]; Marc Paulais[4]; Eric Honore[2]; Amanda Patel[2];
1: Imperial College London, The Magdi Yacoub Heart Science Centre, London, UK
2: Institut de Pharmacologie Moleculaire et Cellulaire, LabEx ICST, UMR 7275 CNRS, Universite de Nice Sophia Antipolis, Valbonne, France
3: Laboratoire de Physiomedecine Moleculaire, Universite de Nice Sophia Antipolis,Nice, France
4: UPMC Universite Paris 06, UMR 872 CNRS, Laboratoire de Genomique, Physiologie et Physiopathologie Renales, Paris, France

Mechanical forces associated with fluid flow and/or circumferential stretch are sensed by renal epithelial cells and contribute to both adaptive or disease states. Non-selective stretch-activated ion channels (SACs), characterized by a lack of inactivation and a remarkably slow deactivation, are active at the basolateral side of renal proximal convoluted tubules. Knockdown of Piezo1 strongly reduces SAC activity in proximal convoluted tubule epithelial cells. Similarly, overexpression of Polycystin-2 (PC2) or, to a greater extent its pathogenic mutant PC2-740X, impairs native SACs. Moreover, PC2 inhibits exogenous Piezo1 SAC activity. PC2 coimmunoprecipitates with Piezo1 and deletion of its N-terminal domain prevents both this interaction and inhibition of SAC activity. These findings indicate that renal SACs depend on Piezo1, but are critically conditioned by PC2.

P-02 : not present

P-03 THE ERYTHROID ANION EXCHANGER (AE1) FUNCTION IS MODULATED BY STOMATIN

Youcef Chouali[1]; Sandrine Genetet[1]; Yves Colin-Aronovicz[1]; Corinne Armari-Alla[2]; Patricia Aguilar-Martinez[3]; Pierre Ripoche[1]; Claude Lopez[1]; Isabelle Mouro-Chanteloup[1];
1: Inserm UMR_S1134/INTS Paris-France
2: CHU hopital de jour Hopital Couple Enfant Grenoble-France
3: Laboratoire d'hématologie Hôpital Saint Eloi Montpellier-France

AE1 (anion exchanger 1 or Band 3), a member of the SLC4A (solute carrier) family, is expressed in the plasma membrane of RBCs (red blood cells) and α-intercalated cells of the kidney collecting duct. In RBCs, AE1 is the most abundant integral membrane protein which plays a major role in CO2 transport and in the integrity of the membrane structure. Recent biochemical studies suggest an interaction of erythroid AE1 with stomatin (protein 7.2b), an ubiquitous membrane protein involved in the down-regulation of different transporters and ionic channels.
In this study, the ability of stomatin to modulate the Cl-/HCO3- exchange activity of AE1 using stopped-flow spectrofluorometry was evaluated. First, we analyzed the functional characteristics of AE1 protein normally expressed on RBCs of OHSt (overhydrated hereditary stomatocytosis) or CHC (cryohydrocytosis) patients, which lack stomatin and harbor mutations on RhAG (ammonium transporter) and Glut1 (glucose transporter) proteins, respectively. RBC ghosts were resealed in the presence of pyranine, a pH-sensitive fluorescent probe and submitted to inward HCO3- and outward Cl- gradients.
Alkalinization rate constants deduced from stopped-flow analysis allowed the determination of apparent permeabilities of AE1 protein, which were 50% lower for patient RBCs compared to controls.
By a similar approach, the function of recombinant AE1 expressed using an inducible system in HEK293 cells was tested in the presence of either an overexpression of recombinant stomatin or a partial inhibition of endogenous stomatin expression by interfering RNAs. Cells were loaded with the BCECF pH-sensitive fluorescent probe before analysis. Consistent with RBC studies, apparent permeabilities associated with AE1 activity were found 33% higher in HEK293 cells overexpressing stomatin and 30% lower in those with a reduced endogenous expression, compared to cells with normal endogenous stomatin expression.
These results show, for the first time, a positive modulation by stomatin of the activity of a transporter protein, AE1. Future investigations will aim at characterizing the interaction between the two proteins and deciphering the molecular mechanisms of a stomatin-based modulation of the AE1 function.

P-04 REGULATION OF TRPC AND ORAI1 CHANNELS BY CARDIAC MINERALOCORTICOID RECEPTOR: GENOMIC EFFECT OF ALDOSTERONE

Jessica Sabourin; Ana-Maria Gomez; Jean-Pierre Benitah;
Signalisation et Physiopathologie Cardiaque Inserm UMR-S 769 - LabEx LERMIT.5 rue Jean-Baptiste Clément 92296 Châtenay-Malabry

Although aldosterone and mineralocorticoid receptor (MR) activation have been involved in the pathogenesis and progression of cardiac hypertrophy and heart failure, their cellular mechanism of action on cardiomyocyte function is not yet completely elucidated. Given that alterations of Ca2+ fluxes have been described as a key step of cardiac MR deleterious effects, we hypothesized that TRPCs, Orai channels and their main activator STIM1 might be regulated by aldosterone/MR signaling in cardiomyocytes.
Using a combination of molecular biological and biochemical approaches, we found that TRPC1, C3-6 isoforms and Orai1/STIM1 are expressed at transcript and protein levels in neonatal rat ventricular cardiomyocytes (NRVCs). Interestingly, mRNA expression of TRPC1, C5, and Orai1 were increased in ventricular cardiomyocytes treated for 24h with 100nM and 1μM of aldosterone while TRPC3, C4, C6 and STIM1 were not affected. The selective MR antagonist, RU28318, prevented the aldosterone-enhanced TRPC1, C5 and Orai1 expression. Increased protein expression is also detectable for TRPC1, TRPC4 and STIM1 in treated cardiomyocytes. Additionally, stimulation of MR signaling by aldosterone during 24h significantly enhanced Store-Operated Ca2+ entry (SOCE measured by Fluo-4 and Fura-2 fluorescence) activated by Ca2+ stores depletion (with thapsigargin plus caffeine in presence of nifedipine) compared to untreated ventricular cardiomyocytes. The common TRPCs and Orai channels inhibitors, SKF-96365 and BTP2, as well as the overexpression of the dominant-negative Orai1 or TRPC1 significantly reduced SOCE, suggesting that TRPCs and/or Orai1 family are involved in the aldosterone-increased SOCE. Moreover, the modulation of SOCE appears to be mainly mediated by SGK1 and MR activation since SGK1, which expression is upregulated by aldosterone treatment, inhibition by GSK650394 or MR blockade by RU28318 abolished the aldosterone-increased SOCE. Finally, by functional approach, we showed that aldosterone induced diastolic Ca2+ overload dependent on TRPC/Orai channels activity.
These findings showed that aldosterone/MR activation enhances TRPC1, C4, C5 and Orai1/STIM1 expression and SOC entry in NRVCs, and that this may provide evidence for a novel pathway whereby Ca2+ entry and cardiac function are altered.

P-05 IDENTIFICATION OF TWO NATIVE NAV1.5 PHOSPHORYLATION SITES AS POTENTIAL DETERMINANTS OF THE INCREASED LATE SODIUM CURRENT IN HEART FAILURE

Fabien Coyan[1]; Sophie Burel[1]; Matthew Meyer[2]; Cheryl Lichti[3]; Joan Brown[4]; Flavien Charpentier[1]; Jeanne Nerbonne[5]; Reid Townsend[6]; Lars Maier[7]; Céline Marionneau[1];
1: L'Institut du Thorax, Nantes, France
2: Department of Medicine, Washington University Medical School, Saint Louis, MO, USA
3: Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA
4: Department of Pharmacology, University of California, San Diego, CA, USA
5: Department of Developmental Biology, Washington University Medical School, Saint Louis, MO, USA
6: Department of Internal Medicine and Cell Biology and Physiology, Washington University Medical School, Saint Louis, MO, USA
7: University Hospital Regensburg, Regensburg, Germany

Voltage-gated Na+ (Nav) channels are key determinants of myocardial excitability and defects in Nav channel functioning or regulation, associated with inherited and acquired cardiac disease, increase the risk of life-threatening arrhythmias. In heart failure, the inactivation properties of Nav1.5 channels are altered, resulting in decreased channel availability and increased late Na+ current. Although previous studies have suggested roles for CaMKII and CaMKII-dependent Nav1.5 phosphorylation, the global native phosphorylation pattern of Nav1.5 channels associated with these alterations is unknown. Phosphoproteomic analyses were undertaken to identify and quantify in situ the native phosphorylation sites on the Nav1.5 proteins purified from wild-type and CaMKIIδc-overexpressing (CaMKIIδc-Tg) mouse ventricles. A total of 18 phosphosites were identified, 8 of which are novel compared with our previous MS analyses. Of these 18 phosphosites, the C-terminal phosphoserines pS1937/pS1938 are present in the CaMKIIδc-Tg IPs (n=3/4) and absent in the WT IPs (n=0/4). In addition, pS1989 is 9-fold more represented ($p<0.05$, n=4 in each condition) in the CaMKIIδc-Tg, than in the WT, IPs. To explore the possibility that phosphorylation at these C-terminal sites regulates the gating properties of Nav1.5 channels, the orthologous human (serine to glutamate) double phosphomutant Nav1.5-S1933E-S1984E was generated and investigated by whole cell voltage-clamp analyses in HEK293 cells. These analyses revealed that the relative percentage of the TTX-sensitive late Na+ current, compared with the peak Na+ current, is significantly ($p<0.01$) higher for the double phosphomutant (0.07 ± 0.007%, n=24), compared with the WT (0.04 ± 0.004%, n=20), currents. In addition, although the fast and slow time constants of inactivation are similar, the relative contribution of the fast inactivating component of the peak Na+ current is significantly ($p<0.05$) lower with the phosphomutant, compared with the WT channels. Together, these analyses provide 8 novel native cardiac Nav1.5 phosphorylation sites, 2 of which are in the C-terminus of Nav1.5 and selectively modulate the late component of Na+ current, suggesting a role for modulation at these sites in heart failure.

