The 22nd Ion Channel Meeting, September 2011, France

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Keywords: ion channels, cell death, membrane excitability, extracellular matrix, channel regulation, synaptic plasticity, ion channel distribution, channelopathies

The 22nd Ion Channel Meeting was organized by the French Ion Channel Society (Association Canaux Ioniques) from the 25th to the 28th of September 2011 on the French Riviera (Gien). This year, again, more than one hundred researchers from France, Europe and extra-European countries gathered to present and discuss their recent advances and future challenges in the ion channels and transporters field. The scientific committee organized a plenary lecture and five thematic symposia by inviting international researchers to present their recent outstanding work on themes as diverse as muscular channelopathies, regulation of channels by extracellular matrix, receptor-channels interactions, localization and distribution of ion channels, their involvement in the cell life and death, and finally how they participate in the evolution and adaptability of cellular excitability. These presentations are summarized in this meeting report. Two sessions of oral communications selected from submitted abstracts and two poster sessions were also organized to present the ongoing work of young researchers worldwide.

Introduction

The French Ion Channel Society (Association Canaux Ioniques) held its 22nd meeting from the 25th to the 28th of September 2011. As every year, this conference brought together more than 100 researchers on the Gien peninsula coming from France, the United States, Japan, Germany, Italy, the United Kingdom, Canada, Switzerland, Poland, Ireland and Belgium. This meeting provides a unique opportunity of discussion between senior researchers and Ph.D., students or postdoctoral researchers like in a summer school. All attendees gathered in a pleasant resort by the sea for three days and housed at the conference site with special attractive low-fees proposed for students. The meeting was organized around one plenary lecture given by an international specialist, five symposia involving young and senior researchers, two sessions of oral presentations of selected abstracts and two poster sessions. This report will focus on the plenary lecture and the five thematic symposia.

Plenary Lecture

Channelopathies of skeletal muscle. The plenary lecture from Karin Jurkat-Rott (Division of Neurophysiology; Ulm University; Germany) put into perspective the clinical observations that have defined skeletal muscle myopathies with molecular studies that helped define the molecular and electrophysiological parameters affected in these diseases. These channelopathies show how molecular modifications induced by mutations cause changes in the electrophysiological properties of ion channels, finally resulting in a particular physiological dysfunction. Karin Jurkat-Rott explained how hereditary muscle channelopathies with weakness are caused by mutations that alter the electrical bistability of the muscle fiber membrane. Bistability means that the resting membrane potential of normal fibers can jump between two stable values P1 and P2. While P1 follows the predictions of the Goldman-Hodgkin-Katz equation (-83 mV at 4 mM [K+]o and -99 mV at 1 mM [K+]o), P2 is about -60 mV and its value (but not its frequency) is largely independent of [K+]o. P1 fibers are excitable and can generate action potentials initiating contraction. In contrast, P2 fibers are unexcitable and paralyzed. At the resting potential, the hyperpolarizing currents (outward K+, inward Cl-) outbalance the depolarizing inward Na+ current. Therefore weakness may be caused by (i) reduction of the hyperpolarizing Cl- current (transient weakness in myotonia congenita) (ii) increase of depolarizing Na+ current aberrantly as in hypokalemic periodic paralysis, or through the pore as in hyperkalemic periodic paralysis and paramyotonia congenita (iii) reduction of the hyperpolarizing Kir2.1 K+ current as in the Andersen-Tawil Syndrome. An increase in the P2 fraction due to an enhanced Na+ inward current results in an intracellular Na+ accumulation and edema as shown by 23Na-MRI and by fat-suppressed 1H-MRI in-vivo. Aldosterone antagonists and carbonic anhydrase inhibitors are able to both shift P2 to P1 and to reduce the Na+ and H2O accumulation, thereby recovering muscle strength. This lecture clearly showed how ion channel mutations can modify...
electrophysiological properties in vivo leading to pathological phenotype, thus helping to propose therapeutic perspectives.

