The 21st Ion Channel Meeting
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On September 12–15, 2010 the French Ion Channels Association organized its annual scientific meeting on the French coast of Mediterranean Sea. This meeting takes place in an attractive location and provides a great opportunity for principal investigators as well as young researchers to present and discuss their recent advances and future challenges in the field of ion channels and transporters. The French Ion Channels Association was created more than 20 years ago and its goal is to organize an annual meeting and more recently to promote interactions (through the website www.canaux-ioniques.fr) between active members of the international scientific community in the field of ion channels. In this report of the 21st edition of the meeting, we are summarizing the five main symposia that reflect original works and relevant developments in the domain of ions channels and transporters.

Introduction

The 21st edition of the French Ion Channels Association meeting took place on September 12–15, 2010 on the Mediterranean coast (Presqu’île de Giens, France). This year, more than 150 participants attended the meeting with 60 abstracts submitted covering various aspects of the biology of ion channels and transporters. The main goal of the meeting is to attract senior international scientists recognized for their original work and give them opportunity to debate with the scientific community their recent advances in the field of ion channel research. Another objective of the meeting is to promote interactions and collaborations of students and young scientists with international senior scientists. This year, the meeting was organized around five symposia with invited speakers, three oral communication sessions, one plenary lecture and two poster sessions. The present report will focus on the five symposia, which covered: (1) ion channels and toxins, (2) pathology of ion transporters, (3) aquaporins, from microorganisms to mammalians, (4) neuronal excitability and integration and (5) membrane dynamics of ion channels.

Symposium I: Ion Channels and Toxins

Organized by Anne Baron-Foster (IPMC, UMR-6097 CNRS, UniversitNavy Nice Sophia-Antipolis, Valbonne, France).

Since 1975, peptidic toxins isolated from animal venoms have been extensively used to study ion channels. During this symposium, this was illustrated by recent data from ion channel structure-function analysis, pharmacological properties to in vivo physiological roles and new therapeutic perspectives.

Frank Bosmans from the team of Kenton Swartz at the National Institute of Health (NIH) in Bethesda (USA) was the first speaker. He used venom toxins to study voltage-sensors and Na1.9 channel function, thus leading to new perspectives in pain therapy. Using a protein engineering approach, the contribution of the four voltage sensors was determined both in channel function and pharmacology. Like canonical Na channels, Na1.9 channels possess functional voltage sensors interacting with scorpion and tarantula toxins, but also specific structural motifs that contribute to the unusually slow gating properties of Na1.9 channels and to specific voltage-sensor pharmacology.

Enzo Wanke, the second invited speaker, is a Professor of Physiology in Milano (Italy). He presented new concepts of promiscuity and selectivity of toxins and of vulnerability of ion channels. Pharmacological profiles of five different tarantula toxins were studied on human voltage-dependent Na+ and K+ channels. Tarantula toxins display a multi-mode mechanism: a single toxin could affect a voltage-dependent channel in different ways, acting as a pure pore blocker but also as a gating-modifier with different potencies and affinity. Tarantula toxins are also target-promiscuous ion channel modulators, since a single toxin could affect several ion channels with similar potencies. On the other hand, the concept of vulnerability of ion channels was illustrated by the ability of different toxins to induce similar current blockade. These data and new concepts should help structure-function studies and new pharmacological developments.

The third speaker, Ines Ibañez-Tallon, came from the Max Delbrück Center (MDC) in Berlin (Germany) where her group is working on cell-surface tethered toxins. She showed how...
powerful these toxins are as tools to study neuronal circuitry and physiological roles of ion channels in vivo. The expression of membrane tethered toxins in transgenic mice (lentiviral vector injection) leads to various behavioral impairments. The inducible expression of tethered ω-conotoxin MVIIA, targeting presynaptic Ca$_{2.2}$ channels, induces long term silencing of neurotransmission in hippocampal neurons, as well as an inhibition of dopamine release by substantia nigra neurons. Under the control of a nociceptor-specific gene (Na$_{1.8}$ channels), the induction of tethered conotoxin MrVIa expression leads to a decrease of inflammatory and thermal pain due to the inhibition of TTX-resistant Na$^+$ channels in transgenic mice. Tethered toxins could thus lead to innovative applications for manipulating neuronal and tissue-specific networks, as well as therapeutic strategies.