P-06 AGGRAVATION OF CARDIAC MYOFIBROBLAST ARRHYTHMOGENEICITY BY MECHANICAL STRESS

Teddy Grand[1]; Nicolò Salvarani[2]; Florian Jousset[2]; Stephan Rohr[2];
1: Institut de Biologie de l'ENS (IBENS), Neuroscience Section CNRS UMR8197, INSERM U1024 Ecole Normale Supérieure 46 rue d'Ulm, 75005 Paris, France
2: Department of Physiology, University of Bern, Bühlplatz 5, CH-3012 Bern, Switzerland

Lowly polarized cardiac myofibroblasts (MFBs) induce arrhythmogenic slow conduction and ectopic activity by causing partial depolarization of electrotonically coupled cardiomyocytes (CMCs) in-vitro. We hypothesize that mechanical stress may aggravate this condition by activation of depolarizing stretch activated channels (SACs) in MFBs. Experimental preparations were obtained from neonatal rat ventricular cells. Acutely blocking SACs with streptomycin (SM; 50 μmol/L) had no effect on Vm of single CMCs (ctl: -75.7±1.5mV; SM: -75.4±1.3mV), but caused MFBs to hyperpolarize from -29.4 ± 7.8 mV to -34.3±7.4 mV suggesting the presence of active SACs in MFBs under control conditions. Accordingly, SM had no effects on impulse conduction (θ) in CMC cell strands (ctl: 336.3±24.4 mm/sec; SM: 329.5±21.4 mm/sec) but increased θ from 173.8±68.2 mm/sec to 224.9±63.3 mm/sec in strands of CMCs coated with MFBs. Modulation of θ by MFB-SACs was observed by subjecting cell strands cultured on silicone membrane to acute length changes. In CMC-only strands, θ was positively correlated with strand length (overall elongation by 10.5% caused an increase of θ from 325.7.6±26.4 mm/s to 348.8±39.9 mm/s) which can be explained by changes in cell geometry. In contrast, θ of CMC-MFB strands decreased with increasing strand length (from 291.4±48.7 mm/s; to 255.0±46.6 mm/s) suggesting that stretch caused activation of SACs in MFBs that ultimately led to partial depolarization of coupled CMCs. The results demonstrate that acute stretch differentially affects θ depending on the cellular composition of cardiac tissue. Slowing of conduction is observed only in presence of MFBs suggesting that arrhythmogenic consequences of mechanical stress may be aggravated by MFBs in fibrotically remodelled myocardia.

P-07 CARDIOMYOCYTES DIFFERENTIATED FROM URINE-DERIVED PLURIPOTENT STEM CELLS CAN BE USED AS MODELS TO RECAPITULATE ELECTROPHYSIOLOGICAL CHARACTERISTICS OF CARDIAC ARRHYTHMIAS

Mariam Jouni; Karim Si-Tayeb; Zeineb Es-Salah-Lamoureux; Xénia Martin Latypova; Anaïs Rungoat; Flavien Charpentier; Gildas Loussouarn; Patricia Lemarchand; Nathalie Gaborit;
l'institut du thorax - UMR Inserm 1087/CNRS 6291 IRS-UN, 8 quai Moncousu, BP 70721 44007 Nantes cedex 1, France

Purpose: Channelopathies associated with cardiac arrhythmias have essentially been studied in heterologous systems or animal models, independently of the patients' genetic background. However, the alteration of channel functions may lead to remodelling of other proteins, which would be missed in such models. Because sources for human cardiomyocytes are extremely limited, the use of urine samples to derive cardiomyocytes would be a non-invasive and elegant way to get a clearer overview of the affected cardiac electrical functions that lead to pathologies, while expressing the patient gene pool. We set up an experimental approach to obtain and characterize cardiomyocytes differentiated from urine-derived pluripotent stem cells (UiPS-CMs), from a patient with Long QT syndrome. This patient presents a mutation on hERG KCNH2 gene (p.A561P).
Methods: Cells obtained from urine sample from the A561P patient and his asymptomatic (not mutated) mother were reprogrammed using the episomal-based method. Urine-derived iPS cells were then differentiated into cardiomyocytes using the matrix sandwich method with modifications. Gene and protein expressions of ventricular-specific markers were analyzed, and optical recording of action potentials was performed using the high throughput CellOptiq system. Voltage-clamp and current-clamp experiments were also conducted to record hERG ionic currents as well as action potentials from UiPS-CMs.
Results: UiPS cells could be differentiated into functional cardiomyocytes with proper expression of ventricular cytoskeletal proteins and ion channels. These UiPS-CMs were electrically functional, with nodal-, atrial- and ventricular-like action potentials recorded using both CellOptiq and patch-clamp techniques. Application of ajmaline, 4-aminopyridine, nifedipine, 293B or E-4031 to the differentiated cardiomyocytes confirmed the contribution of INa, Ito, ICa, IKs and IKr currents, respectively, to shape the action potentials. Comparing hERG channels expression from the patient's UiPS-CMs to those from the mother's allowed to establish that the mutation led to a trafficking defect that resulted in a reduced IKr current. This phenotype was accompanied by lengthened action potentials that sometimes resulted in observable arrhythmias (early afterdepolarizations).
Conclusion: Urine-derived pluripotent stem cells from ion channels mutation-bearing patients can be used as novel models to differentiate functional cardiomyocytes that recapitulate cardiac arrhythmia phenotypes.

P-08 TRAFFICKING DEFECTIVE MUTATIONS MODULATE NAV1.5 N GLYCOSYLATION STATES.

Aurélie Mercier; Romain Clément ; Patrick Bois; Aurélien Chatelier;
Laboratoire Signalisation et Transports Ioniques Membranaires (STIM), ERL CNRS 7368, Université de Poitiers, Poitiers, France.

Voltage gated sodium channels are membrane proteins that play a critical role in electrical signaling of excitable cells. Amongst this family, the isoform Nav1.5 is responsible for the initiation and propagation of cardiac action potentials. As most of membrane proteins, Nav1.5 is well known to be a glycoprotein with ~5% of its total weight corresponding to carbohydrates. To date, it has been shown that Nav1.5 glycosylations such as sialylations influence biophysical properties of this channel protein. However, whereas N glycosylation are well known post-traductional modifications that modulate surface localization of many ionic channels, little is known about these maturation impacts on voltage gated sodium channels. Perturbation of Nav1.5 trafficking is a well characterized phenomenon occurring in pathology such as Brugada syndrome. Our laboratory previously revealed that trafficking defective mutants of Nav1.5 exert a dominant negative effect upon wild type protein surface localization. The objective of this study was (i) to characterize N glycosylations of Nav1.5 during its membrane trafficking and (ii) to investigate these maturations in the context of the dominant negative effect exerted by Nav1.5 trafficking defective mutations.

P-09 THE SKELETAL MUSCLE ClC-1 CHANNEL ACCOUNTING FOR MACROSCOPIC RESTING CHLORIDE CONDUCTANCE IS A TARGET OF ANGIOTENSIN-II VIA AT-1 RECEPTOR AND NADPH OXIDASE

Anna Cozzoli; Antonella Liantonio; Elena Conte; Maria Cannone; Sabata Pierno; Giulia Maria Camerino; Annamaria De Luca;
Dept. Pharmacy-Drug Sciences, University of Bari

Skeletal muscle chloride channels ClC-1 account for a large resting chloride conductance (gCl) that plays a key role in electrical stability of sarcolemma. gCl is a sensitive biomarker of inflammation and altered mechano-trasduction in various pathophysiological conditions, such as dystrophinopathies. We presently assessed the involvement of Angiotensin-II (Ang-II) in modulating muscle chloride channels. By means of intracellular microelectrode recordings we found that Ang-II application to mouse extensor digitorum longus (EDL) muscle fibers reduced gCl in the nanomolar range (EC50 = 60 nM) and in a concentration-dependent manner, meanwhile increasing potassium conductance (gK). The effect was inhibited by the AT-1-receptor antagonist losartan and the protein kinase C-inhibitor chelerythrine; no antagonism was observed with the AT-2 antagonist PD 123,319. The scavenger of reactive oxygen species (ROS) N-acetyl cysteine and the NADPH-oxidase (NOX) inhibitor apocynin also antagonized Ang-II effects on gCl. Ang-II also lowered the voltage threshold for mechanical activation, suggesting the involvement of calcium homeostasis. Ang-II and the AT1 agonist L162,313, did increase the intracellular cytosolic calcium, measured by fura-2, with a two-step pattern. These latter effects were not observed in the presence of losartan, of the phospholipase C inhibitor U73122, and in absence of extracellular calcium, disclosing a Gq-mediated calcium entry mechanism, likely via the activation of a TRP-like channel. The increase in cytosolic calcium may contribute to the Ang II signaling on gCl by co-activation of PKC. qPCR analysis showed that AT-1 receptor, angiotensinogen and angiotensin-converting enzyme are expressed in EDL muscle along with NOX2. The data show for the first time that AT1-mediated Ang-II pathway, also involving NOX and ROS, directly modulates chloride channel conductance and calcium homeostasis in adult myofibers (supported by Italian MIUR-PRIN project n° 20108YB5W3_004).

P-10 not present

P-11 MODIFICATION OF CHLORIDE CONDUCTANCE AND CLC-1 CHLORIDE CHANNEL EXPRESSION DUE TO STATIN TREATMENT IS WORSENED BY AGING IN RAT SKELETAL MUSCLE

Sabata Pierno; Giulia Maria Camerino; Michela De Bellis; Maria Cannone; Antonella Liantonio; Elena Conte; Kejla Musaraj; Adriano Fonzino; Diana Conte Camerino;
University of Bari, Italy

Skeletal muscle is a target of statin side effects, indeed patients undergoing this therapy complain muscle disorders ranging from myalgia to severe myopathy. Advanced age patients, suffering from senile muscle atrophy and loss of performance, experience an increased risk of statin-induced muscle disorder. We previously demonstrated that statin administration to adult rats reduces resting chloride conductance (gCl) in skeletal muscle. This parameter is sustained by the ClC-1 muscle channel and controls the resting membrane potential and excitability. Indeed, a large reduction of gCl produces myotonic-like symptoms. Resting gCl is also reduced in skeletal muscle of aged rats. The aim of our study was to investigate whether statin therapy has higher influence on the aging-associated changes in skeletal muscle performance by evaluating the resting gCl, by two-intracellular microelectrode technique and the resting intracellular calcium (restCa) level by FURA-2 in the extensor digitorum longus (EDL) muscle of 6-months-old adult and 29-months-old aged animals chronically treated with 10mg/kg/day atorvastatin. Gene expression was also evaluated by Real-Time-PCR analysis. Resting gCl was found to be strongly reduced in the aged treated animals with respect to the adult-treated ones. Indeed, it was 1319±70μmicroS/cm2 (n=42) and 1840±99μmicroS/cm2 (n=45) in treated and untreated aged rats and was 1650±111μmicroS/cm2 (n=27) and 2512±44μmicroS/cm2 (n=16) in treated and untreated adult rats, respectively. In parallel to the decrease of gCl, the Real-Time-PCR analysis revealed a significant reduction of ClC-1 mRNA expression more potently in aged animals treated with atorvastatin. Since the restCa was increased by statin in vitro application we evaluated this parameter in EDL muscle of atorvastatin-treated aged rats. No modification of restCa was found between treated and untreated fibers of aged rats suggesting that this parameter was not involved in gCl reduction. These data suggest that the alteration of gCl may be one of the significant causes of muscle damage in elderly patients under statin therapy.