Symposium I

“Regulations of ion channels by the extracellular matrix” organized by Sabine Lévi (Institut du Fer à Moulin, UMR839, INSERM-UPMC, Paris, France). This symposium highlighted the key role of extracellular matrix (ECM) components in the regulation of ion channels and their physiological consequences. First, Alexander Dityatev (Department of Neuroscience and Brain technologies, IIT, Genova, Italy) showed the importance of tenascin C and hyaluronic acid, two key ECM molecules, in synaptic plasticity involving L-type voltage-dependent Ca2+ channels. First, he reported the genetic deletion of tenascin-c or the enzymatic removal of hyaluronic acid with hyaluronidase reduced long-term potentiation (LTP) at CA3-CA1 synapses. Furthermore, removal of hyaluronic acid impaired the activity of L-type voltage-dependent Ca2+ channels, prevented somatic translocation of the phosphorylated extracellular signal-regulated kinases ERK1 and ERK2 in area CA1, prevented activation of the cyclic AMP-responsive element-binding protein (CREB), and finally reduced hippocampus-mediated contextual fear conditioning. The ECM also regulates synaptic efficacy by acting on receptor surface trafficking. Using single particle tracking (SPT), Renato Frischknecht (Leibnitz Institute for Neurobiology; University of Magdeburg; Germany) reported that the perineuronal net, a meshwork of ECM glycoproteins and proteoglycans, reduces lateral diffusion of AMPA receptors in hippocampal neurons. Preventing lateral diffusion of AMPA receptors induced paired-pulse depression (PPD), a phenomenon that was blocked by hyaluronidase treatment. The perineuronal net is mainly associated with parvalbumin-positive GABAergic interneurons. He then showed a selective increase in the synaptic mobility of AMPA receptors on aspiny neurons. The regulation of ion channels by the ECM is not restricted to neurons. Annarosa Arcangeli (Department of Experimental Pathology and Oncology; University of Florence; Firenze, Italy) highlighted the functional interaction between β1 integrin and K+ channel in tumor cells. The K+ channel K11.1 also known as hERG1 is aberrantly expressed in several human cancers where it controls different aspects of the neoplastic cell biology. Using immunoprecipitation and fluorescence resonance energy transfer (FRET) microscopy, her group clearly demonstrated direct interaction between β1 integrin and hERG1 channels in living cells. This complex is localized at the plasma membrane of tumor cells. The development and analysis of mutants allowed them to conclude that intracellular epitopes of both the β1 integrin and the hERG1 channel are involved in mediating the complex formation. The identification of these epitopes represents an important step toward design and production of therapeutic molecular tools, such as bifunctional antibodies, capable of targeting, and possibly, unlocking the complex. Finally, David Crottès (Institut de Biologie du Développement et Cancer; Nice, France) represented the regulation of hERG-β1 integrin interaction by the sigma1 receptor. The silencing of sigma1 receptors decreased hERG current. This was correlated with a decrease in the mature form of hERG and a reciprocal increase in the immature form of the channel. Therefore, the Sigma1 receptor controls leukemic cell adhesion by regulating hERG post-translational expression. This may represent a therapeutic target to reduce the activity of membrane signaling channel macrocomplexes involved in cancer progression.