The last speaker of this symposium was Rocio Finol-Urdaneta who presented her post-doctoral work carried out in the group of Robert French from Calgary (Canada). By comparing the pharmacological profiles of two conotoxins on K$_{1.2}$-containing homomeric or heteromeric channels expressed in Xenopus oocytes, she showed that the cardioprotective effect of conotoxin kM-RIIIK against ischemia involves specific heteromeric K$^+$ channels—the heteromeric K$_{1.2}$/1.7 channels—rather than homomeric K$_{1.2}$ channels.

**Symposium II: Pathologies of Ion Transporters**
Organized by Mallorie Poët (TIANP, UMR-6907 CNRS, Université de Nice Sophia-Antipolis, Nice, France). This symposium focused on the involvement of various ion transporters in physiopathology.

The first speaker was Christian Hubner who heads the department of Human Genetics at the University Hospital of Jena (Germany). His presentation was an overview of his work on the SLC4 membrane proteins. To investigate the role of bicarbonate transporters in vivo, his group generated a collection of knockout mouse models for several members of the SLC4A family. He presented some in vivo analysis of mouse models. In particular, he showed that SLC4A8 invalidated animals have problems in Na$^+$ reabsorption by renal collecting ducts. SLC4A10 KO animals show reduced neuronal excitability whereas SLC4A3 KO mice present reduced seizure threshold. His work pointed out the critical role of this transporter family for neuronal and kidney homeostasis. In addition, analysis of these mouse models allowed him to unravel unexpected roles in the secretion of cerebrospinal fluid, in inner ear physiology, as well as kidney and retina functions.

The second speaker was Harad Sitte from the Institute of Pharmacology of the Center for Biomolecular Medicine and Pharmacology at the Medical University of Vienna (Austria). His main interest is to understand the function of monoamine transporters—the NSS family—and their central role in motor function, reward and cognition. One main question was whether altered monoamine transporter structure or regulation may serve as the basis for clinical disorders linked to compromised monoamine neurotransmitter signaling, including autism, depression, drug abuse schizophrenia and attention deficit hyperactive disorder (ADHD). He presented recent advances in the molecular mechanisms underlying the psychostimulant activity of clinically relevant substances like amphetamines. He also illustrated the effect of structural changes of these transporters that can convert their function from transporters to channels.

Raul Estevez was our third speaker. He is Professor of Physiology in the Department of Physiological Sciences at the University of Barcelona and presented his recent results on the molecular basis of megalencephalic leukoencephalopathy with subcortical cysts. This disease is a rare type of leukodystrophy mainly caused by mutations in the MLC1 gene. The function of MLC1 is still unknown but its homology to transporter and its localization to the plasma membrane suggest that it could mediate substrate/ion translocation across the cell surface. Disease-causing missense mutations are responsible for a dramatically decreased level of MLC1 at the plasma membrane in Xenopus oocytes and mammalian cells. Raul Estevez was able to show that MLC1 mutations reduce protein levels in vivo and proposed pharmacological strategies that improve MLC1 expression (low temperature and glycerol) to treat MLC patients.

Finally, Ingrid Chamma from the Institut du Fer à Moulin in Paris presented her Ph.D., work on the membrane dynamic properties of the K/CI transporter KCC2 using Single Particle Tracking (SPT) with quantum dots (QDots). In mature hippocampal neurons, this transporter is clustered in dendritic spines and spines near excitatory synapses. Using SPT, they found that 60% of the QD-bound KCC2 remained at synapses for long periods of time. Since KCC2 is modulated by neuronal activity they then investigated the effect of neuronal activity on the membrane dynamics of the transporter. Application of 4-aminopyridin that increases neuronal activity led to an increase in KCC2 lateral mobility. These results suggest that KCC2 anchoring near excitatory postsynaptic densities is influenced by neuronal activity.