P-12 KCNK3 CHANNEL PARTICIPATES TO THE DEVELOPMENT OF PULMONARY ARTERIAL HYPERTENSION.

Fabrice Antigny; Philippe Jourdon; Marc Humbert; Frederic Perros;
Inserm U999 Le Plessis Robinson

Pulmonary Arterial Hypertension (PAH) is a severe and progressive disease characterized by an abnormal elevation of the pulmonary arterial pressure (PAP) due to strong remodeling of small pulmonary arteries (PA), which involves endothelial dysfunction, an excessive proliferation of pulmonary arterial smooth muscle (PASMC) and endothelial (EC) cells. Recently, KCNK3 has been identified as a new predisposing gene for PAH. Last years, 6 mutations from 6 PAH patients are identified in KCNK3 gene, encoding for a non-voltage dependent outward rectifier K+ channel, leading to a loss of function. However, the pathophysiological role of KCNK3 in PAH is poorly understood.

In present study, we assessed the involvement of KCNK3 channel in well-established rat pulmonary hypertension (PH) model induced by monocrotaline (MCT) injection. MCT-treated rat developed a rapid and progressive PH in 3 weeks. In this context, rat intrapulmonary arteries were isolated from control and MCT rats. Then K+ currents were recorded from freshly isolated PASMCs and EC cells using the whole-cell patch-clamp technique. KCNK3 expression and localization were evaluated by Western blot, immunostaining and Q-PCR.

We showed that KCNK3 current is slightly decreased 7 days after PH induction in PASMC and dramatically reduced after 14 and 21 days. Decreased KCNK3 current is also correlated to decrease protein and mRNA levels of KCNK3, in rat lung tissues and in isolated pulmonary arteries, respectively. In same time, we showed that PASMC are progressively depolarized during PH establishment. Interestingly, in freshly isolated pulmonary EC, KCNK3-current is significantly decreased 7 days after MCT-treatment suggesting that decreased activity of KCNK3 played a key role in the initiation of PH.

We further demonstrate that human lung tissue and cultured hPASMC from idiopathic PAH patients exhibit a dramatic reduction of KCNK3 mRNA leading to excessive proliferation Similarly, KCNK3 knockdown by siRNA increased hPASMC proliferation compared to control.

In conclusion, we showed for the first time that KCNK3 expression and activity are strongly reduced in primary PASMC and EC in rat PH model, demonstrating that KCNK3 channels could participate to the development of PH.

P-13 CAVEOLAE ARE INVOLVED IN STRETCH-INDUCED CA2+ SIGNALING IN PULMONARY HYPERTENSION

Guillaume Gilbert; Thomas Ducret; Jean-Pierre Savineau; Roger Marthan; Jean-François Quignard;
Centre de Recherche Cardiothoracique de Bordeaux

Introduction: Vascular smooth muscle cells are submitted to stretch forces exerted by blood pressure. Pulmonary arteries transduce a mechanical stimulus of stretch into a biological response of contraction, a mechanism called myogenic tone which involves Ca2+ stretch-activated channels (SAC). As membrane plasticity and shape are important for the SAC activity, we investigate the role of caveolae in the Ca2+ and contraction response to stretch of pulmonary arterial smooth muscle cells (PASMC).
Methods and results: Isometric contractions showed that pulmonary arteries from pulmonary hypertensive rats induced by a chronic hypoxia (CH rats) are hyper-responsiveness to stretch, as compared to normal rats. This is mediated by a higher SAC activity and Ca2+ response to stretch which was measured on freshly isolated PASMC with a patch-clamp setup coupled to indo-1. Interestingly, CH rats exhibit Ca2+ and contraction response to stretch in absence of extracellular Ca2+. These responses involve the sarcoplasmic reticulum Ca2+ (measured by a confocal microscope with the fluo-5N probe) and caveolae since a pharmacological approach using methyl-β-cyclodextrin (MβCD, a caveolae disrupter) inhibit these responses. By contrast, MβCD has no effect on normal rats. Finally, by immunostainings on vessels, we showed that caveolae are actually present on PASMC from normal and CH rats.
Conclusion: Caveolae are important for stretch-induced calcium response in CH rats via a new subcellular organization between caveolae and intracellular calcium stores.

P-14 INFLUENCE OF CELLULAR ENVIRONMENT ON PULMONARY ARTERIAL SMOOTH MUSCLE CELLS.

Thibaud Parpaite; Guillaume Cardouat; Marthe Mauroux; Eva Delbrel; Jennifer Gillibert-Duplantier; Jean-François Quignard; Arnaud Courtois; Roger Marthan; Jean-Pierre Savineau; Thomas Ducret;
Univ. Bordeaux, Centre de Recherche Cardio-Thoracique de Bordeaux, INSERM U1045, F-33000 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 (HPV) 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). It has been previously shown that SAC activity is increased in pulmonary arterial smooth muscle cells (PASMC) from chronically hypoxic rats (animal model for PH) but whether this feature is directly caused by hypoxia alone or is a consequence of stretch induced by the HPV is a matter of debate.
In the present study, we thus investigated the respective contribution of mechanical and hypoxic stresses observed in the context of PH. These points were evaluated as part of the calcium response, as well as altered physiological responses in this disease (such as migration, cytoskeleton reorganisation, and cell realignment). We isolated PASMC from normoxic rats and mice and exposed these cells to in vitro hypoxia (1 % O2 during 48h), or uniaxial cyclic stretch (10 %, 1 Hz).
First, as calcium homeostasis perturbation in PASMC is a major characteristic during PH establishment, we measured the SAC-induced calcium response. Using calcium measurement experiments (indo-1 or fura-PE3), we showed that this conditioning significantly increased the hypotonic-induced calcium response. Then, we tested the impact of in vitro hypoxia on cell migration and cytoskeleton rearrangement. We showed that hypoxia induced a reorganization of the F-actin cytoskeleton, the tubulin and intermediate filament networks (immunostaining experiments), potentially associated with an enhanced serotonin-induced pro-migratory response (wound-healing assay). Finally, we demonstrated that an in vitro cyclic stretch regulate the orientation of PASMC: most of the cells (> 90 %) reoriented perpendicular to the main axis of the applied strain within one hour after force application.
In conclusion, these data point out, for the first time, both a direct effect of hypoxia and stretch on smooth muscle cells in pulmonary circulation.

P-15 TIAGABINE IMPROVES HIPPOCAMPAL LONG-TERM DEPRESSION DEFICITS INDUCED BY MATERNAL IMMUNE STRESS IN RATS

Aline Rideau Batista Novais; Nadine Crouzin; Mélanie Cavalier; Mathilde Boubal; Janique Guiramand; Catherine Cohen-Solal; Marie-Céleste de Jésus Ferreira; Gilles Cambonie; Michel Vignes; Gérard Barbanel;
UMR5247 IBMM Université Montpellier 2

Rat maternal immune challenge with lipopolysaccharide (LPS, 500 μg.kg-1 ip on gestational day 19) led to an early disturbance of glutamatergic synaptic plasticity in the hippocampus of male offspring characterized by a premature loss in the ability to exhibit long-term depression following low frequency stimulation (LFS-LTD), between postnatal days (PD) 12 and 25 and by the outbreak of an aberrant long-term potentiation induced by paired-pulse low frequency stimulation (ppulse-LTP). Prenatal LPS challenge also induced a significant loss in GABAergic neurons, associated with a presynaptic deficiency in GABAergic transmission assessed with patch clamp recordings. Increasing ambient GABA by impairing GABA reuptake with tiagabine did not significantly interact with the occurrence of LTD in control animals, but both rescued the impaired LFS-LTD and prevented the aberrant ppulse-LTP in LPS-challenged rats. Thus, deficiency in tonic actions of GABA seems to be central to the dysregulation of synaptic plasticity observed after prenatal immune challenge and modulating GABAergic tone may be a possible therapeutic strategy for the cognitive impairment associated with this condition.

P-16 IMPLICATION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID-1 AND -4 CHANNELS IN INTRACELLULAR CALCIUM SIGNALING TRIGGERED BY ENGINEERED NANOPARTICLES

Ninon Hontans[1]; Thibaud Parpaite[1]; Thomas Ducret[1]; Jean-François Quignard[1]; Isabelle Baudrimont[1]; Stéphane Mornet[2]; Roger Marthan[1]; Bernard Muller[1]; Jean-Pierre Savineau[1]; Arnaud Courtois[1];

1: Université de Bordeaux ; INSERM U1045, Centre de Recherche Cardio-Thoracique de Bordeaux, F-33076 Bordeaux, France

2: CNRS UPR 9048, Institut de Chimie de la Matière Condensée de Bordeaux, F-33608 Pessac, France

Background: Nanotechnology promotes the use of nanomaterials, especially engineered nanoparticles (NPs, aerodynamic diameter under 100 nm). This is expecting to bring many human benefits, but is perceived with apprehension for human health risks. Indeed, engineered NPs possess some similar characteristics to ultrafine environmental particles, and were shown to exert deleterious health effects, especially on cardiovascular functions as they could exert pro-hypertensive effects. In this context, the effects of NPs on calcium signaling which is often involved in hypertensive diseases remain poorly described.

Objective: We assessed the Silicium dioxide NPs (SiO2 NPs) effects on calcium signaling and proliferation in rat pulmonary artery smooth muscle cells (PASMC) under no stretch or cyclic mechanical stretch that mimic wall pressure found in hypertensive diseases. Implication of Transient Receptor Potential Vanilloid (TRPV) channels was seek as they are involved in some hypertensive diseases, such as pulmonary hypertension, and some NPs detrimental effects.

Methodology: Freshly dissociated rat PASMC were subjected or not to a cyclic mechanical stretch (20 % over resting length, 1 Hz, for 24 to 48 h). Intracellular calcium concentration ([Ca2+]i) measurements were performed using FURA-PE3 imaging and proliferation was assessed by BrdU incorporation.

