The 20th Ion Channel Meeting
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The French Ion Channel society has existed since 1989 and its main goal is to annually organize a scientific meeting. This meeting, which gathers young and senior French scientists, provides a great opportunity for exchange and interaction among the ion channel research community. Additionally, for many years, the French ion channel meeting has attracted a significant number of scientists from different European countries, promoting the discussion of new insights and advances, as well as aiding in the establishment of collaborations. In this report, we summarize the five symposia selected for their novelty and importance in human channelopathies, neuroplasticity, ion channel regulations, intracellular ion channels and plant physiology.

Introduction

The French Ion Channel society celebrated its 20th anniversary during its annual meeting held on the Mediterranean coast of France (Giens Peninsula) from the 20th to the 23rd of September 2009. Initially, the main goal of this annual meeting was to resemble young and senior researchers from France who investigate the role, regulation, and the function of ion channels. Additionally, for many years, the French Ion Channel meeting has gathered a significant number of scientists from different European countries, elevating the discussion of new insights and advances in this particular field. In 2009, 150 participants from ten countries (Great Britain, Switzerland, Germany, Italy, Belgium, Denmark, USA, Canada, Portugal and France) participated in the success of the 20th Ion Channels Meeting which included a plenary lecture from W.A. Catterall, 29 oral presentations and 42 poster communications. In this report, we summarize the five symposia selected for their novelty and importance in human channelopathies, neuroplasticity, ion channel regulations, intracellular ion channels and plant physiology.

Plenary Lecture: Sodium and Calcium Channels, Electrical Signalling and Neuroplasticity

The plenary lecture from W.A. Catterall (University of Washington, Seattle, WA USA), entitled “Sodium and Calcium Channels, Electrical Signalling and Neuroplasticity” gave an overview of the most recent insights on the sodium channel structure and the involvement of Na+ and Ca2+ channels in neuronal plasticity. In the first part of his talk, the structural features of Na+ channel activation were discussed. His last set of data, based on electrophysiological experiment with scorpion toxins and modelling, showed that the structure of S3-S4 segments remains at the cell surface, reinforcing the validity of the sliding helix model of gating. Next, he presented on the regulation of brain Na channels by cAMP-dependent protein-kinase (PKA) bound by A Kinase Anchoring Protein-15 (AKAP15) and by protein kinase C-epsilon (PKCe) associated via a Receptor for Activated C Kinase (RACK). Interestingly, Dr. Catterall showed the synergetic and voltage-dependence of the inactivation of Na+ channels regulation by PKA and PKCe, making Na+ channels unavailable for activation by depolarization. In addition, during trains of action potentials, the progressive entry of Na+ channels into a slow-inactivated state is stimulated by the phosphorylation process. This mechanism provides an unexpected form of cellular plasticity, which controls the frequency and pattern of neuronal firing in response to G protein-coupled receptors and protein phosphorylations. The last part of this plenary lecture concerned the regulation of Ca2.1 channels by binding of calmodulin (CaM) to the C-terminal domain, containing an IQ-like motif and a CaM binding domain (CBD). Calcium regulation of P/Q-type Ca2+ currents consist of facilitation and depression of synaptic transmission. Moreover, Ca2+ and voltage-dependent regulation of Ca2.1 channels through interaction with Ca2+ sensor proteins contributes substantially to short-term synaptic plasticity. W.A. Catterall highlighted that two complementary forms of neuronal plasticity co-exist: a cellular plasticity of action potential firing and a short-term plasticity of synaptic transmission. Both result from regulation of Na+ and Ca2+ channels by distinct signaling complexes.

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Symposium I: “Neurons, Ion Channels and Human Disorders”

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The first session illustrated the contribution of neuronal ion channels in human disabilities ranging from monogenic diseases to complex behaviors. The first speaker was Philippe Marambaud (The Feinstein Institute for Medical Research, North Shore, Manhasset, NY USA), who presented the novel Ca\(^{2+}\) channel CALHM1 (for calcium homeostasis modulator 1) as a risk factor for late-onset Alzheimer’s disease (AD). The variant P86L of CALHM1 is genetically-associated with AD in case-control studies. P. Marambaud demonstrated that CALHM1 is a homotetrameric transmembrane glycoprotein that generates calcium selective cation currents at the plasma membrane in transfected cells. He further showed that CALHM1 expression negatively controls amyloid β (Aβ) levels in APP-transfected mouse neuroblastoma N2a cells. According to the association of P86L with AD, the P86L variant promoted Aβ accumulation by interfering with CALHM1-mediated Ca\(^{2+}\) permeability. CALHM1 may therefore be considered as a new target for clinical management of AD.