**Symposium III: Aquaporins from Microorganisms to Mammalians**
Organized by Olivier Pichon (EA 2106 Biomolécules et Biotechnologies Végétales, Université François Rabelais, Tours, France). This symposium presented different aspects of the role of these water channels across the different kingdoms of life, from bacteria and plants to mammals.

The First speaker, Stefan Hohmann from the University of Göteborg (Sweden) spoke about the involvement of aquaporins in the control of developmental programs in yeast. The presentation was focused on two highly homologous aquaporins from *Saccharomyces cerevisiae* that were controlled by different signals. *AQY1* was implicated in normal spore formation. Indeed its expression was stimulated when cells enter a resting stage, especially during the formation of ascospores. *AQY1* probably promoted water exit from cells during spore maturation. *AQY2* affected cell surface properties and its expression was controlled by signaling pathway involved in the behavior of yeasts in colonies and growth on surfaces. The presence of aquaporins in yeast appeared to be either an advantage under some conditions or a disadvantage under other conditions and highly controlled by the
pressure of evolution. This may explain why some yeast strains express active aquaporins while others do not.

The second speaker, François Chaumont from the University of Louvain (Belgium) presented the role of plasma membrane aquaporins (PIP) in plants as a highly regulated plumbing system. Plant aquaporins constitute a large and divergent protein family. Some aquaporins can facilitate the movement of a large array of substrates. In maize, thirteen PIP genes are expressed. The group of François Chaumont has quantified and localized the PIP expression and activity in Zea mays roots and leaves at both RNA and protein levels. PIP expression was highly regulated: mRNA and protein expression levels were dependent on the development and environmental conditions and were correlated with changes in the cell membrane water permeability. Plasma membrane PIP trafficking was regulated by hetero-oligomerization, different motifs including the di-acidic signal in N-terminus, and the SNARE SYP121. Gating of aquaporins was controlled by phosphorylation and was dependent on the environmental conditions.

The next invited speaker, Jerome Badaut from Loma Linda University (California, USA) spoke about the physiological role of aquaporins in mammalian brains. Aquaporins are now regarded as multi-functional channels permeable to ions, gas and small solutes such as glycerol, urea and monocarboxylates and appear to be involved in several physiological and pathophysiological processes. AQP4 is localized on astrocytic end-feet and significantly contributes to water movement within brain tissues. AQP4 expression is highly modified in several brain disorders and could play a key role in the cerebral edema formation. AQP9 is expressed in astrocytes and in catecholaminergic neurons. Jerome Badaut’s group has shown that AQP9 expression is negatively regulated by high concentrations of insulin and that a decrease in AQP9 expression in vitro led to decreased glycerol entry accompanied with an increase in glucose consumption. AQP9 could be involved in brain energy metabolism acting as a pipeline for energy substrates.

The “Aquaporin session” ended with Pascal Jourdain from Lausanne Federal Polytechnic School (Switzerland) who presented the application of a noninvasive optical technique in “water imaging”—Digital Holographic Microscopy. This technique allows one to study, at a single-cell level and without the use of dyes, the optical signature of specific proteins involved in the transmembrane water transport. This methodology provided quantitative phase images of living cells. Specifically, the phase signal was mainly dependent on the variation of the intracellular refractive index which, in turn, was mostly reflects the protein content of the cell. In these conditions, an entry of water dilutes the intracellular protein content resulting in a decrease in the phase signal while an exit of water leads to a phase increase.

Symposium IV: Neuronal Excitability and Integration

Organized by Desdemona Fricker (CNRS UMR 7225, CRICM, Paris, France). The four talks in this symposium addressed ion channels used for drug delivery, light switched ion channels, neurons signaling spatial information, and ion channels in epilepsy. Bruce P. Bean (Harvard, USA) presented a clever way of targeting local anesthesia through TRP Channels. TRPV1 and TRPA1 channels have large pores allowing permeation of small molecules into the cell. QX-314, a cationic derivative of lidocaine can enter TRPV1—expressing neurons and block voltage-dependent sodium channels from the cytoplasmic side. TRPV1 channels are preferentially expressed in primary sensory neurons that sense pain. Bruce presented data showing that peri-sciatic injections of capsaicin plus QX-314 can produce a long-lasting block of responses to painful stimuli with minimal effects on motor behavior. Lidocaine itself is also a TRPV1 agonist, and the co-application of lidocaine with QX-314 produces peripheral analgesia that is far longer lasting (8–24 hours) than that produced by lidocaine alone.