Results: In PASMC, expressing TRPV-1 and -4, acute exposure to SiO2 NPs, from 1 to 500 μg/mL, produced a dose dependent increase in [Ca2+]i. In unstretched PASMC, this rise in [Ca2+]i was significantly reduced by capsazepine or HC067047, specific TRPV-1 and -4 inhibitors, respectively. In 24 h stretched cells, the basal [Ca2+]i was significantly enhanced and acute exposure to 50 μg/mL SiO2 NPs produced an significantly higher increase in [Ca2+]i than the one observed in unstretched cells. In contrast to unstretched PASMC, the TRPV inhibitors did not inhibit this [Ca2+]i increase upon NPs exposure. Finally, stretching for 48 h by itself induced PASMC proliferation, as did exposure to 200 μg/mL of SiO2 NPs both in unstretched and stretched cells.

Conclusion: We provided evidence that SiO2 NPs increased [Ca2+]i that was dependent to some extent to TRPV channels activation. Those NPs induced smooth muscle cell proliferation, and thus could participate to the pathophysiology of hypertensive diseases.

P-17 CHARACTERIZATION OF APIS MELLIFERA VOLTAGE-GATED CA2+ CHANNELS IN APIS NEURONS AND AFTER EXPRESSION IN XENOPUS OOCYTES

Thierry Cens[1]; Matthieu Rousset[1]; Claude Collet[2]; Mohamed Chahine[3]; Jean-Christophe Sandoz[4]; Pierre Charnet[1];
1: CRBM, CNRS UMR 5237, Montpellier, Fr.
2: INRINRA UMR 406, Abeilles et Environnement, Avignon, Fr.
3: Université Laval Robert-Giffard, QUEBEC, Canada
4: LEGS, UPR 9034; CNRS, Gif sur Yvette

Insecticides have been suspected to participate in abnormally elevated mortalities occurring in domestic honeybee apiaries worldwide. Among the currently commercialized pesticides, Pyrethroids are synthetic insecticides broadly used for their major neurotoxic action on the insect nervous system. These chemically engineered compounds, mainly described as targeting Na+ channels, have also been suspected to inhibit invertebrate and vertebrate voltage-gated Ca2+ channels. However, the molecular tools needed to precisely study the impact of these drugs on the physiology and toxicology of the honeybee are still lacking.
We have recently identified in the genomic honeybee database and cloned three pore-forming CaV-alpha subunits that could belong to the CaV1, CaV2 and CaV3 families, a single CaV-beta and three CaV-alpha2-delta subunits. We report here the cloning, expression and characterization of these subunits, their tissues distribution in brain and muscle, as well as the characterization of endogenous Ca channels in honeybee muscle cells, olfactory receptor neurons, antennal lobes neurons and Kenyons cells. These results may help to understand the functional diversity of voltage-gated Ca2+ currents recorded in honeybee muscle and neurons and provide interesting tools to characterize the role of these channels in honeybee physiology and toxicology.

P-18 PYRETHROIDS DIFFERENTIALLY ALTER VOLTAGE-GATED SODIUM CHANNELS FROM THE HONEYBEE CENTRAL OLFACTORY NEURONS

Aklesso Kadala[1]; Mercédès Charreton[1]; Ingrid Jakob[1]; Thierry Cens[2]; Matthieu Rousset[2]; Mohamed Chahine[3]; Yves Le Conte[1]; Pierre Charnet[2]; Claude Collet[1];

1: INRA, UR406 Abeilles et Environnement, Toxicologie Environnementale, F-84000 Avignon, France

2: CNRS, UMR 5237, Centre de Recherche de Biochimie Macromoléculaire, Université Montpellier 2, F-34293 Montpellier

3: Department of medicine, Laval University, Canada

The sensitivity of neurons from the honey bee olfactory system to pyrethroid insecticides was studied using the patch-clamp technique on central 'antennal lobe neurons' (ALNs). In these neurons, the voltage-dependent sodium currents are characterized by negative potential for activation, fast kinetics of activation and inactivation, and the presence of cumulative inactivation during train of depolarizations. Perfusion of pyrethroids on these ALN neurons submitted to repetitive stimulations induced (1) an acceleration of cumulative inactivation, and (2) a marked slowing of the tail current recorded upon repolarization. Cypermethrin and permethrin accelerated cumulative inactivation of the sodium current peak in a similar manner and tetramethrin was even more effective. The slow-down of channel deactivation was markedly dependent on the type of pyrethroid. With cypermethrin, a progressive increase of the tail current amplitude along with successive stimulations reveals a traditionally described use-dependent recruitment of modified sodium channels. However, an unexpected decrease in this tail current was revealed with tetramethrin. If one considers the calculated percentage of modified channels as an index of pyrethroids effects, ALNs are significantly more susceptible to tetramethrin than to permethrin or cypermethrin for a single depolarization, but this difference attenuates with repetitive activity. Further comparison with peripheral neurons from antennae suggest that these modifications are neuron type specific. Modeling the sodium channel as a multi-state channel with fast and slow inactivation allows to underline the effects of pyrethroids on a set of rate constants connecting open and inactivated conformations, and give some insights to their specificity. Altogether, our results revealed a differential sensitivity of central olfactory neurons to pyrethroids that emphasize the ability for these compounds to impair detection and processing of information at several levels of the bees olfactory pathway.

P-19 INVOLVEMENT OF POTASSIUM CHANNELS IN CIRCUMNUTATION OF THE INFLORESCENCE STEM IN ARABIDOPSIS THALIANA

Maria STOLARZ[1]; Halina DZIUBINSKA[1]; Jean-Baptiste THIBAUD[2]; Kazimierz TREBACZ[1];

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

2: BPMP, UMR 5004 CNRS-INRA-SupAgro-UM2, Campus INRA-SupAgro, 2 place Viala, 34060 Montpellier cedex 2, France

In plants, potassium and anion-selective channels are involved in cell turgor maintenance and volume changes. In particular, these ion transport systems are believed to play an essential role in the mechanism of circumnutation, a kind of slow motion of some plant organs that would result from co-ordinated cell volume changes. Molecular identity of the potassium channels involved in these processes, however, still remains unclear. To address this issue, time-lapse video recordings of Arabidopsis thaliana wild type (WT) and potassium channel mutant plants knocked out at either the AKT2 or the GORK locus (both encoding K+-selective voltage-gated channels) were used to study the kinetics of the inflorescence stem circumnutation. The period, trajectory length, rate, shape and direction of circumnutation were measured. The period of circumnutation both in the WT and in the akt2- and gork- KO mutants was approx. 85 min. No effect of potassium channel mutations on the shape and direction of circumnutation was observed. On the contrary, the trajectory length and the rate of circumnutation were significantly ($p<0.01$) lower in gork- plants than in the WT and akt2- plants. These data provide the first evidence that the depolarization-activated outward-rectifying (GORK) but not the weakly inward-rectifying (AKT2) potassium Shaker-like channels are involved in the circumnutation mechanism of the inflorescence stem in Arabidopsis thaliana.

The research was supported by the grant POLONIUM: "Disclosing molecular basis of electrical signalling in plants".

P-20 BINDING SITE AND INHIBITORY MECHANISM OF THE MAMBALGIN-2 PAIN-RELIEVING PEPTIDE ON ACID-SENSING ION CHANNEL 1A

Salinas Miguel; Besson Thomas; Delettre Quentin; Diochot Sylvie; Boulakirba Sonia; Douguet Dominique; Lingueglia Eric;
CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, UMR 7275, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France

Acid-sensing ion channels (ASICs) are neuronal proton-gated cation channels associated with nociception, fear, depression, seizure, and neuronal degeneration, suggesting roles in pain and neurological and psychiatric disorders. We have recently discovered black mamba venom peptides called mambalgin-1 and mambalgin-2, which are new three-finger toxins that specifically inhibit with the same pharmacological profile ASIC channels to exert strong analgesic effects in vivo. We now combined bioinformatics and functional approaches to uncover the molecular mechanism of channel inhibition by the mambalgin-2 pain-relieving peptide. Mambalgin-2 binds mainly in a region of ASIC1a involving the upper part of the thumb domain (residues Asp-349 and Phe-350), the palm domain of an adjacent subunit, and the β-ball domain (residues Arg-190, Asp-258, and Gln-259). This region overlaps with the acidic pocket (pH sensor) of the channel. The peptide exerts both stimulatory and inhibitory effects on ASIC1a, and we propose a model where mambalgin-2 traps the channel in a closed conformation by precluding the conformational change of the palm and β-ball domains that follows proton activation. These data help to understand inhibition by mambalgins and provide clues for the development of new optimized blockers of ASIC channels.

P-21 UNDERSTANDING THE FUNCTIONAL COUPLING BETWEEN SUR AND KIR6.2 SUBUNITS IN THE ATP-SENSITIVE POTASSIUM CHANNEL

Maria Antonietta Principalli; Jean Revilloud; Julien Dupuis; Michel Vivaudou;
71 Avenue des Martyrs 38044 Grenoble, France

The ATP-sensitive potassium channel (KATP) is a multimeric membrane complex that is ubiquitously expressed in excitable cells and links cell metabolism to membrane excitability. The full-length complex is made of two different proteins: the sulfonylurea receptor (SUR), which belongs to the ABC protein family and the inward rectifier K+ channel Kir6.2. Both subunits associate to form a heterooctamer (4SUR/4Kir6.2) of ~950 kDa. SUR regulates channel gating as a function of internal ATP and ADP concentrations. It is also the target of pharmaceutical KATP channel openers (KCO) and blockers which respectively up- and downregulate the gating of Kir6.2.

How are SUR and Kir6.2 functionally coupled? In particular, how does SUR translate the binding of physiological and pharmacological ligands into changes in Kir6.2 gating?

It has been previously found (Dupuis et al. J Physiol 2008) that three neighbouring residues (1305, 1310, 1313) in the loop region between transmembrane domain 2 (TMD2) and nucleotide-binding domain 2 (NBD2) of SUR2A are implicated in the activation pathway linking binding of openers to SUR2A and opening of Kir6.2.

The isoforms SUR1 and SUR2A are expressed in different organs: SUR1 is predominant in the pancreas, SUR2A in the heart. In pancreatic β-cells, KATP channels are partly active at rest while in cardiomyocytes they are mostly closed. This divergence of function may be associated with differences at the molecular level between the two SUR isotypes. We are currently trying to understand if the same residues are implicated in the isoform SUR1 and, in this case, whether the mechanism of activation is the same.

The strategy used to understand this mechanism is based on: 1) the creation of chimeric KATP channels, taking advantage of the homology between SUR1, SUR2A and MRP1 and 2) the characterization of these by patch clamp technique in xenopus oocytes. Our preliminary results reveal differences in the way SUR1 and SUR2A interact with Kir6.2 although the same region underlies this interaction.

