Meeting Report

The 19th Ion Channel Meeting

September 2008, France

Delphine Bichet,1,* Christophe Duranton,2 Hélène Hirbec,3 Stephan Kellenberger,4 Jean Mérot,5 Lauriane Ulmann6 and Fabien Van Coppenolle7

1Institut de Pharmacologie Moléculaire et Cellulaire; CNRS UMR 6097; Université de Nice Sophia Antipolis; Valbonne, France; 2Université de Nice Sophia Antipolis; CNRS-FRE 3093; Parc Valrose, Nice, France; 3Institut des Neurosciences de Montpellier; INSERM U583; Hôpital St Eloi; Montpellier, France; 4Département de Pharmacologie et de Toxicologie; Université de Lausanne; Lausanne, Switzerland; 5Institut du Thorax; INSERM U915; Nantes, France; 6Institut de Génomique Fonctionnelle; CNRS UMR 5203; INSERM U661; Université Montpellier 1-2; Montpellier, France; 7Université Lyon 1; UMR CNRS 5123; Villeurbanne, France

Key words: ion channels, membrane proteins, epithelial channels, protein trafficking, channelopathies

Introduction

From the 21st to the 24th of September 2008, the French Ion Channels Society organized its annual scientific meeting on the Giens peninsula, on the coast of the Mediterranean Sea (France). As every year, the meeting had two primary goals: to attract internationally recognized senior scientists and give them the opportunity to share their latest advances in the field of ion channel research with the scientific community, and to encourage interactions of students and young researchers with senior scientists who serve as role models for pursuing successful careers in science.

This year, more than 150 scientists attended the meeting organized around five symposia of invited speakers, three thematic sessions of selected communications, two poster sessions and one “out of frame” conference. The present report will focus on this year’s five symposia on aspects of the ion channel field from fundamental to clinical research. The meeting is an opportunity for leading investigators as well as young researchers to present and discuss their recent advances and future challenges in the ion channel field.

Symposium I

“Recent advances to explore membrane proteins,” organized by Christophe Duranton, CNRS FRE 3093, Université de Nice Sophia-Antipolis, Nice, France. The topic of the first session of the 19th meeting of Ion Channels was “Recent advances in the exploration of membrane proteins.” The first presenter was Valentina Emiliani (Université Paris Descartes, Paris, France). Valentina presented a new technique for optic microscopy based on wave front engineering. The combination of a liquid-crystal spatial light modulator matrix and a confocal microscope allows holographic imaging and generates 3D multiple excitation spots. An example of 3D glutamate decaging by photolysis performed on slice brain was presented and confirmed the efficiency of this novel approach. Finally, Valentina concluded that the holographic microscope provides an extremely flexible method for activation of various photosensitive proteins and small molecules.

The second speaker, Herman Schillers (University of Munster, Germany) described the use of atomic force microscopy to investigate the three-dimensional structure of the anionic channel CFTR in its native environment. Based on atomic force microscopy observations performed on CFTR-transfected oocytes Herman proposed that CFTR molecules are organized in tail-to-tail dimeric structures with a central pore inside the plasma membrane. Applying atomic force microscopy to human erythrocytes Herman found that the distribution of CFTR molecules is comparable to that of epithelial cells. Herman finally concluded that atomic force microscopy experiments performed on isolated plasma membranes allow not only quantification and localization of membrane proteins but also provide insight in their dynamics at a single-molecule level.

The last speaker, Sergio Grinstein (Hospital for Sick Children, Toronto, Canada) talked about cellular phospholipid remodeling and the targeting of signaling proteins. Beside the fact that the lipid distribution of biological membranes is highly asymmetric, the uneven distribution of anionic lipids generates electrostatic surface potentials that differ markedly between membranes. By using fluorescent biosensors of the lipid membrane composition, Sergio tracked the remodeling of the lipid membrane during phagocytosis in macrophages. Interestingly, both the lipid composition and the
surface charge changed rapidly during phagosome formation and maturation rendering the phagosomal vacuole similar to endosomes and lysosomes.