Symposium II

“Direct cross talk between ion channels and receptors: just a kiss?” organized by Laurent Groc (Institut Interdisciplinaire de Neurosciences, UMR5297 CNRS-Université de Bordeaux; Bordeaux, France). The goal of this symposium was to gather prominent neuroscientists to discuss our current understanding of the functional interplay between membrane neurotransmitter receptors in neurons. The first speaker was Fang Liu (CAMH; University of Toronto; Canada), who discussed the multiple functions of the complex composed of G-protein coupled dopamine D1 and ionotropic glutamate NMDA receptors. Based on the pioneering work from her laboratory, F. Liu emphasized the functional role of the D1-NMDA receptor complex in cellular brain processes, such as synaptic plasticity, and in cognitive functions, such as the working memory in rodents. She convincingly demonstrated that the formation of such a D1/NMDA receptor complex regulates the interplay between different neurotransmitter systems, i.e., glutamatergic and dopaminergic signaling. The second speaker, Mike Edwardson (University of Cambridge; Cambridge, UK), presented fascinating work from his laboratory on the determination of the architecture of receptors and ion channels using atomic force microscopy (AFM) imaging. This method gives access to the determination of the arrangement of subunits within multimeric proteins, such as ionotropic receptors and ion channels. In his presentation, M. Edwardson focused on the structure of an ionotropic receptor, i.e., the P2X receptor, and an ion channel, i.e., the epithelial sodium channel (ENaC). He showed convincing evidence that in addition to assembling as trimers, both proteins form higher-order structures, shedding new light on the membrane organization of native receptors. The third speaker was Julie Perroy (CNRS IGF; Montpellier, France). She presented exciting work on the remodeling of the postsynaptic scaffold during long-term potentiation. Specifically, she reported that the postsynaptic protein, Homer, is dynamically tuned in synapses, and such assembly/disassembly governs physical and functional crosstalk between the glutamate ionotropic NMDA and metabotropic mGluR5 receptors. This astonishing data clearly indicate that the direct interaction between membrane receptors, and their subsequent intracellular signaling, is physiologically regulated by the presence of interacting scaffold proteins. The fourth, and last speaker of the symposium, was a selected presentation of Thomas Boulin (ENS; Paris, France). He presented his recent data, obtained in the laboratory of Dr. Bessereau, on the allosteric modulation of an ionotropic acetylcholine receptor by a novel transmembrane protein, MOLO-1. This protein is a transmembrane protein with a conserved extracellular DUF477 domain, and is localized in neuromuscular
Symposium III

“Ion channels in localized subcellular domains” organized by Massimo Mantegazza (IPMC, LabEx ICST, UMR 7275 CNRS-Université de Nice Sophia-Antipolis; Valbonne, France). The topic of this symposium was on mechanisms of subcellular distribution of voltage-gated ion channels and on their functions in these domains. The first two talks focused on two neuronal subcellular domains: dendrites and the axon initial segment (AIS). Dendritic voltage-gated ion channels can boost post-synaptic potentials generated by synaptic inputs and are important for generating dendritic action potentials, which in turn can modulate synaptic inputs and plasticity. Synaptic inputs pre-integrated in dendrites undergo a final integration and are converted into frequency-modulated action potential discharges in the AIS, where there is a high density of sodium (Na) and potassium (K) channels, which then transfer information forward to synaptic terminals and back-propagate into dendrites.