P-22 THIK2 ASSOCIATES WITH THIK1 TO FORM FUNCTIONNAL HETERODIMERS

Sandy Blin[1]; Dawon Kang[2]; Franck Chatelain[1]; Sylvain Feliciangeli[1]; Maud Larroque[1]; Donghee Kim[2]; Florian Lesage[1]; Bichet Delphine[1];

1: LabEx ICST, Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, and Université de Nice Sophia Antipolis, 660 Route des Lucioles, 06560 Valbonne, France

2: Department of Physiology and Biophysics, Chicago Medical School, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, Illinois 60064

Tandem pore domain halothane-inhibited K+ channel 1 (THIK1) produces background K+ currents, but not THIK2 despite good sequence conservation with THIK1. In a previous article (Chatelain, F.C, Bichet, D., Feliciangeli, S., Larroque, M.M., Braud, VM and Lesage, F. 2013), we show that this lack of THIK2 current activity is due to a unique combination of intracellular retention and weak basal activity in the plasma membrane. Inactivation of the retention/retrieval signal and/or activating mutations in the pore region of THIK2 give channels producing measurable currents, demonstrating that THIK2 is a functional channel. Here, we show that THIK1 and THIK2 can form functional heteromers. Coinjecting THIK1 with THIK2 strongly reduces THIK1 activity in a dose dependent manner. We also designed THIK subunits containing dominant-negative mutations (THIK1DN and THIK2DN) and co-expressed them with the wild type subunits. THIK2DN mutant was able to inhibit THIK1 currents and reciprocally THIK1DN was able to inhibit an active form of THIK2 (THIK2-A155P-I158D) whereas TREK1 was not inhibited by THIK1DN nor THIK2DN. Using fluorescent approaches based on close proximity between both proteins, we demonstrated THIK1 and THIK2 are colocalizing in cells. Finally, we expressed covalent tandems of THIK proteins to obtain expression of pure homomeric and heteromeric channels (named Td-THIKx-THIKx). Td-THIK1-THIK2 produces K+ currents of amplitude similar to Td-THIK1-THIK1 but with a noticeable difference in the current kinetics. Besides, Td-THIK1-THIK2 shows particular electrophysiological properties. Unlike Td-THIK2-THIK2 that is mainly detected in the endoplasmic reticulum like THIK2, Td-THIK1-THIK2 distributes at the plasma membrane indicating that THIK1 can mask the ER retention/retrieval motif of THIK2. Altogether, these results show that THIK1 and THIK2 form functional homomeric and heteromeric channels with specific properties, further expanding the known repertoire of K+ channels.

P-23 GENETICALLY ENCODED PHOTO-CROSS-LINKER TO STUDY INTERFACES FOR POSITIVE ALLOSTERIC REGULATION OF GLUTAMATE-GATED ION CHANNELS

Meilin TIAN[1]; Pierre PAOLETTI[2]; Shixin YE-LEHMANN[2];
1: École Normale Supérieure, Institut de Biologie de l'ENS(IBENS), Paris, 75005 France. Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University (ECNU), Shanghai, China
2: École Normale Supérieure, Institut de Biologie de l'ENS(IBENS), Paris, 75005 France

Genetic code reprogramming and expansion with unnatural amino acids (UAA) enables the incorporation of amino acids with unique chemical properties absent in natural amino acids into proteins, which can serve as chemical probes. Recently we have applied this site-directed mutagenesis technology to investigate structure-function of N-methyl-D-aspartate receptors (NMDARs). NMDARs are glutamate-gated ion channels widespread in the CNS and regulating excitatory synaptic transmission and plasticity; they are also targets of therapeutic interest in neuronal disorders. NMDARs are tetrameric complexes composed of two obligatory GluN1 subunits and two GluN2 (2A to 2D) subunits, assembling into a dimer-of-dimer architecture. Each GluN2 subunit imparts distinct gating and pharmacological properties. Recent studies have revealed that the N-terminal domains (NTDs), large extracellular clamshell-like domains preceding the agonist-binding domain, play crucial role in controlling the functional diversity of NMDAR subtypes. Although the NTD of GluN2B receptors is relatively well understood, little is known about GluN2A NTDs and its subunit interfaces. Here we systematically map the dimer interface at the NTD levels in both GluN2A and GluN2B containing receptors by incorporating the photo-cross-linker UAA p-azido-L-phenylalanine (AzF) at the NTD dimer interfaces, one residue at a time in GluN2B and GluN2A subunits. By combining live cell photo-treatment and electrophysiological measurements, we characterized the functional changes of AzF mutant receptors following UV illumination. Western blotting has been also performed to determine the possibility for inter-subunit crosslinking after UV treatment. F114AzF in GluN2B was discovered as a residue that dimerizes with GluN1 under UV illumination, causing enhanced channel activity. These findings indicate that the close association of NTDs in GluN2B receptors acts as a mean for positive regulation of channel activity, while at GluN2A receptors, GluN2A and GluN1 NTDs interact less tightly. Further steps include characterizing the NTD dynamic domain interactions by identifying photo-cross-linking partners at different activity states.

P-24 FUNCTIONAL ASSESSMENT OF CRYSTALLIZATION-OPTIMIZED G PROTEIN-COUPLED RECEPTORS USING ION CHANNEL-COUPLED RECEPTORS

Katarzyna Niescierowicz[1]; Lydia Caro[2]; Vadim Cherezov[3]; Michel Vivaudou[1]; Christophe Moreau[1];

1: IBS/Channels, Institut de Biologie Structurale, 71 Avenue des Martyrs, 38044 Grenoble, France

2: Present address: University of Toronto, Toronto, ON, M5S 1A8, Canada

3: The Scripps Research Institute, La Jolla, CA 92037, USA

Ion Channel-Coupled Receptors (ICCRs) are artificial ligand-gated ion channels created by fusion of G protein-coupled receptors (GPCRs) to a K+ inward rectifier channel (Kir6.2) such that the channel is a direct reporter of the receptor conformational changes. The GPCR-channel communication proceeds without any involvement of G proteins and the electrical signal amplitude correlates with the ligand concentration. This concept has been validated with 4 prototypical GPCRs: the M2 muscarinic, the D2L dopaminergic, the β2 adrenergic and the opsin receptors [Moreau et al. 2008. Nature Nanotechnol 3:620; Caro et al. 2011. PloS ONE 6:e18226; Caro et al. 2012. PloS ONE 7:e43766].

The intrinsic instability of the GPCRs has proved a challenge to crystallographic studies. A successful approach, introduced by Cherezov et al [2007. Science 318:1258] and subsequently applied to obtain 12 GPCR structures, consists in the insertion of the T4 phage lysozyme domain (T4L) in the 3rd intracellular loop of the receptors. However, this modification abolishes G protein binding and prohibits related functional assays. Current characterization of crystallization-optimized GPCR(T4L) is performed by radiolabeled ligand assays or localized FRET techniques. Requiring no biochemical steps, ICCRs are an alternative tool to functionally characterize modified GPCRs that are unable to bind or activate G proteins and not amenable to most GPCR functional assays, with the added benefit of reporting not only ligand binding, but also ligand-induced conformational changes. We demonstrate here [Niescierowicz et al. 2014. Structure. 22:149] the validity of this tool with 3 different GPCRs (M2-muscarinic, β2-adrenergic and oxytocin receptors).

P-25 AUTOMATED MICROFLUIDIC TECHNOLOGY FOR SCREENING OF ACTIVITY OF NANOPORES AND ION CHANNELS USING DROPLET INTERFACE BILAYERS

Tomasz Kaminski[1]; Magdalena Czekalska[1]; Slawomir Jakiela[1]; Tanuj Sapra[2]; Hagan Bayley[2]; Piotr Garstecki[1];

1: Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

2: Department of Chemistry, University of Oxford, Oxford, OX1 3TA, UK

We demonstrate an automated microdroplet technology that enables fast, simple and reproducible in vitro screening of inhibitors against membrane proteins and ion channels. The system automates i) formation of a lipid bilayer at the interface between a pair of nanoliter aqueous droplets submerged in oil, ii) exchange of one droplet to form a new bilayer between compartments comprising new chemistry, and iii) measurements of electrical current through single protein channels.

We introduce a new microfluidic architecture - a hydrodynamic trap designed to localize the droplets with respect to each other and with respect to the electrodes. Recently, few microfluidic platforms utilizing Droplet Interface Bilayers (DIBs) technique were developed for formation of artificial lipid bilayers. However they do not allow for both high-throughput, automated generation of bilayers and electrophysiological measurements at the same time. Efficient and repetitive reconstitution of functional lipid bilayers was possible thank to the Droplet on Demand method, which provides high degree of control over composition and location of each droplet.

The accuracy of our system was tested in the screening experiment of activity of various concentrations of gamma-cyclodextrin on single alpha-hemolysin pore. We were able to perform several subsequent repetitions of screening of 6 concentrations (1-50 μM) of inhibitor . Using the traces obtained for 10-50 μM we calculated dissociation constant Kd value for binding of alpha–cyclodextrin to a–hemolysin to be equal 61± 7μM.

Our approach can be applied to screen the activity of membrane proteins at the single-molecule level. The technique that we report here allows for automation of these measurements and for additional operations, such as washing and exchange of both the inhibitor solution and of the protein.

P-26 THE VOLTAGE-GATED SODIUM CHANNEL IN THE PACIFIC OYSTER CRASSOSTREA GIGAS : ITS SENSITIVITY TO PARALYTIC SHELLFISH TOXINS (PST) PRODUCED BY ALEXANDRIUM MINUTUM.