**Symposium II**

“Epithelial ion channels,” organized by Stephan Kellenberger, Département de Pharmacologie et Toxicologie, Université de Lausanne, Switzerland. The subcellular location and cell surface density of different inwardly rectifying potassium (Kir) channels is precisely controlled. In the symposium on epithelial ion channels, Paul Welling (Baltimore, USA) presented data on trafficking mechanisms of Kir1.1 and Kir2.1. In the renal outer medullary K+ channel Kir1.1 (ROMK) an endocytotic signal motif on the C-terminus interacts with clathrin-adapter proteins and promotes endocytosis, while a motif in the N-terminus acts as a Golgi-export signal, once a serine residue within the motif is phosphorylated. These trafficking events are regulated by WNK kinases and the serum/glucocorticoid-regulated kinase. To illustrate the relevance of K+ channel trafficking, Paul provided information on the mechanism of a disease mutation in the Kir2.1 channel that blocks Golgi export, and indicated how WNK kinases, when mutated in pseudohypoaldosteronism type II, alter K+ secretion as a possible consequence of interference with K+ channel trafficking.

Heidi Fodstad (Lausanne, Switzerland) used a mouse cortical collecting duct (CCD) cell line to investigate the regulation of the ROMK-mediated K+ secretion by aldosterone and K+ concentration. Exposure to aldosterone significantly decreased ROMK mRNA levels, while it did not affect the amplitude of the barium-sensitive K+ conductance, which, however, was increased by an overnight exposure to a high K+ concentration. Thus, in the CCD cells the ROMK-mediated K+ conductance is regulated according to the basolateral K+ concentration, while aldosterone stimulates K+ secretion through its effect on Na’ transport in spite of a small decrease in ROMK expression.

Joost Hoenderop (Nijmegen, Netherlands) discussed different mechanisms of regulation of the TRPV5 Ca2+ channel. The fine-tuning of Ca2+ excretion in the kidney takes place in the distal convoluted and connecting tubule, where Ca2+ is actively reabsorbed via the transcellular pathway, for which Ca2+ entry through the TRPV5 channel constitutes the rate-limiting step. Ablation of the TRPV5 gene in mice seriously disturbs renal Ca2+ handling, the TRPV5 channel constitutes the rate-limiting step. Ablation of the TRPV5 gene in mice seriously disturbs renal Ca2+ handling, and lysosomes.

**Symposium III**

“Dynamic regulation of ion channel trafficking,” organized by Delphine Bichet, CNRS UMR 6097, Université de Nice-Sophia Antipolis, Valbonne, France. Regulation of receptor and ion channel trafficking is a key cellular function. It does not only control the number of channels at the cell surface, but also regulates their precise sub-cellular localization. This function is particularly important for polarized systems such as neurons and was nicely illustrated by the three invited speakers of this symposium.

The first illustration came from Lily Jan (University of California San Francisco, USA) who presented her recent studies on the regulation of potassium channel trafficking in neurons. Focusing on a G-protein-activated inwardly rectifying potassium (GIRK) channel, Lily showed that neuronal activity regulates their surface density. Indeed, activation of NMDA glutamate receptors increased GIRK surface expression at synaptic sites on soma and dendrites. Interestingly, this activity-dependent regulation of trafficking is dependent on the phosphorylation of an internalization motif present in GIRK, where dephosphorylation promotes channel delivery from recycling endosomes. This regulation of GIRK channel number reveals a powerful way to dynamically modulate neuronal excitability.

The second speaker, Bénédicte Dargent (Université de la Méditerranée, INSERM UMR 641, Marseille, France) presented mechanistic evidences for the distribution of ion channels and their associated proteins in specific neuronal compartments. Focusing on the axonal initial segment (AIS) and on the nodes of Ranvier, Bénédicte illustrated the targeting and clustering of voltage-gated sodium (NaV) channels in these two highly specialized axonal sub-domains. She then showed that this accumulation of Nav channels is mainly due to their specific attachment to the dense sub-membrane cytoskeleton and especially to ankyrin G. Indeed, mutations in the ankyrin-binding motif of Nav channels abolished their AIS accumulation whereas overexpression of ankyrin robustly decreased Nav channel lateral mobility. These mechanisms are crucial in maintaining the neuronal polarity that is necessary to action potential initiation and propagation.