Heinz Beck (Department of Epileptology; University of Bonn Medical Center; Bonn, Germany) presented a study of the integrative properties of the dendrites of the principal cells of the hippocampus, done using a combination of dual somatodendritic patch-clamp recordings and multiphoton glutamate uncaging. Several principal neurons display forms of active dendritic signal propagation, mediated by precisely regulated levels of different voltage-gated channels. In these dendrites, synchronous inputs can induce nonlinear integration modes directly triggering dendritic action potentials and thus overcoming dendritic voltage attenuation. The data showed that granule cell of the dentate gyrus exhibit a fundamentally different type of dendritic integration. In fact, because of very strong dendritic voltage attenuation, the impact of individual synapses on granule cell output is low. However, the integration is linearized by voltage-dependent Na and Ca channels, which can boost synaptic inputs, and it is only weakly affected by input synchrony and independent of input location. These experiments show that dentate granule cell dendritic properties are optimized for linear integration and strong attenuation of synaptic input, which may contribute to the sparse activity of granule cells in vivo. The talk of Bénédicte Dargent (CRN2M; UMR7286 CNRS; Marseille, France) was about the molecular organization of the AIS and mechanisms of localization of Na channels at the AIS. Na channels accumulate at the AIS by interacting with the scaffolding protein ankyrin-G (ank-G). Interestingly, four casein kinase-2 (CK2) phosphorylation sites are present in the ank-G binding site of Na channels, and CK2 accumulates in the AIS. Surface plasmon resonance experiments have shown that CK2 phosphorylation can strengthen the interaction between ank-G and Na channels. Moreover, western-blot and immunocytochemistry experiments with a phosphospecific antibody demonstrate that the serine 1112 in the Na ank-G binding site is phosphorylated in cultured hippocampal neurons. Other experiments have shed light on the molecular link between ank-G and axonal microtubules. End binding (EB) proteins were reasonable candidates because they accumulate in the AIS, in which they bind to microtubules and have low mobility. In fact, EB3 binds to ank-G with high affinity, as shown by surface plasmon resonance, recruits ank-G to microtubules in the COS cell line, and its knock-down with shRNAs downregulates ank-G and Na targeting at the AIS. Therefore, ank-G is essential for stabilizing the multi-protein AIS complex and anchors Na channels to axonal microtubules through EB proteins; its interactions with Na channels can be modulated by CK2. Hugues Abriel (Department of Clinical Research; University of Bern; Bern, Switzerland) showed that distinct macromolecular complexes determine membrane localization of Na,1.5 sodium channels in ventricular cardiomyocytes. These cells have at least three plasma-membrane sub-domains: intercalated disks, lateral sarcolemma and T-tubules; Na,1.5 is mainly targeted to intercalated disks and lateral sarcolemma. Dystrophin, a cytoplasmic protein important for connecting the cytoskeleton with the extracellular matrix, is found in macromolecular complexes with Na,1.5 and syntrophin, a dystrophin associated protein which binds to a PDZ binding motif (SIV) located in the C-terminus of Na,1.5. Dystrophin interaction with Na,1.5 depends on the C-terminal domain of the channel and is mediated by syntrophin. Cardiomyocytes from mdx mice, spontaneous dystrophin deficient mutants, show reduced Na,1.5 protein expression and sodium current. Interestingly, dystrophin and syntrophin are not found in intercalated disks and, consistently, Na,1.5 signal in immunocytochemistry experiments is reduced only in the lateral sarcolemma of mdx mice. Notably, the membrane-associated guanylate kinase (MAGUK) SAP97 is localized in the intercalated disks and interacts through its PDZ domains with the SIV motif of Na,1.5; silencing of SAP97 with shRNAs reduces sodium currents in cardiomyocytes. Therefore, the type of Na,1.5 macromolecular complex depends on the subcellular domain. The functional role of these differential interactions remains to be elucidated. Katell Fablet, Ph.D., student in Michel De Waard laboratory (Grenoble Institute of Neuroscience; Grenoble, France), showed that during neuronal development in vitro the Ca,β4 accessory cytoplasmic subunit can translocate into the nucleus, and analysis of gene expression profiles in lethargic (lh) mice, a spontaneous knockout model for Ca,β4, displayed deregulated levels in comparison with the wild type. In particular, tyrosine hydroxylase (TH) was strongly upregulated in lh mice, thus probably downregulated by β4. In fact, subsequent experiments have shown that β4 can interact in the nucleus with the thyroid receptor α (Trα) transcription factor, B56D (a regulatory subunit of protein phosphatase 2A), and, in the presence of B56D, with HP1γ (heterochromatin protein 1 gamma). This complex interacts with the TH promoter.
and downregulates its expression by inhibiting Trx action and by dephosphorylation of histone H3 and chromatin remodeling.

