Potassium channels still hot

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Potassium channels are important for the regulation of cell function in excitable as well as non-excitable cells. By opening, they stabilize the membrane potential and in excitable cells such opening will set the resting potential, repolarize action potentials and terminate periods of action potential firing. In non-excitable cells K^+ channels also have a role in transmembrane transport, volume regulation, signal transduction as well as maintaining cell resting potential. The combined use of electrophysiological and molecular biological techniques in the past ten years has demonstrated a much larger diversity of K^+ channels in comparison to other ion channel families. Structure–function relationships have identified the features of the channels that are responsible for particular channel functions (Fig. 1). In addition, a growing awareness of certain K^+ channels as possible targets for therapeutic intervention has increased interest in the field of K^+ channel research. Recently, an International Potassium Channel Conference* (IPCC), took place to bring together many experts in the field of K^+ channels to present and discuss the most recent developments in every aspect of research in K^+ channel biology. Presentations incorporated structure/function, expression/localization as well as regulation, modulation and pharmacology**. The conference also gave the opportunity to participants for poster presentations of their own results on K^+ channels or related topics.

Voltage-gated K^+ channels

The scientific programme started with a session on the gating of voltage-gated K^+ channels. Gary Yellen (Harvard Medical School and Massachusetts General Hospital, Boston, USA) opened the session with a description on how voltage-gated K^+ channels respond to changes in membrane potential: they open and close their K^+ selective pores. By substituting cysteines in *Shaker* channels at several positions in S6 and chemically modifying the sulfhydryl groups he could identify positions in the intracellular pore of the channel that lie behind the intracellular activation gate. Amino acid residues in positions 470 and 474 apparently hardly move at all during gating, whereas the amino acid in position 476 appeared to be involved directly in gating movement since Cd^{2+} binding could hold the channel in an open configuration.

Voltage-gated K^+ channels undergo at least two different mechanisms of inactivation. These two mechanisms are regulated by different structural domains of the channel molecules and are termed N-type and C-type inactivation (Fig. 1). The molecular mechanism for N-type inactivation is caused through regions close to the N-terminus of the channel[1,2]. This part of the channel forms an inactivation particle that can block the pore of the channel from the cytoplasmic site. Removal of this inactivation mechanism through removal of the inactivation particle uncovers another, slower type of inactivation. Since different *Shaker*-splice variants with different C-termini showed corresponding differences in this second, slower type of inactivation, it was termed C-type inactivation. The exact molecular mechanisms of the C-type inactivation process may involve structures that are close to the extracellular mouth of the channel since extracellular tetraethylammonium (TEA^+) can modify this type of inactivation[3]. In addition, mutations in the putative TEA^+ binding site as well as changes in $[K^+]_o$ can also drastically change C-type inactivation[4].

A new and exciting view on the C-type inactivated *Shaker* channels was presented by Stefan Heinemann (Max-Plack-Gesellschaft, Molecular & Cellular Biophysics, Jena, Germany). Through excellent measurements removing the K^+ concentration on both sides of the membrane he revealed that instead of being shut and impermeant, C-type inactivated channels change their permeability to become selective for Na^+ and Li^+ over K^+. Moreover, the channels are then blocked efficiently by K^+.

The last speaker in the session on gating was Ehud Isacoff (University of California, Berkeley, USA), who described experiments using voltage clamp fluorimetry to study rearrangement movements of a K^+ channel during gating, i.e. environmentally sensitive fluorophores were conjugated to cysteines that had been introduced at specific positions on S4 of the channel protein. The hypothesis was that if the cysteine labelled with the fluorophore would change its environment during gating the fluorophore would rapidly report a shift in fluorescence emission. Some of his results could be explained by Diane Papazian (University of California, Los Angeles, USA), who raised the possibility that the S4 segment of *Shaker* channels might not have the same orientation as the S2 and S3 segments but may instead be slightly tilted.