Floriane Boullot[1]; Evelyne Benoit[2]; Justine Castrec[1]; Maëva Leitwen[1]; Antoine Beuzit[1]; Hélène Hégaret[1]; Pierre Boudry[1]; Philippe Soudant[1]; Caroline Fabioux[1];

1: UMR 6539 CNRS / UBO / IRD / IFREMER. Laboratoire des sciences de l'Environnement MARin (LEMAR), Institut Universitaire Européen de la Mer, rue Dumont d'Urville, 29280 Plouzané, France

2: Unité de Recherche Neurobiologie et Développement, UPR 3294, Institut de Neurobiologie Alfred Fessard, Campus CNRS-Gif sur Yvette, 1, Avenue de la Terrasse, Bât. 32-33, 91198 Gif sur Yvette, France

French coasts are regularly affected by harmful algal blooms (HAB), including blooms of dinoflagellates Alexandrium spp. These micro-algae produce paralytic shellfish toxins (PST), as saxitoxin (STX), which bind to voltage-gated sodium channels (Nav) and block conduction of muscle and nerve fibers. Filter feeder marine organisms like oysters can accumulate high levels of PST, making them toxic and unsuitable for human consumption. The toxin load has been shown to vary by a factor of 80 between oysters Crassostrea gigas from a same population exposed experimentally to A. minutum, suggesting a high variability in PST tolerance. The purpose of this study is i) to characterize voltage-gated sodium channels in C. gigas and ii) to determine whether Nav and its potential isoforms play a role in the differential sensitivity of C. gigas to PST. Among three proteins annotated as "Nav" in the C. gigas genome, only Nav9 has all the characteristics of a true Nav and was selected as candidate in this study. The tissular and cellular expression pattern of Nav9 was characterized by real time PCR and in situ hybridization. Nav9 appeared mainly expressed in neurosecretory cells of the visceral ganglia, in nerve fibers of the skeletal muscle and in epithelial cells of labial palps and mantle. First electrophysiological measurements performed on nerves of oysters showed a significant decrease of the global action potential when exposed to 10-7g/mL of STX. These first results indicated a medium sensitivity of oyster to PST, which must be confirmed by further electrophysiological experiments. Alternative splicing transcripts of Nav9 are being characterized as they could be the source of the variability of sensitivity to PST in C. gigas, as demonstrated in some insects for resistance to pyrethroids.

Aguettaz Elizabeth
Laboratoire de Signalisation et Transferts IoniquesMembranaires
1, rue Georges Bonnet Bât B
86000 Poitiers - France
Tel: 0662619234 - Fax:
elizabeth.aguettaz@univ-poitiers.fr

Allard Bruno
Université Lyon 1
UMR 5534 CNRS/Université Lyon 1 43 bd du 11 Novembre 1918
69622 Villeurbanne - France
Tel: 06 20 94 02 19 - Fax:
bruno.allard@univ-lyon1.fr

Alloui Abdelkrim
INSERM U1107
69675 BRON - FRANCE
Tel: 04 73 17 82 30 - Fax: 04 73 17 82 33
abdelkrim.alloui@udamail.fr

Antigny Fabrice
Inserm U999
Centre chirurgical Marie Lannelongue
92350 Le Plessis Robinson - France
Tel: 331 40 94 25 15 - Fax:
antignyfabrice@gmail.com

Balycheva Marina
University of Verona, Cardiosurgery Department
P.le A. Stefani, 1
37126 Verona - ITALY
Tel: +393289048537 - Fax:
marina.balycheva@univr.it

Belaia Irina
N/A
UCL Institute of Neurology University College London
WC1N 3BG London - U.K.
Tel: +447879608545 - Fax:
d_rusakov@yahoo.com

Ben Soussia Ismail
Institut de pharmacologie moléculaire et cellulaire
660 Route des Lucioles
06560 Valbonne - France
Tel: +33493957777 - Fax: +33493957708
bensoussia@ipmc.cnrs.fr

Berrou Mikael
World Precision Instruments
1 Hunting Gate
SG4 0TJ Hitchin - United Kingdom
Tel: +33 9 70 44 90 00 - Fax: +44 1462 424 701
mberrou@wpi-europe.com

Bescond Jocelyn
University of Poitiers
Equipe Transferts Ioniques et Rythmicité Cardiaque (TIRC) Laboratoire STIM, ERL CNRS 7368 Pôle Biologie Santé, Bâtiment B36/B37 1 Rue Georges Bonnet, TSA 51106
86073 POITIERS - Cedex 9 - FRANCE
Tel: +33549453528 - Fax: +33549454014
jocelyn.bescond@univ-poitiers.fr

Besson Thomas
IPMC CNRS UMR7275
660, route des Lucioles Sophia Antipolis
06560 Valbonne - FRANCE
Tel: 04 93 95 34 22 - Fax: 04 93 95 77 08
besson@ipmc.cnrs.fr

Blin Sandy
Institut de Pharmacologie Moléculaire et Cellulaire
660 route des lucioles Sophia-Antipolis
06560 Valbonne - France
Tel: 0645596314 - Fax:
blin@ipmc.cnrs.fr

Bobak Nicole
Institut de Pharmacologie Moléculaire et Cellulaire
660 Route des Lucioles Sophia Antipolis
06560 Valbonne - France
Tel: 0493957732 - Fax:
bobak@ipmc.cnrs.fr

Boffen (Pessah) Beatrice
3104 Oyster Bay Avenue Davis, CA
95616 Davis - USA
Tel: 530-400-5900 - Fax:
laughingmoon@sbcglobal.net

Bois patrick
Université de Poitiers
Equipe Transferts Ioniques et Rythmicité Cardiaque (TIRC) Laboratoire Signalisation et Transports Ioniques Membranaires (STIM), ERL CNRS 7368 Université de Poitiers, UFR SFA Pôle Biologie Santé, Bâtiment B36/B37 1 Rue Georges Bonnet, TSA 51106
86073 Poitiers - France
Tel: 0549453900 - Fax: 0549454014
patrick.bois@univ-poitiers.fr

Borie Amélie
IGF
141 Rue de la Cardonille
34094 Montpellier - France
Tel: +33 4 34 35 92 95
amelie.borie@igf.cnrs.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 de Bordeaux Institut des maladies neurodégénératives (IMN) CNRS UMR 5293 Université Bordeaux 146 rue Léo Saignat
33076 Bordeaux - France
Tel: 0557575763 - Fax: 0557575760
eric.boue-grabot@u-bordeaux.fr

Boullot Floriane
LEMAR - IUEM
Rue Dumont d'Urville
29280 PLOUZANE - France
Tel: 0678830258 - Fax:
floriane.boullot@univ-brest.fr

Bourinet Emmanuel
Institut de Génomique Fonctionnelle
141, Rue de la Cardonille
34094 Montpellier - France
Tel: 04 34 35 92 48 - Fax:
emmanuel.bourinet@igf.cnrs.fr

Chahine Mohamed
Laval University
Centre de recherche en neuroscience Institut universitaire en santé mentale de Québec 2601 chemin de la Canardière
G1J 2G3 Québec - Canada
Tel: 1 (418) 663-5747 #47 - Fax: 1 (418) 663-8756
mohamed.chahine@phc.ulaval.ca

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

Chataigneau Thierry
UMR 7199 CNRS/ Université de Strasbourg, Laboratoire de Conception et Application de Molécules Bioactives Equipe de Chimie et Neurobiologie Moléculaire 74 route du Rhin
67400 Illkirch - France
Tel: (+33) 03 68 85 41 33 - Fax: (+33) 03 68 85 43 06
thierry.chataigneau@unistra.fr

Chatelier Aurelien
CNRS/Poitiers University
STIM Laboratory, Equipe Transferts Ioniques et Rythmicité Cardiaque, ERL CNRS 7368, UFR SFA Pôle Biologie Santé, Bâtiment B36/B37 1 Rue Georges Bonnet, TSA 51106
86073 Poitiers - Cedex 9- France
Tel: +33 (0)549 453 747 - Fax: +33(0) 549 454 014
aurelien.chatelier@univ-poitiers.fr

Cognard Christian
Lab. Signalisation et Transports Ioniques Membranaires (STIM)
ERL CNRS/Université de Poitiers n°7368 Pôle Biologie Santé Bât B36/B37 1 rue Georges Bonnet TSA 51106
86073 POITIERS CEDEX 9 - France
Tel: 0549454064 - Fax: 0549454014
christian.cognard@univ-poitiers.fr

Collet Claude
INRA
UR406, Abeilles et Environnement, Toxicologie Environnementale. Domaine St Paul, Site Agroparc, CS 40509, cedex9
84914 Avignon - France
Tel: 0432722649 - Fax: 0432722602
claude.collet@avignon.inra.fr

Compans Benjamin
IINS CNRS UMR 5297
146 rue leo saignat Institut Magendie
33077 Bordeaux - France
Tel: 33557574080 - Fax:
benjamin.compans@wanadoo.fr

Connolly Christopher
University of Dundee
Division of Neuroscience Medical Research Institute Ninewells Medical School
DD1 9SY Dundee - UK
Tel: +44 (0)1382 383105 - Fax:
c.n.connolly@dundee.ac.uk

Courtois Arnaud
INSERM U1045
Université de Bordeaux 146, rue Léo Saignat
33076 Bordeaux - France
Tel: 05 57 57 95 13 (off. - Fax: 05 57 57 16 95
arnaud.courtois@u-bordeaux.fr

De Luca Annamaria
Department of Pharmacy - Drug Sciences, University of Bari
Via Orabona 4, Campus
70125 Bari - Italy
Tel: 0039 080 5442245 - Fax:
annamaria.deluca@uniba.it

Desaphy Jean-Francois
University of Bari
Dept. Pharmacy & Drug Sciences via Orabona 4 - campus
70125 Bari - Italy
Tel: +39-0805442217 - Fax:
jeanfrancois.desaphy@uniba.it

Desarmenien Michel
CNRS
IGF 141 Rue de la Cardonille
34094 Montpellier Cedex - France
Tel: +33 4 34 35 92 94 - Fax:
mdesarmenien@igf.cnrs.fr

Deval Emmanuel
IPMC, UMR 7275 CNRS/Université de Nice Sophia Antipolis
660 route des lucioles Sophia Antipolis
06560 Valbonne - France
Tel: 04.93.95.34.18 - Fax:
deval@ipmc.cnrs.fr

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

Duprat Fabrice
INSERM
IPMC 660 route des lucioles
06560 Valbonne - France
Tel: +33493957723 - Fax: +33493957708
duprat@ipmc.cnrs.fr

Dziubińska Halina
University Maria Curie-Sklodowska of Lublin (Poland)
Akademika 19
20-033 Lublin - Poland
Tel: 815375931 - Fax: 815375902
Halina.Dziubinska@umcs.lublin.pl

Entcheva Emilia
Stony Brook University
Dept. Biomedical Engineering BST6-140B
Institute for Molecular Cardiology
11790 Stony Brook - United States
Tel: +1 631-220-4444
emilia.entcheva@stonybrook.edu

Es-Salah-Lamoureux Zeineb
l'institut du thorax Unité Inserm
UMR1087/CNRS UMR6291
IRS-UN, 8 Quai Moncousu BP 70721
44007 Nantes cedex 1 - France
Tel: 33 (0)228 080 110 - Fax: 33 (0)228 080 130
zeineb.lamoureux@inserm.fr

Faivre Jean-François
Université de Poitiers
CNRS-ERL 7368 Université de Poitiers UFR SFA - Pôle Biologie Santé Bâtiment B36/B37 1 Rue Georges Bonnet 86000 POITIERS France
Tel: 0549453672 - Fax: 0549454014
jean-francois.faivre@univ-poitiers.fr