Symposium IV

“Evolution and adaptability of membrane excitability” organized by Jean-Marc Goaillard (INSERM U641; Université de la Méditerranée; Marseille, France). How did evolution shape the action potential (AP) and the function of the ion channels underlying it? Although working on very different models, the four scientists invited to speak in this symposium all addressed these questions that are central to our understanding of cellular physiology. Henrik Alle (Charité-Universitätsmedizin; Berlin, Germany) first presented his work investigating how the energy expenditure associated with APs is minimized in specific types of mammalian cortical neurons. He showed that the overlap between the opposing currents generated by the fast sodium and the delayed rectifier potassium channels is minimal in hippocampal mossy fibers because of the matching kinetics of these two channels. In addition to the optimization at the level of ion current kinetics, this type of axon also relies on potassium channels with gating properties that optimize their recruitment during the AP, such that a minimal number of potassium channels is needed to repolarize the axon’s membrane potential. Therefore, AP energy efficiency seems to rely on structural properties of single channels and on the association of channels with matching kinetics. Interestingly, as Jeremy Niven (University of Cambridge; Cambridge, UK) then demonstrated, energy efficiency is not common to all APs in the animal kingdom. Using computational approaches, he showed that, while the AP is optimized in hippocampal interneurons (as described by H. Alle), thalamocortical relay neurons and cerebellum granule cells; it is far from being optimal in cortical fast-spiking interneurons, squid giant axons, crab motor axons or honeybee Kenyon cells. Again, matching kinetics between the depolarizing and the hyperpolarizing currents involved in the AP seem to be the most critical factor, but minimizing the amplitude of the currents also helps to reduce the energetic cost of the AP. This lecture raised the question: why is energy efficiency optimized in some systems while being far from optimal in others? David McKinnon (Stony Brook University; NY USA) developed the idea that the fitness of the cardiac AP between mammalian species of growing size (from mouse to human) might be achieved essentially by regulatory evolution rather than structural evolution. Indeed, although the duration of the cardiac AP faithfully scales with body size, the properties of the underlying ion channels (including their kinetics, voltage-dependence, etc.) are essentially invariant between these different species. The increase in duration in larger species seems rather to be explained by the decrease in amplitude of several potassium currents involved in the repolarization of the AP. Analysis of the sequences (coding and non-coding) of the genes also demonstrated that most changes are located in the regulatory regions, not in the coding regions of the genes, confirming the hypothesis that evolution of cardiac AP in mammalian species relies on regulatory evolution rather than structural evolution. Finally, Tracy Ann Cuin (Institut de Biologie Intégrative des Plantes Claude Grignon; Montpellier, France) presented surprising data about the properties of APs in the plant Arabidopsis thaliana. Although little is known about the identity of the channels involved in the generation of the very slowly propagating AP in this plant species, she demonstrated that the properties of the AP significantly vary between different Arabidopsis ecotypes (populations adapted to specific ecosystems), due to changes in the activation state of two types of potassium channels, AKT2 and GORK. In summary, these four presentations underlined the importance of the regulation and the co-adaptation of ion channel properties for preserving information transfer or energy efficiency.