K^+ channel evolution

A good case for using data from the nematode *Caenorhabditis elegans* genome-sequencing project as a tool for the discovery of novel K^+ channel genes was emphasized by Larry Salkoff (Washington University School of Medicine, St Louis, USA), who gave an overview of all the K^+ channel genes that had been identified during this project so far. Interestingly and most intriguing was the fact that *C. elegans*, although a simple organism with only 302 neurones, has a genome consisting of between 20 000 and 50 000 genes. By way of comparison, the human organism, whilst containing 10^{11} neurones, has been estimated to have

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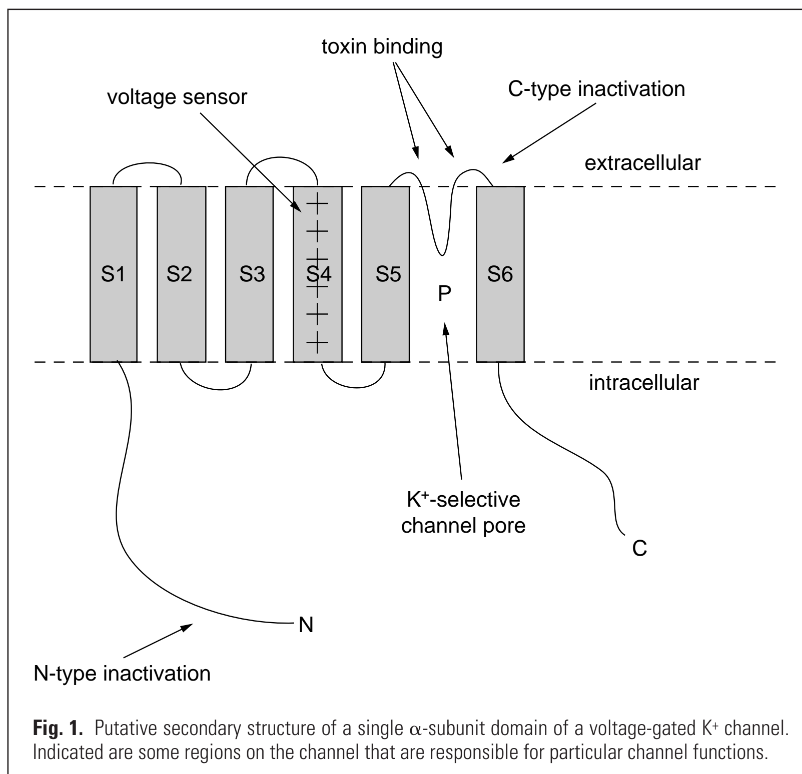

Fig. 1. Putative secondary structure of a single α-subunit domain of a voltage-gated K^+ channel. Indicated are some regions on the channel that are responsible for particular channel functions.

only 80 000–100 000 genes in its genome (a factor of only 2–5 times more than *C. elegans*). Within its genome, *C. elegans* has genes coding for the whole set of K^+ channels also used in mammals (Fig. 2). The questions about why *C. elegans* has all the K^+ channel genes, especially in the light of the dramatic differences in the apparent functional complexity of the nervous system in mammals compared to *C. elegans,* was enthusiastically discussed. The expression of a family of K^+ channels with two putative pore domains and one of their possible functional roles was discussed and reported by Steve Goldstein (Yale University School of Medicine, New Haven, USA). He suggested them to be responsible for generating a resting membrane potential (K^+-selective open rectifier or leak channels).

Very elegant studies illustrating the large structural and potentially functional diversity of channels in the brain were described first by Oliver Dolly (Imperial College, London, UK) and later by Jochen Röper (Center for Molecular Biology, Hamburg, Germany). Dolly had a vast body of data that showed a huge range of subunit combinations in different brain regions. Dolly confined the creation of diversity of K^+ channel subtypes through subunit combinations not only to the α- but also to the different β-subunits in mammalian brain. Jochen Röper concentrated on the functional consequences of interaction between α- and β-subunits and identified an N-type inactivation prevention (NIP) domain, which he also called IND (inactivation-neutralization domain), in Kv1.6 that could prevent the interaction of the inactivation particle of Kvβ1.1 with the α-subunit of Kv1.6 and could pinpoint this IND to a couple of amino acids in the N-terminus of the α-subunit of Kv1.6.