Jean Christophe Poncer (Inserm and UPMC, Institut du Fer à Moulin, Paris) described the functional impact of human mutations in the γ2 subunit of the GABA\(_A\) receptor, associated with generalized epilepsy syndromes with febrile seizures. When expressed in hippocampal neurons, the K289M mutation accelerates the decay of synaptic currents without modifying the membrane trafficking or synaptic aggregation of γ2. In contrast, the R43Q mutation specifically reduces tonic currents evoked by low concentrations of GABA, reflecting decreased membrane expression of α5-containing extrasynaptic receptors. Therefore, two mutations affecting the same gene lead to a very similar epilepsy syndrome yet by affecting distinct modes of GABAergic signaling. J.C. Poncer then carried on with an exquisite analysis of the membrane dynamics of K289M mutant using molecular imaging. He showed the K289M mutation reduces the dwell time of GABA\(_A\) receptors at the synapse and increases its endocytosis, specifically in conditions of increased neuronal activity such as hyperthermia. This phenotype might further promote seizures that occur during febrile episodes. This talk was a beautiful example that refined characterization of monogenic mutations is still required to develop pertinent pharmacotherapies for genetically homogeneous diseases.

Steven N. Treistman (Institute of Neurobiology, University of Puerto Rico, San Juan, Puerto Rico) spoke about the implication of the calcium- and voltage-gated BK channel in alcohol acute tolerance. He showed that a neuronal cell body responds to alcohol by a mechanism that simultaneously downregulates BK channel gene expression and switches splice variants from alcohol-sensitive to alcohol-insensitive α subunit of BK channel, the latter involving the expression of mi-R9. Persistence of striatal neuron alcohol tolerance is also dependent upon pattern of ethanol presentation. Dr. Treistman showed that while 1 or 3 hours of alcohol exposure led to tolerance that was reversed within 5 hours of withdrawal, a 6-hour exposure time to 20 mM ethanol (legal intoxication) led to persistent insensitivity of striatal neurons to ethanol for at least 24 hr, probably as a result of changes in BK isoform composition. Finally, Dr. Treistman showed that the physical properties of lipid bilayers, such as thickness, modify the basic gating and ethanol sensitivity of the BK channel and then alter the time course of the ethanol response of the BK channel. Acute tolerance of the BK channel may then result from different actions of alcohol on multiple excitatory and inhibitory sites, underlying the complexity of the individual response to alcohol drinking.

The first symposium ended with Jacques Barhanin (CNRS and Université de Nice Sophia Antipolis, Valbonne Sophia Antipolis, France), who showed evidence for a key role of Task2 K\(^+\) channels in setting central respiratory CO\(_2\) and O\(_2\) sensitivity. J. Barhanin and collaborators demonstrated that Task2 expression in the central nervous system is strictly restricted to a few brainstem nuclei, particularly to the retrorubral/parafacial region, which is a chemosensitive structure. Task2-/- mice had lost the long-term hypoxia-induced respiratory decrease in plethysmographic experiments and showed hypersensitivity to low CO\(_2\) concentrations leading to hyperventilation. Finally, Task2 currents were activated by reactive oxygen generated during hypoxia in COS7 cells in vitro. Altogether, these data argue for a role of TASK2 channels as central O\(_2\) sensors.

Symposium II: “Intracellular Ion Channels”

Jean-Luc Morel
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The topic of this session was the regulation of intracellular Ca\(^{2+}\) concentration via mobilization of intracellular calcium stores. The first part of the session focused on the nicotinic acid adenine dinucleotide phosphate (NAADP) pathway. Andreas Guse (University Medical Centre, Hamburg-Eppendorf, Germany) explained that NAADP was synthesized in T cells via TCR/CD3 stimulation and was acting as a calcium releasing factor. NAADP was able to induce localized as well as propagated calcium signals in T cells. The target of NAADP was localized on intracellular compartments, probably on the endoplasmic reticulum. The ryanodine receptor 1 (RYR1) could be the receptor of NAADP as it increased the binding of \[^{3}H\]ryanodine. Furthermore, BZ194 that inhibited the NAADP-dependent calcium signals also decreased the binding of \[^{3}H\]ryanodine on RYR1 expressed in T cells.