Feliciangeli Sylvain
CNRS
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

Feltz Anne
IBENS, institut de biologie de l'Ecole Normale Supérieure
46, rue d'Ulm
75005 Paris - France
Tel: 0671412488
feltz@ens.fr

Feske Stefan
New York University Medical Center
Department of Pathology 550 First Avenue, SRB 316
10016 NEW YORK - USA
Tel: +1-212-263-9066
FESKES01@nyumc.org

Fourbon Yann
INSERM UMR 1069 N2C TOURS
Faculté de Médecine Bât. Dutrochet, 2ème étage 10 boulevard Tonnellé 37032 Tours Cedex (France)
37032 TOURS - FRANCE
Tel: 02.47.36.60.24 - Fax: 02.47.36.62.26
yann.fourbon@laposte.net

Gailly Philippe
University of Louvain
University of Louvain. Institute of Neuroscience. Laboratory of Cell Physiology. 53, av. Mounier, bte B1.53.12
1200 Brussels - Belgium
Tel: 3227645542 - Fax: 3227645580
philippe.gailly@uclouvain.be

Gautier Helene
University of Cambridge
Department of Physiology, Development and Neuroscience University of Cambridge Downing Street, Anatomy Building
CB2 3DY Cambridge - UK
Tel: +44(0)1223 333 762 - Fax:
hg295@cam.ac.uk

Gilbert Guillaume
INSERM U1045
146 rue Léo Saignat Bâtiment TP 2ème étage
33076 BORDEAUX Cedex - BORDEAUX Cedex
Tel: 0688490213 - Fax: 0557571694
guillaume.gilbert138@hotmail.fr

Gorelik Julia
Imperial College London
Du Cane rd
W12 0NN London - UK
Tel: 0044 (0) 207594 2736 - Fax: 0044 (0) 207594 3653
j.gorelik@imperial.ac.uk

Grand Teddy
Ecole normale supérieure
Institut de Biologie de l\'ENS (IBENS), Neuroscience Section CNRS UMR8197, INSERM U1024 Ecole Normale Supérieure 46 rue d\'Ulm
75005 Paris - FRANCE
Tel: 01 44 32 38 92 - Fax:
teddy.grand@ens.fr

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

Guizouarn Hélène
CNRS-Université Nice Sophia Antipolis
Institut de Biologie Valrose Bâtiment de Sciences Naturelles 28 av. Valrose
06100 Nice - France
Tel: 0492076837 - Fax: 0492076834
guizouar@unice.fr

Hans Michael
Institute of Physiology II
University of Bonn Nussallee 11
53115 BONN - Germany
Tel: +49-228-73-60123 - Fax: +49-228-73-60119
michael.hans@ukb.uni-bonn.de

Harkat Mahboubi
UMR 7199 CNRS/ Université de Strasbourg, Faculté de Pharmacie
Laboratoire de Conception et Application de Molécules Bioactives Equipe de Chimie et Neurobiologie Moléculaire UMR 7199 CNRS/ Université de Strasbourg 74 route du Rhin
67400 Illkirch - France
Tel: (+33) 03 68 85 41 16 - Fax: (+33) 03 68 85 43 06
mahboubi.harkat@etu.unistra.fr

Hirbec Helene
IGF
141 rue de la cardonille
34094 MONTPELLIER - France
Tel: 0434359304 - Fax:
helene.hirbec@igf.cnrs.fr

Hosy Eric
CNRS
Institut Magendie, IINS 146 rue Leo saignat
33077 Bordeaux - France
Tel: 05 57 57 40 99 - Fax:
eric.hosy@u-bordeaux.fr

Jarman Duncan
Cambridge Bioscience
Munro House Trafalgar Way Bar Hill
CB23 8SQ Cambridge - UK
Tel: +44 7766 114 031 - Fax: +44 1954 781 323
duncan.jarman@bioscience.co.uk

Kaminski Tomasz
Institute of Physical Chemistry
Kasprzaka 44/52
01-224 Warsaw - Polska
Tel: 0048223433247 - Fax: 0048223433333
tkaminski@ichf.edu.pl

Kreir Mohamed
Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich - Germany
Tel: +4989218997981 - Fax:
mohamed@nanion.de

Landoulsi Zied
Institut Pasteur de Tunis
13, place Pasteur, B.P. 74
1002 Tunis - Tunisia
Tel: +216 22 89 48 26 - Fax: +216 71 791 833
landoulsi.z@gmail.com

Legros christian
Lab. of Neurovascular an Mitochondrial Integrated Biology
CNRS UMR6214, Inserm U1083, Univ. Angers
Faculty of Medicine Rue Haute de Reculée
F-49045 ANGERS - FRANCE
Tel: +33-2-41-73-58-34 - Fax: +33-2-41-78-58-95
christian.legros@univ-angers.fr

Lesport Pierre
Institut de Génomique Fonctionnelle
141, Rue de la Cardonille
34094 Montpellier - France
Tel: 04 34 35 92 48 - Fax:
pierre.lesport@igf.cnrs.fr

Levenes Carole
CNRS
CNRS UMR8119 45 rue des St Pères
75006 Paris - France
Tel: 33 (0)142 864 157 or : 33 (0)142 862 138
carole.levenes@gmail.com

Magaud Christophe
Laboratoire Signalisation & Transports Ioniques Membranaires
ERL7368 bat.36 1,rue George Bonnet TSA 51106
86022 Poitiers - France
Tel: 0549453891 - Fax: 0549454014
christophe.magaud@univ-poitiers.fr

Malecot Claire O.
CNRS ERL 7368, Laboratoire Signalisation et Transports Ioniques Membranaires
CNRS ERL 7368, Laboratoire STIM, Groupe Physiologie des Cellules Cardiaques et Vasculaires,Faculté des Sciences, Parc de Grandmont
37200 TOURS - FRANCE
Tel: 02-47-36-70-09 - Fax:
claire.malecot@univ-tours.fr

Marionneau Céline
L'Institut du Thorax
IRS-Université de Nantes 8 quai Moncousu BP 70721
44007 Nantes - France
Tel: 02-28-08-01-63 - Fax: 02-28-08-01-30
celine.marionneau@univ-nantes.fr

Marra Sébastien
IPMC/CNRS UMR7275
660 route des Lucioles - Sophia Antipolis
06560 Valbonne - France
Tel: 0493953418 - Fax:
marra@ipmc.cnrs.fr

Martinez Audrey
Institut des Maladies Neurodégénératives-CNRS UMR 5293
146, rue Léo Saignat Bat 1B 3ème étage.
33076 Bordeaux - France
Tel: 0557575765 - Fax: 0557575760
audrey.martinez@u-bordeaux2.fr

Mawet Jacques
ACTELION Pharmaceuticals
16 Gewerbestrasse
4123 Allschwil - Switzerland
Tel: +41615656478 - Fax:
jacques.mawet@actelion.com

Mazars Christian
CNRS
Laboratoire de Recherche en Sciences Végétales UMR CNRS-UPS 5546 Pôle de Biotechnologie Végétales,24 Chemin de Borde Rouge Auzeville BP 42617
31326 Castanet-Tolosan - France
Tel: (+33)534323836 - Fax: (+33)534323802
mazars@lrsv.ups-tlse.fr

Meli Albano
Inserm U1046 - University of Montpellier I & II
CHU Arnaud de Villeneuve 371 avenue du Doyen G. Giraud
34295 Montpellier - France
Tel: +33695232855 - Fax: +33467415242
albano.meli@inserm.fr

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

Mouro-Chanteloup Isabelle
INSERM U1134/INTS
6 Rue Alexandre Cabanel
75015 Paris - France
Tel: +33 (0)1 44 49 31 50 - Fax: +33 (0)1 43 06 50 19
isabelle.mouro-chanteloup@inserm.fr

Moxon Suzanne
Scientifica
1a Kingfisher Court Brambleside Bellobrook Industrial estate Uckfield
TN22 1QQ East Sussex - United Kingdom
Tel: 01825 749933 - Fax: 01825 749934
leah.hunt@scientifica.uk.com

Pardo Luis
Max-Planck-Institute of Experimental Medicine
Hermann-Rein-Str. 3
37075 Göttingen - Germany
Tel: +495513899646 - Fax: +495513866644
pardo@em.mpg.de

Parpaite Thibaud
Centre de Recherche Cardio-Thoracique de Bordeaux, INSERM U1045
Université de Bordeaux, 146, rue Léo-Saignat (case 13)
33076 Bordeaux - France
Tel: 0678948885 - Fax:
thibaud.parpaite@u-bordeaux.fr

Pessah Isaac
UC Davis
Department of Molecular Biosciences 1089 Veterinary Medicine Drive School of Veterinary Medicine Davis, CA
95616 Davis - USA
Tel: 530-752-6696 - Fax:
inpessah@ucdavis.edu

Peyronnet Remi
Imperial College London
Heart Science Centre, Hill End Road
UB9 6JH Harefield - United Kingdom
Tel: 00441895453805 - Fax:
r.peyronnet@imperial.ac.uk

Pierno Sabata
University of Bari
Dept. Pharmacy & drug Sciences via Orabona 4 - campus
70125 Bari - Italy
Tel: +39-0805442793 - Fax:
sabata.pierno@uniba.it

Principalli Maria Antonietta
Institut de biologie structurale
71 AVENUE DES MARTYRS CS 10090 38044 GRENOBLE CEDEX 9
38044 Grenoble - France
Tel: +33 4 57 42 85 86 - Fax:
maria-antonietta.principalli@ibs.fr

Quignard Jean-Francois
Université Bordeaux INSERM U1045
146 rue Leo Saignat
33074 BORDEAUX Cedex - France
Tel: 05 57 57 95 71 - Fax:
jean-francois.quignard@u-bordeaux.fr

Rapetti-Mauss Raphael
Institut de Biologie Valrose - IBV
Institut de Biologie Valrose UNS - CNRS UMR7277 - Inserm U1091 Faculté des Sciences
Parc Valrose
06108 Nice - France
Tel: +33 492076553 - Fax:
Raphael.RAPETTI-MAUSS@unice.fr

Revilloud Jean
Institut de Biologie Structurale
6 rue jules Horowitz
38042 Grenoble - FRANCE
Tel: 04 57 42 85 91 - Fax: 04 76 50 18 90
jean.revilloud@ibs.fr

Rode Baptiste
University of Leeds
University of Leeds LIGHT/DCDR Garstang 6.44
UK
LS2 9JT Leeds - United Kingdom
Tel: +44 (0) 113 34 34707 - Fax:
b.m.rode@leeds.ac.uk