The third speaker, Daniel Choquet (Université de Bordeaux, CNRS UMR 5091, France) presented his work on ion channel mobility at neuronal excitatory synapses. Using single particle tracking, Daniel demonstrated the rapid surface movement of ionotropic AMPA glutamate receptors at individual synapses. The movement corresponds to fast lateral diffusion of the receptor and allows the exchange of desensitized with functional receptors at the post-synaptic density. Moreover, preventing this diffusion through cross-linking, endogenous clustering or calcium rises induced by high frequency stimulation slows down the recovery from synaptic
depression. The data nicely illustrate the functional relevance of this new concept implicating channel lateral diffusion in fast excitatory synaptic transmission.

The last speaker of the symposium, Hamed Nazzari (University of British Columbia, Vancouver, Canada), presented his PhD studies carried out in Eric Accili’s laboratory on the role of N-linked glycosylation in modulating the trafficking and surface expression of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. In his presentation, Hamed showed that mutation of a putative N-glycosylation site (N380Q) in HCN2 eliminates channel maturation and trafficking to the cell surface. The glycosylation site is absent in the sea urchin HCN ortholog but is conserved among mammalian HCN isoforms, suggesting that N-glycosylation of HCN channels has probably emerged during evolution as a new regulatory mechanism of surface trafficking.

Symposium IV

“Ion channels in cellular interactions,” organized by Lauriane Ulmann, CNRS UMR 5203, Université de Montpellier, France. Sylvia Cohen-Kaminsky (CNRS, Université Paris Sud, France) presented evidence for the involvement of glutamatergic transmission in the formation of immunological synapses between dendritic cells (DCs) and T lymphocytes. As a presynaptic element, VGLUT is present in DC cells, which release glutamate through calcium-dependent exocytosis, while T cells express a large repertoire of GluRs. During the formation of the immunological synapse, the PSD95 protein, an essential component of the NMDA receptor-signaling complex, is recruited in the contact zone where it co-localizes with NMDAR. Furthermore, in vivo silencing of the NR1 subunit of the NMDAR inhibits T cells intracellular calcium increase triggered by immune synapse formation. These data suggest that, similarly to CNS synapses, glutamate signaling is involved in the intercellular communication during the immunological synapse formation.

In the second presentation of the session, Andrea Volterra (University of Lausanne, Switzerland) detailed the mechanisms by which astrocytes fine-tune synaptic transmission. In the hippocampus, firing of perforant path (PP) afferents results in a [Ca^{2+}] increase in the astrocytes of the dentate molecular layer mediated by P2YR1 activation. This calcium signal triggers glutamate release from astrocytes, which in turn, activates pre-synaptic NR2B-containing NMDA receptors of PP-granule cell synapses. Andrea presented convincing experiments showing that TNFα modulates this glio-transmission. On acute hippocampal slices, application of 2MeSADP, a P2Y1R agonist, increases mEPSCs frequency that is inhibited by the NR2B inhibitor ifenprodil. In contrast, the effects of 2MeSADP are not observed in TNFα KO mice. Interestingly, application of TNFα in WT mice induces an increase of mEPSC frequency that is blocked by ifenprodil. Thus, TNFα release by astrocytes may contribute to the modulation of synaptic efficacy at pre-synaptic levels. This regulation may have important implications in neurodegenerative diseases where elevated secretion of TNFα is observed.

In the third presentation, Richard Robitaille (University of Montreal, Canada) presented data about the ability of perisynaptic Schwann cells (PSC) to decode patterns of neuronal activity. At the neuromuscular junction (NMJ), these glial cells are able to detect and modulate synaptic activity. However, the ability of glial cells to decode patterns of neuronal activity was unknown. By electrically stimulating the motor nerve of the soleus muscles and simultaneously recording the calcium response of PSC and end plate potentials in the muscle, Richard showed that PSC respond with different calcium firing patterns to different patterns of electrical nerve activity. For example, a continuous 20 Hz stimulation triggers a single calcium response in PSC and induces post-tetanic potentiation while bursting 20 Hz stimulation triggers an oscillatory calcium response and post-tetanic depression. In both cases, blocking calcium elevation in glial cells inhibits short-term synaptic plasticity and conversely, elevating calcium in glial cells induces plasticity. Thus, glial cells are able to decode different patterns of electrical activity but also to adapt their feedback control of synaptic efficacy.