K^+ channels in disease

K^+ channel dysfunction has been shown so far to be linked to a small number of disease states. So-called 'K^+ channelopathies' have been identified so far for the Long QT-Syndrome type 1 and 2 with mutations found in the voltage-activated long Q–T channel-1, KvLQT1, and the human ether-à-go-go related channel HERG as well as for episodic ataxia with myokymia (EA1) in which mutations were found in Kv1.1. During this conference at least one more disease was introduced that might also be explained through a dysfunction of K^+ channels. George K. Chandy (University of California, Irvine, USA) reported on a new *Shaker*-family member, Kv1.7. An overexpression of Kv1.7 in pancreatic islet cells of diabetic mice might influence insulin secretion and cause diabetes. Chandy's model predicted that during glucose application, functional K_{ATP} would be blocked leading to a slow membrane depolarization. At the threshold, voltage-gated Ca^{2+} channels would frequently open and close, Ca^{2+} would rush into the cell leading to an increase in $[Ca^{2+}]_i$ that in turn would then induce insulin secretion. Overexpression of Kv1.7, a rapidly activating voltage-gated K^+ channel, could rapidly repolarize the membrane during the Ca^{2+} spikes, would therefore determine the frequency of Ca^{2+} spikes during the depolarization and ultimately reduce Ca^{2+}-influx. As a result of reduced Ca^{2+} influx, the increase in $[Ca^{2+}]_i$ would be less and would lead in turn to less insulin secretion.

Cloning discoveries

The seemingly never-ending story about the cloning of new K^+ channel types was told by John Adelman (Oregon Health Sciences University, Portland, USA). He started by describing the functional characteristics of the cloned small conductance Ca^{2+}-activated K^+ channel, SK, that is apamin-sensitive, followed by the characterization of a newly cloned Ca^{2+}-activated K^+ channel with intermediate conductance, IK, which is sensitive to block by charybdotoxin (CTX) and might represent the Gardos channel. The apamin-sensitive SK channel might also be involved in certain muscle diseases since it had been shown (and Adelman confirmed this by northern blot analysis) that these channels appear after denervation in adult muscle. The question of how the upregulation of apamin-sensitive SK channels might cause pathologies such as myotonic dystrophy remains, however, unanswered.