The second speaker, Antony Galione (Department of Pharmacology, University of Oxford, GB) stated that the NAADP-activated receptor remained enigmatic during a long period of time. His group showed very recently (work published...
in 2009) that a two-pore channel (TPC) localized on acidic organelles but not in the endoplasmic reticulum was this receptor. This channel, a member of the voltage-gated cation channel family, consisted of 12 transmembrane domains organized in two homologous parts similar to the transmembrane domains of Ca²⁺ and Na⁺ voltage channels.

Both speakers further developed the pharmacological approach of NAADP pathway that was essential to investigate the function of NAADP receptor in physiological functions. They are both associated with the discovery of small organic molecules. BZ194 was used by the group of Andreas Guse: BZ194 was shown to inhibit the NAADP-dependent mobilization of calcium stores but neither interfered with other Ca²⁺ mobilization by d-myo-inositol 1,4,5-trisphosphate or cyclic ADP-ribose, nor with capacitative Ca²⁺ entry. The second molecule was Ned-19, used by the group of A. Galione: Ned-19 inhibits both NAADP-mediated calcium release and NAADP binding. In intact T cells, Ca²⁺ mobilization evoked by NAADP was implicated in cellular translocation of the “nuclear factor of activated T cells” (NFAT), as well as T cell receptor-driven interleukin-2 production, and proliferation of antigen-experienced CD4⁺ effectors T cells. All of these steps in immunological synapse establishment between primary effector T cells and astrocytes were inhibited by BZ194. For these reasons, NAADP antagonists constitute an interesting way to specifically and effectively modulate T-cell activation and have a high potential for the therapy of autoimmune diseases.

The knowledge of the RYR1 channel is particularly important because it is expressed in skeletal muscle and is one of the most important molecular actors of the excitation-contraction coupling. Paul Allen (Harvard Medical School, Boston, USA) and collaborators explore the implication of RYR1 in the physiopathology of skeletal muscle and increase the knowledge on RYR1. Firstly, P. Allen reviewed the interaction between RYR1 and the voltage-gated L-type Ca²⁺ channel family, consisting of 12 transmembrane domains organized in two homologous parts similar to the transmembrane domains of Ca²⁺ and Na⁺ voltage channels.

symposium III: “Ion Channels in Plants”

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This symposium presented different roles of ion channels in plants: electric signaling (a weakly explored field in plants), Ca²⁺ signaling upon plant interaction with microorganisms, and roles of plant homologs of cyclic nucleotide-gated channels and glutamate receptors. First, Patrick Favre from the University of Fribourg (Switzerland) spoke about electric signaling in plants using two examples, the primitive liverwort Conocephalum conicum and the higher plant Arabidopsis thaliana (a model plant in molecular physiology and functional genomic studies). Although action potentials (AP) have been recorded in plants (e.g., in the carnivorous Venus flytrap plant) for more than a century, plant excitability has not been extensively evaluated. Dr. Favre showed, using methods allowing AP recordings in Arabidopsis with high reproducibility, that in A. thaliana as well as in C. conicum, AP can be triggered by different stimuli (e.g., light, voltage pulses, wounding and local change in KCl concentration). Detailed analysis of AP in the two plants revealed a similar propagation velocity (close to 1 mm min⁻¹), but a refractory period longer in Arabidopsis. These advances in AP recording in Arabidopsis open the way to molecular identification of channels involved in AP and to a better understanding of the role of electric signaling in plant physiology.

Giles Oldroyd from the John Innes Centre (Norwich, UK) talked about Ca²⁺ signaling upon the establishment of symbiosis in legume plants. Most plant species have developed beneficial interactions with microorganisms that facilitate the uptake of mineral nutrients. Legume plants can form symbiotic interactions with both mycorrhizal fungi and rhizobial bacteria. Genetic dissection of the pathways involved in the recognition of symbiotic signals and establishment of symbiotic interactions in the legume Medicago truncatula, revealed a number of conserved genes. A calcium- and calmodulin-dependent protein kinase (CaMK) was shown to be required in this common symbiosis pathway and oscillations in calcium were observed. Dr. Oldroyd showed that the nature of the calcium oscillations differs between rhizobial and mycorrhizal interactions, not in frequency but in the shape of the oscillations, potentially allowing CaMK to decode the calcium signals. Observation of co-localization of CaMK and calcium oscillations at the nucleus further supports this hypothesis.