Richard H. Kramer (Berkeley, USA) focused on photochemical tools for controlling neural activity with light. He presented a method that confers light sensitivity onto voltage-gated K+ channels by acute application of small molecules, such as AAQ. The AAQ “photoswitch” has been used in retinas from blind rd1 mice that are unable to respond to light. Intraocular injection of AAQ can restore the pupillary light reflex. Another photo-switch molecule is named QAQ. Richard presented data showing that in the trans configuration QAQ functions as an intracellular blocker of voltage-gated K+, Na+ and Ca2+ channels, but blockade is alleviated by switching to cis with 380 nm light and restored by switching to trans with 500 nm light. The net effect is that neuronal activity can be silenced in a light-reversible manner. QAQ may be delivered into cells through ion channels and in this way allows optical control over firing in nociceptive neurons that possess TRPV1 channels and P2X receptors.

Matthew Nolan (Edinburgh, UK) addressed the function of neuronal circuits that mediate spatial cognition. The discovery of topographic organization of spatial encoding by neurons in layer II of the medial entorhinal cortex (MEC) inspired Matthew to investigate cellular mechanisms that are important for computation in this neuronal circuit. In his presentation he illustrated that the tuning of synaptic integration in layer II neurons follows a topographical organization similar to the resolution of spatial firing fields in behaving animals. He concluded that synaptic response tuning is crucial for models that explain generation of grid firing fields.

Camille Liatutard presented her Ph.D., studies carried out in Massimo Mantegazza’s laboratory (University of Nice-Sophia Antipolis, France) on hippocampal network excitability in a severe myoclonic epilepsy of an infancy mouse model. In her presentation, Camille showed that hippocampal slices from Na1.1 knock-out mice are hyperexcitable during the epileptogenic period and in young adult mice, with an increased number of spikes in the after-discharges induced by tetanic stimulation. Paired pulse stimulation in CA1 had shown decreased depression of the population spike for short interpulse intervals and the appearance of a double spike after the second stimulus for longer interpulse intervals.

Symposium V: Membrane Dynamics of Ion Channels

Organized by Jean Christophe Poncer (Institut du Fer à Moulin, Paris, France). Pioneer experiments using Fluorescence Recovery
After Photobleaching (FRAP) in the late seventies have revealed biological membranes as fluid mosaics in which proteins move within lipid bilayers. Yet, how lateral diffusion of proteins in the membrane influences their function has remained elusive until the recent development of single-particle tracking (SPT) techniques. Unlike FRAP which is greatly limited by its spatial resolution, SPT has revealed the diffusion properties of individual ion channels at the submicrometric level, and allowed the mechanisms of their modulation to be addressed with unprecedented refinement. SPT using fluorescent nanoparticles was first used in Neuroscience to study the membrane dynamics of postsynaptic receptors and their contribution to synaptic function and plasticity. However, it is now becoming a technique of choice to study the lateral diffusion of both ligand- and voltage-gated channels, as well as other transmembrane proteins such as transporters. This symposium was aimed at illustrating the recent advances in this promising field and comparing the diffusive properties of various ion channels and transporters as well as their molecular determinants.