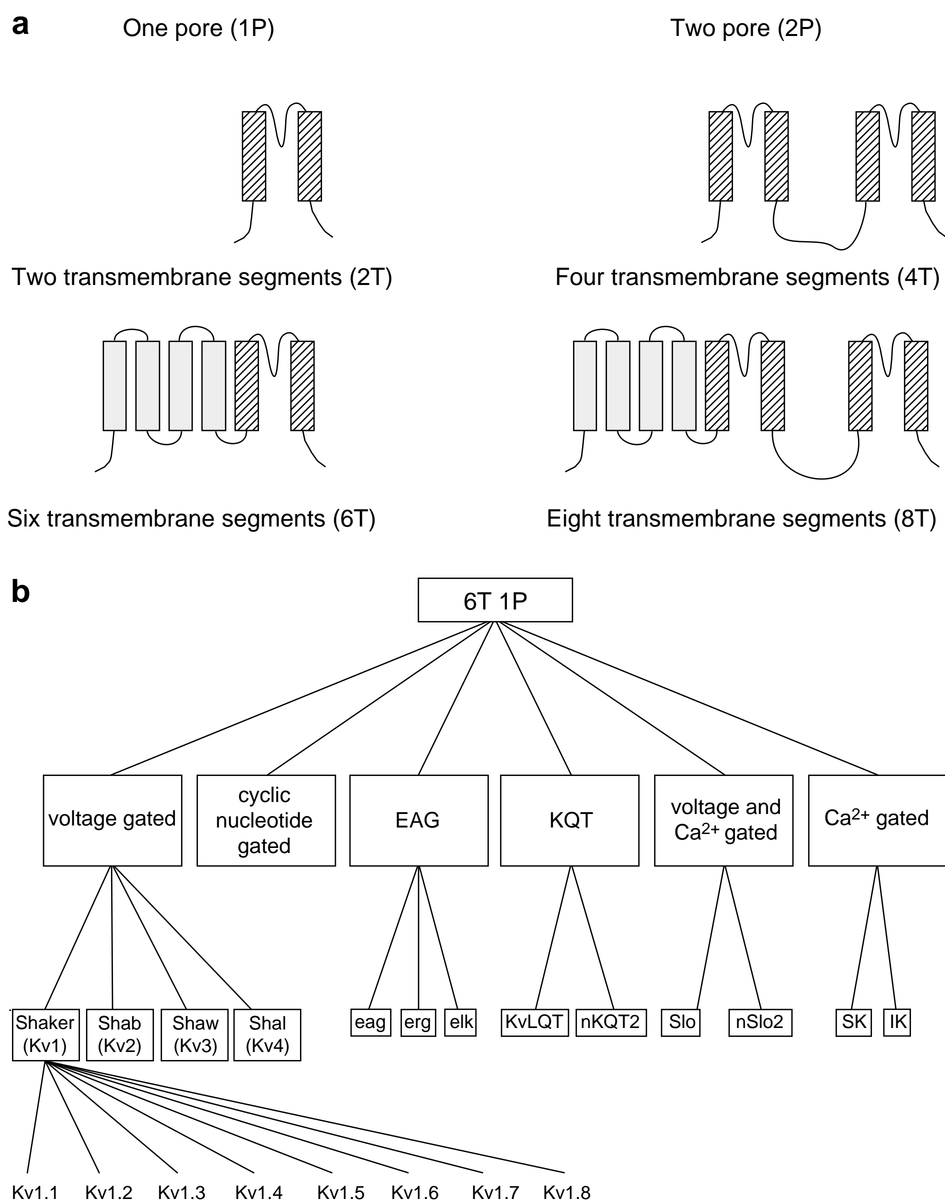

Fig. 2. Structural classes of K^+ channels. **a:** K^+ channels can be grouped into four main structural classes on the basis of their transmembrane (TM) and pore (P) segments. **b:** Hierarchical classification for the 6T 1P structural classes of K^+ channels. Adapted from Ref. 5.

Similar Ca^{2+}-activated K^+ channels could be responsible for the slow afterhyperpolarization (sAHP) observed in hippocampal pyramidal cells following action potential depolarizations. Paola Pedarzani (Max-Planck-Institute for Experimental Medicine, Göttingen, Germany) showed that although these sAHP are not sensitive either to CTX or apamin, the channels might be other members of the SK or IK family as yet not cloned. Interestingly the sAHP in these cells is not only highly sensitive to a variety of neurotransmitters but might be regulated by an ongoing phosphorylation/dephosphorylation process even in the absence of neurotransmitters.

Ca^{2+}-activated K^+ channels

The session about Ca^{2+}-activated high conductance K^+ channels (BK) led to new information of the functional consequences of the interaction of the α- and β-subunit. Maria Garcia (Merck Research Laboratories, Rahway, USA) emphasized the weaker CTX binding kinetics on membranes containing only α-subunits compared to membranes containing α + β-subunits, suggesting that the β-subunit influences CTX binding. Ligia Toro (University of California, Los Angeles, USA) reported and presented evidence that the N-terminus of the α-subunit is extracellular and therefore possesses an additional transmembrane segment, S0. The coupling of the two subunits might be sensitive to $[Ca^{2+}]_i$ and the region of interaction in the α-subunit is most likely to be the S0 segment.

The question whether BK channels can play a functional role in cell physiology, since their activation voltage seems very depolarized even

at high $[Ca^{2+}]_i$, was explicitly answered by Soeren-Peter Olesen (NeuroSearch, Glostrup, Denmark). Using a cell line that stably expresses *hslo* α + β-subunits in combination with transient voltage ramps that simulated action potentials, he showed that these channels could be activated and could therefore be responsible for the fast hyperpolarization observed in excitable cells.