Symposium V

“Ion channels in cell life and death” organized by Fabrice Matifat (JE-2530 “Canaux Ioniques et Cancer du Sein,” UFR Sciences; Amiens, France). Over the past few years, an increasing number of ion channels has been shown to be involved in a range of physiological and pathological processes that regulate cell life and death. Among all of these channels, plasmalemmal Transient Receptor Channels (TRP) channels, mitochondrial ion channels and also intracellular Ins(1,4,5)P₃ receptors expressed in the endoplasmic reticulum were chosen to illustrate these phenomena. The 5th session on the 22nd Ion Channel Meeting was opened by Natalia Prévarskaya (Université de Lille 1; Lille, France) who presented the involvement of TRP channels in Ca²⁺ signaling in prostate tumors. Especially, she showed that TRPM8 can be expressed in short isoforms that can interact with the C-terminal loop of the full-size TRPM8 channel and modulate it. More precisely, she showed that the expression of the short isoform can affect conformational changes during channel activation, change its responsiveness to modulators (menthol and cold) and modify the temperature-dependence of the full-length channel. The second speaker was Kathleen W. Kinnally (New York University; NY USA) who gave an overview of the channels involved in the control of the mitochondrial membrane permeabilization that is a key step in cell death via either apoptosis or necrosis. On the basis of results obtained recently and due to the developments in the pharmacology of mitochondrial channels, the examination of the role of the Permeability Transition Pore (PTP) in the inner membrane and the Mitochondrial Apoptosis-induced Channel (MAC) in the outer membrane in cell death enabled a better understanding of the crosstalk between these two channels. Jan B. Parys (Katholieke University Leuven; Leuven, Belgium) presented on the newly discovered role of Ins(1,4,5)P₃ receptor in controlling cell apoptosis and autophagy through its molecular interaction with anti-apoptotic Bcl-2 protein. His very interesting results show that Bcl-2 inhibits Ins(1,4,5)P₃-induced Ca²⁺ release by binding to its BH4 domain in the regulatory domain of the Ins(1,4,5)P₃R. Interestingly, the anti-apoptotic protein Bcl-Xₐ, despite its functional and structural homology with Bcl-2, does not interact with the Ins(1,4,5)P₃R through its BH4 domain and therefore does not inhibit Ins(1,4,5)P₃-induced Ca²⁺ release. So, it appears that the modulation of the interaction between the Ins(1,4,5)P₃R and Bcl-2-family members may, through interference with the initiation of apoptotic or autophagic processes,
provide new possibilities for the treatment of cancer or neurodegenerative diseases. The 4th speaker, Christian Mazars (University of Toulouse; Toulouse, France), presented very interesting results concerning the triggering of programmed cell death in tobacco cells by the use of sphingolipids. These compounds are able to elicit both a burst of Reactive Oxygen Species (ROS) and an internal Ca^{2+} elevation from the nucleus but it appeared that, contrary to inhibition of nuclear Ca^{2+} signaling and Ca^{2+} entry, suppression of ROS with the NADPH oxidase inhibitor does not impact on cell death. His work highlights the crucial role of nuclear Ca^{2+} signature in controlling sphingolipid-induced cell death in tobacco cells in a ROS-independent manner and likely through Ca^{2+} channels belonging to the glutamate channel family. Finally, the last oral presentation was selected among submitted abstracts and was from Nadine Khadra (University of Rennes 1; Rennes, France). Her results demonstrate that death receptor CD95 engagement leads to the redistribution of the CRAC channel STIM1/Orai1 into a specialized plasma membrane structure (CD95-CAP) where it allows a localized Ca^{2+} entry that orchestrates the recruitment of PKCb2 and consequently blocks the subsequent apoptotic signal. Overall, the data indicate that the CD95-mediated Ca^{2+} response engages a negative feedback loop, which prevents the initial steps of the CD95 signal.

Announcement

The organizing committee will be pleased to welcome you for the 23rd Ion Channel Meeting that will be held from the 23rd to the 26th September 2012 at the Belambra Club resort of the Giens peninsula (France). The program and registration information is available on the website: www.canaux-ioniques.fr. Abstract submissions are open until the 10th of June and registration is open until the 15th of July 2012.

Acknowledgments

The organizing committee sincerely thanks the Association Française contre les Myopathies (AFM) for its financial support. We also want to thank our partners and exhibitors Dipsi Industrie (France), Scientifica Ltd., (United Kingdom), Nanion Technologies GmbH (Germany), Labtech France (France), Andor Technology (Ireland), Carl Zeiss Microscopie (France), BMG Labtech (France), Harvard Apparatus (France), HEKA Elektronik (Germany), Euromedex (France), Word Precision Instruments (United Kingdom), Sichem GmbH (Germany) and Dominique Dutscher (France). Finally, we want to thank Cyril Sarrauste de Menthière, the webmaster of the website: www.canaux-ioniques.fr and Caroline Strube, the president of the French Ion Channel Society.