Joseph Kittler (University College, London, UK) presented recent data on the Ca\(^{2+}\)-dependent modulation of GABAA receptor clustering at synapses. Using imaging of a super-ecliptic pHluorin-tagged recombinant alpha2 subunit, he showed that activation of NMDA receptors induces a rapid and reversible reduction of GABAA receptor synaptic aggregation. Consistent with this observation, SPT experiments revealed an increased mobility and reduced dwell time of individual GABAA receptors at synapses. A calcineurin antagonist blocked this effect and involved phosphorylation of Ser327 on the gamma2 subunit of the receptor, as evidenced in experiments using mutant gamma2 coexpression with recombinant alpha2. These results illustrate a remarkable crosstalk between excitatory and inhibitory synapses, in which dispersal of synaptic GABAA receptor clusters upon excitatory synaptic activity could act locally to enhance dendritic excitability.

SPT experiments showed that most ionotropic receptors (AMPA, GABAA, glycine...) continuously exchange between synaptic and extrasynaptic compartments and synaptic receptor content is thus regulated by transient diffusion trap of receptors by submembrane cytoskeleton. NMDA receptors were initially thought to be less mobile than other postsynaptic receptors. However, Laurent Groc (CNRS, Université Bordeaux 2, France) presented evidence that membrane diffusion of NMDA receptors actually depends on their subunit composition. In particular, NR2A-containing receptors are more stable than NR2B-containing receptors. Accordingly, the developmental switch in NR2 subunit composition (from NR2B to NR2A) is accompanied by a change in the dwell time of individual NMDA receptors at synapses. The molecular determinants of these subunit-specific diffusion behaviors were elegantly explored by specifically disrupting NR2A interaction with PDZ-domain proteins, using a divalent biomimetic ligand transduced into neurons using a TAT sequence. This approach was used to demonstrate that the mechanisms involved in anchoring distinct NR2 subunits are different. These data therefore disclose an unexpected role of the NR2 subunits in controlling the anchoring and diffusion of NMDA receptors at synapses.

Michael Tamkun (Colorado State University, USA) illustrated a quite different behavior of the diffusion of a voltage-gated ion channel and the regulation of its function. His presentation focused on the K\(_{2.1}\) delayed rectifier channel, which forms large clusters throughout the somato-dendritic membrane in neurons. Here again, high resolution SPT was used to examine the diffusion properties of K\(_{2.1}\) channels in heterologous cells and revealed that clusters are not delineated by a diffusion barrier but may contain obstacles that influence the diffusive behavior of individual channels. Interestingly, non-clustered channels ignore this barrier and freely diffuse over the entire cell surface. Pharmacological data were presented to support the role of submembrane cytoskeleton on K\(_{2.1}\) diffusion properties and clustering. Most strikingly, clustered channels were shown to contain nonconducting channels whereas non-clustered channels were functional. Although the mechanisms linking channel aggregation with their functional state remain to be fully explored, these data raise the questions of the actual role of these large, membrane clusters of non-conducting channels and the physiological conditions of their conversion into non-clustered, functional channels.

Finally, Christophe Letertier (INSERM, Université de la Méditerranée, Marseille, France) presented an elegant study on the molecular constraints that influence membrane diffusion of sodium channels at the axon initial segment (AIS). Using SPT of a recombinant, K\(-Na\) chimeric construct, he showed that ankyrin G impedes the membrane diffusion of the recombinant channel both in neuroblastoma cells and in the AIS of immature neurons where most channels are confined. Point mutations in the ankyrin-binding motif of the chimera channel that decreased its interaction with ankyrin increased the diffusion coefficients of individual channels. Importantly, this interaction was regulated by the protein kinase CK2 that also concentrates in the AIS. These results suggest aggregation of sodium channels in the AIS, which likely influences neuronal excitability, may be modulated in an activity-dependent manner.

**Conclusion**

On Wednesday morning, after the last session and before closing the meeting, the two co-Presidents, Dr. Christophe Duranton (CNRS, University of Nice, France) and Pr. Fabien Van Coppennolle (CNRS, University of Lyon, France), thanked all the attendees for their participation and the speakers for the high quality of symposia and communications.

The organizing committee is pleased to inform you that the 22nd Edition of the Ion Channel Meeting will take place on September 25–28, 2011, in the pleasant venue of Presqu’île de Giens (Bellambra Club Resort) on the French Mediterranean Riviera. Information can be found on http://www.canauxioniques.fr/congress/.