Knocking out a channel

A similar question about the functional role of certain K^+ channels in cell and even animal physiology was brought into discussion by the presentation from Rolf Joho (University of Texas Southwestern Medical Center, Dallas, USA). He described in detail the physiological and behavioral alterations in a mutant mouse deficient for Kv3.1. Several changes in the mutant mice occurred compared to normal mice, i.e. motor skills were less coordinated in the mutant mice, neuronal synchronization and slow-wave activity in the EEG were decreased in the mutant mice, etc. In spite of these changes it was heated discussion whether other channels could rescue some of the functions of Kv3.1 in the mutants and whether it would be feasible to pinpoint the physiological function of a channel by using this approach.

Inward rectifiers

A session on inward rectifier channels started the scientific programme on the last day of the meeting. Matt Kennedy (Harvard Medical School, Boston, USA) concentrated in his presentation on the class of inward rectifier channels that are directly gated by G proteins (GIRK). Coexpression studies with combinations of different GIRK subunits revealed that heteromeric channels might produce much more current than homomeric channels alone. Andreas Karschin (Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany) discussed the differential regulation of different inward rectifier subtypes through neurotransmitter receptors. This form of channel regulation seems very complex since depending on the subunit composition, current can either be reduced or enlarged. For example, in cells which express the $5\text{-}HT_{1A}$ receptor, application of receptor agonist can either reduce or increase current through channels that are composed of Kir3.1 + Kir3.2 or Kir3.1 + Kir3.4 subunits, respectively. Peter Ruppersberg (University of Tübingen, Germany) presented a more biophysical approach on the understanding of the mechanism of the steep inward rectification. As a model he used ROMK1 and the voltage-dependent block by tetrapentylammonium (T5A) since the interaction of the channel and the drug is slow enough to be in a time range that can be studied using rapid application of drug. His results led him to the conclusion that short-range interactions between the blocking and the bulk ions (within the channel) can contribute to the steep inward rectification.

The I_{SK} KvLQT1 channel complex

The last session of the conference focused on studies which showed the formation of a channel complex consisting of KvLQT1 (a classical K^+ channel protein with six transmembrane regions with one pore structure) and another component, termed minK or I_{SK} (a small protein with a single transmembrane domain) resulting in heteromeric channel with new properties. A number of reports have suggested that this channel complex has importance in certain disease states. Michel Lazdunski (CNRS, Valbonne, France) suggested that the C-terminal end of minK can interact with the pore region of KvLQT1 resulting in prolonged openings with smaller single-channel conductance. Andreas Busch (University of Tübingen, Germany) concentrated in his presentation on the pharmacological properties of the channel complex and the contribution of the I_{SK} protein to these properties; for example, KvLQT1 on its own in the membrane is much less sensitive to block by 293B when compared to the channel complex.

One of the highlights of the IPCC was the presentation of the keynote lecture by Hans Meves (University of the Saarland, Homburg, Germany). He reviewed K^+ channel research from his personal viewpoint starting from work in the early 50s about the doubts in the research community whether special channels for K^+ exist to end with the description of single channel, channel structures, as well as specific roles of K^+ channels in cell function. One of his messages was to celebrate the tenth anniversary of the first K^+ channel gene cloned from *Drosophila* independently from three different groups around Lily Jan (University of California, San Francisco, USA), Mark Tanouye (then at CalTech, Pasadena, USA now at the University of California, Berkeley, USA), and Olaf Pongs (then at Ruhr-University, Bochum, Germany now at the Center for Molecular Biology, Hamburg, Germany).

During the poster sessions research was presented on the regulation/modulation of K^+ channels by intracellular components such as ATP, G proteins and β-subunits, etc. Several new toxins and other substances were introduced at the meeting that might help to elucidate channel structure or might be helpful in being used as model drugs to design better and more specific drugs for certain K^+ channel types. This meeting clearly showed that K^+ channel research is still growing and has not reached its peak yet. Future developments will further increase our knowledge about K^+ channels, how they function and what role they play in a variety of different cell types.

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