Rolland Jean-Francois
Axxam SpA
Via A. Meucci 3
20091 Bresso (Milan) - Italy
Tel: +39 02 2105650 - Fax: +39 02 2105602
JeanFrancois.Rolland.JR@axxam.com

Rusakov Dmitri
Univeristy College London
Institute of Neurology Queen Square
WC1N 3BG London - U.K.
Tel: +447879608545 - Fax:
d.rusakov@ucl.ac.uk

Sabourin Jessica
INSERM U769
5 rue Jean-Baptiste Clément
92 296 Chatenay Malabry - France
Tel: 01 46 83 58 17 - Fax: 0146835475
jessica.sabourin@u-psud.fr

Sachs Frederick
SUNY
301 Cary hall
14214 Buffalo - United States
Tel: 716 829 5161 - Fax:
sachs@buffalo.edu

Sachs Frederick
SUNY
301 Cary hall SUNY
14214 Buffalo - United States
Tel: 7168295161 - Fax:
sachs@buffalo.edu

Salinas Miguel
IPMC CNRS UMR7275
660, route des Lucioles Sophia Antipolis
06560 Valbonne - FRANCE
Tel: 04 93 95 34 22 - Fax: 04 93 95 77 08
salinas@ipmc.cnrs.fr

Sarrauste de Menthière Cyril
IGH - CNRS UPR 1142
141, rue de la Cardonille
34396 Montpellier CEDEX 5 - France
Tel: 04 34 35 99 81 - Fax: 04 34 35 99 01
cyril.sarrauste@igh.cnrs.fr

Stroebel David
IBENS / CNRS
S4.9 section Neurosciences IBENS 46, rue d'Ulm
75005 Paris - France
Tel: 0144323892 - Fax:
david.stroebel@ens.fr

Strube Caroline
CRN2M
UMR 7286 Faculté de médecine Nord
13015 Marseille - France
Tel: +334 91698983 - Fax: +334 91688977
caroline.strube@univ-amu.fr

Szabo Ildiko
Department of Biology, University of Padova
viale G. Colombo 3.
35121 Padova - Italy
Tel: 00390498276324 - Fax: 00390498276300
ildi@civ.bio.unipd.it

Szponarski Wojciech
INRA
BPMP INRA-SupAgro 1 Place Viala
34060 Montpellier - France
Tel: 06 22 59 43 93 - Fax:
szponarski@supagro.inra.fr

Thibaud Jean-Baptiste
BPMP
Campus INRA-SupAgro place Viala
34060 Montpellier cedex 2 - France
Tel: 0499612609 - Fax: 0467525737
thibaud@supagro.inra.fr

Tian Meilin
IBENS / CNRS
46, rue d'Ulm
75005 Paris - France
Tel: 0144323892 - Fax:
mtian@biologie.ens.fr

Trebacz Kazimierz
University Maria Curie-Sklodowska of Lublin (Poland)
Akademika 19
20-033 Lublin - Poland
Tel: 815375931 - Fax: 815375902
kazimierz.trebacz@poczta.umcs.lublin.pl

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

Vandier Christophe
University of Tours
Bâtiment Dutrochet 10 boulevard Tonnelle
37032 Tours - France
Tel: 33247366024 - Fax: 3324736226
christophe.vandier@univ-tours.fr

Vignes Michel
UMR5247 IBMM Université Montpellier 2
Laboratoire 'Stress Oxydant et neuroprotection'
CC 090 Université Montpellier 2
34095 Montpellier - France
Tel: 0467143699 - Fax: 0467144251
michel.vignes@univ-montp2.fr

Vivaudou Michel
Institut de Biologie Structurale
IBS, 71 Avenue des Martyrs, CS 10090
38044 Grenoble - FR - FRANCE
Tel: +33 4 57 42 85 85 - Fax:
vivaudou@ibs.fr

Zanou B. Nadege
Université catholique de Louvain
Avenue Hippocrate 55.12 B1
1200 Bruxelles - Belgium
Tel: 003227645594 - Fax: 003227645580
nadege.zanou@uclouvain.be

SPONSORS-EXHIBITORS-AVERTISERS

……

LABEX ISCT
Laboratoire d'Excellence - Ion Channel Science and Therapeutics
Scientific and technical manager: Florian Lesage
Institut de Pharmacologie Moléculaire et Cellulaire
CNRS UMR 7275 Université de Nice Sophia Antipolis
660, route des lucioles
F-06560 Valbonne

……

B'SYS GmbH
Benkenstrasse 254
CH-4108 Witterswil
Switzerland

DUTSCHER s.a.
Jocelyne Cachat
30, rue de l'industrie
BP 62
67172 Brumath
France

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

NANION TECHNOLOGIES
GmbH
Gabrielenstrasse 9
D-80636 Munich
Germany

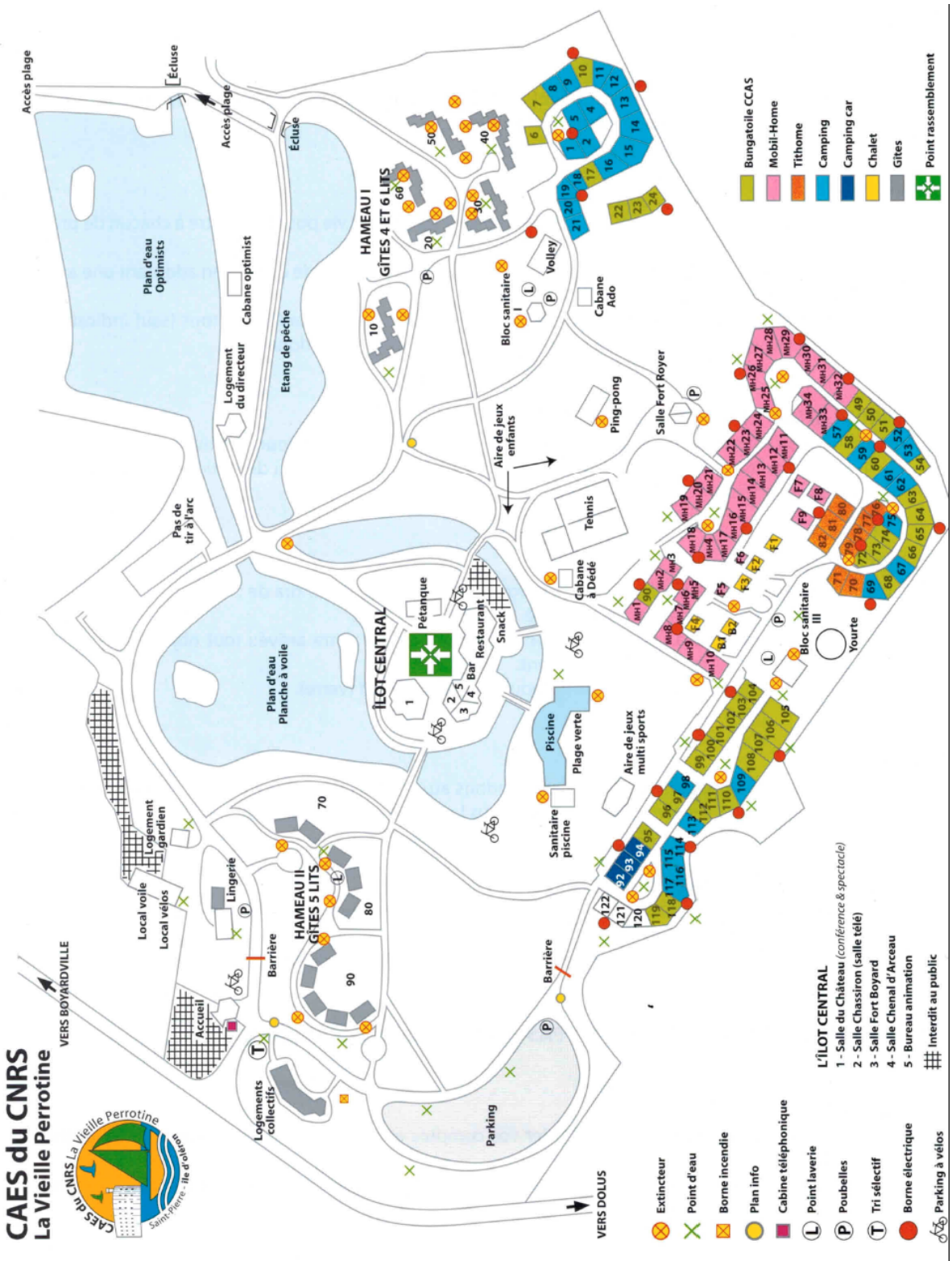
SARSTEDT S.A.R.L.
Route de Gray
Z.I. des Plantes
70150 Marnay
France

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

TEBU BIO Sas
39 Rue de Houdan
78610 Le Perray En Yvelines
France

VWR INTERNATIONAL S.A.S
Le Périgares - Bâtiment B
201, rue Carnot
94126 Fontenay-sous-Bois
France

WORLD PRECISION INSTRUMENTS
Mikael Berrou | Market Manager Be,Fr,Es
World Precision Instruments
1 Hunting Gate
SG4 0TJ Hitchin
United Kingdom


CAES du CNRS
La Vieille Perrotine
VERS BOYARDVILLE
VERS DOLUS
Accès plage
Écluse
Plan d'eau Optimists
Cabane optimist
Logement du directeur
Etang de pêche
Pas de tir à l'arc
Plan d'eau Planche à voile
ÎLOT CENTRAL
Pétanque
Bar
Restaurant
Snack
HAMEAU I
GÎTES 4 ET 6 LITS
Bloc sanitaire I
Volley
Cabane Ado
Aire de jeux enfants
Ping-pong
Salle Fort Royer
Tennis
Cabane à Dédé
Piscine
Plage verte
Sanitaire piscine
Aire de jeux multi sports
Bloc sanitaire III
Yourte
Local voile
Local vélos
Logement gardien
Lingerie
Accueil
Barrière
HAMEAU II
GÎTES 5 LITS
Logements collectifs
Parking
Extincteur
Point d'eau
Borne incendie
Plan info
Cabine téléphonique
Point laverie
Poubelles
Tri sélectif
Borne électrique
Parking à vélos
L'ÎLOT CENTRAL
1 - Salle du Château (conférence & spectacle)
2 - Salle Chassiron (salle télé)
3 - Salle Fort Boyard
4 - Salle Chenal d'Arceau
5 - Bureau animation
Interdit au public
Bungatoile CCAS
Mobil-Home
Tithome
Camping
Camping car
Chalet
Gites
Point rassemblement