Symposium V

“Ion channels and diseases,” organized by Fabien Van Coppenolle, INSERM U800, Villeneuve d’Ascq, France. The fifth session of the conference addressed links between ion channels and diseases: Calcium leak channels and Alzheimer disease; TRPCs channels and smooth muscle cell physiology and pathophysiology; TRPM6 and magnesium disorders and finally Kir2.1 potassium channel and C Andersen’s syndrome.

The first speaker was Ilya Bezprozvanny (UT Southwestern Medical Center, Dallas, USA) who spoke about the role of presenilins as endoplasmic reticulum (ER) calcium leak channels, and its implications for Alzheimer disease. For many years, the nature of ER calcium leak channels was a mystery. Ilya and his team have shown that presenilins, which are known to have a gamma-secretase activity, act also as passive endoplasmic reticulum calcium leak channels. Using double knockout (KO) mouse embryonic fibroblasts, they found that wild type PS1 and PS2 proteins account for 80% of the passive calcium leak from the ER lumen. The ER calcium leak function of presenilins is independent from their gamma-secretase activity. These experiments are of major importance in the understanding of disturbed calcium homeostasis in AD pathogenesis.

In the second talk, Veit Flockerzi (University of Saarland, Homburg, Germany) presented recent findings regarding the functions of TRPC channels of ileal smooth muscle cells in mediating the muscarinic agonist-activated cation current. Veit and his team are particularly interested in the receptor stimulation of phospholipase C and the following activation of TRPC channels and increase in Na⁺ and Ca²⁺ influx (named mICAT). Although the physiological functions of TRPC channels in native cells are still unclear, experiments using TRPC KO mice (like TRPC4 and TRPC6 KO mice) have shown that these channels are involved in cholinergic neurogenic contraction of smooth muscles. 80% and 20% of mICAT are due to TRPC4 and TRPC6, respectively. This study further our understanding of the pathological and physiological roles of TRPC channels.

The third speaker, René Bindels (Radboud University Nijmegen Medical Center, Nijmegen, Netherlands) described the role of the magnesium channel TRPM6 in inherited magnesium disorders. TRPM6 plays a key role in magnesium re-absorption in the kidney and intestine and modulation of TRPM6 expression or TRPM6 activity induces dysregulation of magnesium homeostasis. Recently, a mutation in epidermal growth factor (EGF), which activates TRPM6, was found to be responsible for autosomal recessive hypomagnesia. In conclusion, René and his team have shown that TRPM6 appears...
to be a major channel involved in magnesium homeostasis and in pathological situations.

The last presenter at this symposium was Said Bendahhou (University of Sophia Antipolis, France): “Role of Kir2.1 potassium channels in skeletal muscle excitability”. Said’s work focuses on C Andersen’s syndrome (AS) which is associated with periodic paralysis, cardiac arrhythmia and developmental anomalies. Mutations in the gene encoding the potassium channel Kir2.1 have been linked to AS. Said’s experiments showed that AS myotubes lack a barium sensitive, inward rectifier current which leads to a shift of the resting potential towards depolarizing potentials. This is the first evidence for a functional consequence of AS mutations in human skeletal muscle myotubes.

**Conclusion**

Once again the success of this meeting was achieved through the high scientific quality of the symposia and communications. Moreover, the relaxed atmosphere favored scientific interaction between the attendees that may lead to collaboration and scientific achievement.

Next year the Ion Channel Society will celebrate its 20th anniversary. Do not miss this special event and save the September 20–23, 2009 in your agenda to attend the very special 20th Ion Channel Meeting that will take place in Giens, France.