Dominique Roby from the Laboratoire des Interactions Plantes-Microorganismes (UMR INRA/CNRS, Castanet Tolosan, France) spoke about the role of cyclic nucleotide-gated...
channels in plant defense against bacterial pathogens. Plants possess a large family of cyclic nucleotide-gated channels (e.g., 20 members in Arabidopsis), still weakly characterized but expected to work as Ca\textsuperscript{2+}/cation channels regulated by cyclic nucleotides and calmodulin, as in their animal homologs. Loss of function mutants in two Arabidopsis CNGC genes, CNGC2 and CNGC4, have been shown to be affected in the hypersensitive response (HR, characterized by rapid death of plant cells in contact with pathogens) and/or to spontaneously develop HR-like lesions in the absence of a pathogen attack. Dr. Roby presented a genetic approach enabling the placement of CNGC2 and CNGC4 in the pathways leading to HR/resistance. In addition, expression studies showed that the two CNGC genes are differentially regulated and dependent on the different signaling pathways, suggesting a distinct and complex role of the two CNGC genes in the defense response of plants to bacterial pathogens.

The “plant session” ended with Erwan Michard (Instituto Gulbenkian de Ciência, Oeiras, Portugal) who presented his recent results on the role of plant homologs of animal ionotropic glutamate receptors (GLR) in Ca\textsuperscript{2+} influxes in pollen. An apical, oscillating gradient of cytosolic Ca\textsuperscript{2+} is known to control the morphogenesis of the pollen tube, which extends in a polarized way from the pollen grain cell to carry the male gametes to the ovule. E. Michard gave pharmacological and genetic evidence for a role of plant GLR in pollen tube morphogenesis through an involvement in the building of the apical Ca\textsuperscript{2+} gradient. He showed that Arabidopsis pollen tubes in a mutant lacking expression of a plant GLR displayed apical Ca\textsuperscript{2+} oscillations with much lower amplitude than that in wild-type plants, together with an aberrant growth. Evidence for a possible agonist of such pollen GLR was also presented.

**Symposium IV: “Breakings News”**

**Sébastien Roger**

Inserm U921 “Nutrition, Croissance et Cancer”; Université de Tours; France

The aim of this session was to highlight the importance of recent findings on different types of ion channels. The first presenter, Pr. Thomas Voets, from the University of Leuven (Belgium) focused his talk on Thermo- and Chemo-sensing TRPs and mainly on cold-activated TRPM8 and TRPA1. A model for TRPM8 gating by membrane voltage, cold temperature and by chemical agonists such as menthol was proposed. This channel was shown to be involved in peripheral cold, but not noxious, sensing. While the involvement of TRPA1 in cold-sensing has been debated at length, T. Voet showed that they are gated by “noxious” temperature with a colder threshold than the one observed for TRPM8. They are involved in particular responses to cold in trigeminal neurons and in cold-induced pain. His group recently showed that TRPA1 are also directly activated by nicotine, which could be responsible for the sensory effects of this molecule.

The second speaker, Luis Galietta from the Laboratory of Molecular Genetics, Gaslini Institute, in Genova (Italy) presented his recent findings on the identification of TMEM16A as a Ca\textsuperscript{2+}-dependent chloride channel. In epithelial cells, Ca\textsuperscript{2+}-dependent chloride channels represent a route for chloride secretion together with other chloride channels. While they have been studied for many years, their molecular identity was controversial. His team found that treatment with IL-4 caused an upregulation of calcium-dependent chloride secretion in polarized bronchial epithelial cells through transcriptional effects. Indeed TMEM16A was identified from RNA micro-arrays and characterized as being a plasma membrane protein responsible for Ca\textsuperscript{2+}-dependent chloride currents. Different isoforms of TMEM16A, generated by alternative splicing, have been identified and showed differences in tissue expression and electrophysiological properties.

The last invited speaker of the session, Alan North from the University of Manchester (United Kingdom), talked about his recent identification, cloning and functional studies concerning ATP-gated P2X receptors in single-cell eukaryotes such as the amoeba Dictyostelium discoideum, a photosynthetic alga Ostreococcus tauri, and a choanoflagellate Monoisga brevicollis. Sequences from these newly discovered P2X receptors were compared to those from the trematode worm Schistosoma mansoni, and the vertebrate Rattus norvegicus, and used as the basis for structure-function studies concerning the second transmembrane domain (TM2) of P2X2 receptors. This particular segment is involved in the ion permeation pathway. This analysis is associated with results from site-directed mutagenesis led to the identification of key residues involved in P2X2 gating and ion permeation. A. North presented a schematic for channel opening evoked by ATP.

**Symposium V: Post-transcriptional Modification of Ion Channels and Protein Interaction**

**Hélène Vacher**

Inserm U641; Université de la Méditerranée; Marseille, France

This symposium considered the recent findings of how post-transcriptional modifications and protein-interactions of ion channels can modulate their functions, trafficking properties and membrane endocytosis. The first speaker, James S. Trimmer from the University of California, CA USA, gave a comprehensive overview of how his applied, mass spectrometric-based approaches allowed for identification and quantification of phosphorylation at specific sites on native voltage-dependent K\textsuperscript{+} (K\textsubscript{v}) and Na\textsuperscript{+} (Na\textsubscript{v}) channels, purified from mammalian brain using specific monoclonal antibodies. From his recent results, he showed that, in general, commonly used phosphorylation site-prediction algorithms did not accurately predict these novel in vivo phosphorylation sites. He also illustrated, via several examples, that changes in phosphorylation states at these identified sites impact diverse aspects of K\textsubscript{v} channel biology, including intracellular trafficking, clustering at discrete sites in the neuronal membrane, and channel gating. His studies revealed that phosphorylation provided dynamic functional variability to the
already diverse family of neuronal ion channels whose function underlies neuronal excitability.

Ubiquitylation is a common protein modification that leads to a number of outcomes, including protein degradation, endocytosis, sorting and trafficking of transmembrane proteins. Daniela Rotin, our second invited speaker (The Hospital for Sick Children and University of Toronto, Toronto, Canada) showed that the ubiquitine ligase, Nedd4-2, ubiquitinates the epithelial sodium channel (ENaC) at the plasma membrane and promotes its internalization. Nedd4-2 binds ENaC PY motif via its WW domains. In Liddle syndrome, a hereditary hypertension caused by excessive ENaC cell-surface numbers and activity, this internalization is impaired due to the inability of the mutated PY motif in ENaC to properly bind Nedd4-2. Recently, her team focused its work on studying in vivo functions of the Nedd4 family members. After generating the knockout of Nedd4-1, Rotin observed that the lack of Nedd4-1 in mice leads to heart defects, as well as impaired signalling downstream of tyrosine kinase receptors. She also discussed the effect of knocking out Nedd4-2, specifically in the lung. Her presentation highlighted the crucial role of Nedd4 ubiquitine ligases in regulating ion channel endocytosis and specific signalling pathways, and their link with a number of human diseases.

Finally, the last invited speaker, Stefan Gründer from the Department of Physiology, RWTH Aachen University, Germany, discussed the functional role of N-glycans on the acid-sensing ion channels (ASICs) 1a and 1b, two closely related proton-gated channels. ASIC1a and ASIC1b are derived from the asic1 gene, probably by using alternative promoters. ASIC1a has two consensus sites for N-linked glycosylation in the distal ectodomain, which are common to ASIC1b. In addition to these two distal sites, ASIC1b has two consensus sites for glycosylation in the proximal ectodomain. S. Gründer showed that the two common distal sites are necessary for the full activity and normal proton affinity of ASIC1a and 1b, but that they are not absolutely required for channel activity. Apparently, ASIC1a, devoid of any glycan, can fold properly, assemble into oligomeric channels, traffic to the cell surface, and form functional proton-gated channels. In sharp contrast, the presence of one of the proximal glycans, which is specific to ASIC1b, is an absolute requirement for surface expression of ASIC1b. Gründer assumed that these proximal glycans aided in the proper folding of ASIC1b. If this assumption were correct, this would imply that the structure of the proximal ectodomain of ASIC1b was substantially different from that of ASIC1a.

This year, the French Ion Channels Society will hold its 21st annual meeting at the Belambra resort on the Giens peninsula (French Riviera Côte d’Azur) from the 12th to 15th of September 2010. The 2010 program symposia will focus on ion channels and toxins, transporters and pathologies, excitability and neuronal integration, aquaporins from micro-organisms to mammals and membrane dynamics of ion channels.

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