AuPS/ASB Meeting - Newcastle 2007

Symposium: Potassium channels

Monday 3 December 2007 – Mulubinba Room

Chair: Jamie Vandenberg

Location and function of SK channels in pyramidal neurons in the amygdala

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SK channels are small conductance calcium activated potassium channels. In neurons these channels have traditionally been found to be activated by calcium influx during action potentials and their activation contributes to the control of neuronal firing frequency. Calcium influx during action potentials activated a number of calcium dependent potasisum currents that contribute to action potential repolarisation and the afterhyperpolarisation (AHP) that follows that action potential. SK channels contribute only to the AHP, however, bockade of these channels in amygdala neurons has little effect on neuronal firing properties. We have recently shown that these channels are also expressed at excitatory synapses in lateral amygdala pyramidal neurons where they are activated by calcium influx *via* NMDA receptors. Activation of SK channels at excitatory synapses shunts the synaptic potential and thus reduces synaptic transmission. Blockade of these SK channels with specific blockers reduces the shunt and enhances synaptic transmission and summation of excitatory synaptic potentials. This results in an enhanced ability to induce long term potentiation. Application of neurotransmitters that activate protein kinase A reduced the number of synaptic SK channels and enhanced synaptic transmission and plasticity. These actions of neurotransmitters are mediated by altering the endogenous trafficking of SK channels and their delivery to excitatory synapses. These results show that SK channels, rather than controlling action potential frequency, as in some other cell types, play a key role in controlling the strength of synaptic transmission and contribute to the control of synaptic plasticity.

Potassium channel gating

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K^+ channels are essential for life and exist in countless forms in nature. Major families appear throughout the evolutionary tree, and their diversity reflects both a multitude of cellular functions and tissue/developmental specificity. K^+ channels are distinguished chiefly by the regulatory messages they respond to, and pore gating is in many cases mediated by domains or subunits surrounding the pore in a functional assembly. Although a small number of K^+ channel structures have now been reported, there is still no rational basis on which the molecular conformation observed in a crystal can be classified "closed" or "open" - other than by eyeballing the width of the pore aperture at the intracellular face. Judgements applied on this basis are ill advised, except in truly self-evident cases (*e.g.*, the MthK Ca^{2+}-gated channel). Removal of integral membrane proteins from the lipid bilayer can, and commonly does, lead to structural artefact that confuses the picture.

The research outlined here is aimed at correlating K^+ channel physiology with the structural rearrangement(s) accompanying opening. It will serve as a platform for interpreting the various means by which auxiliary domains of K^+ channels influence action. Our (continuing) experimental goal has been to obtain crystal structures of a K^+ channel of one type in conducting and non-conducting configurations, and, if possible, in intermediate gating states.

A successful collaboration with Dr D.A. Doyle (Oxford, U.K.) in 2003 allowed us to determine and publish the three-dimensional structure of a complete prokaryotic potassium channel assembly of the inward rectifier family (Kuo *et al*., 2003). KirBac1.1 was the first X-ray structure of an integral membrane protein to have been determined in Australia. The pore of KirBac1.1 was unequivocally closed to ion conduction, and the structure established that intracellular regions are coupled to the activation gate of the channel. In collaboration with Doyle we have since determined structures of a close homologue, KirBac3.1, in different conformations (PDB codes for two sets of deposited coordinates: 1XL4 and 1XL6). These provide the first structural data on the binding sites for divalent ions and polycations during current rectification. There are subtle but significant differences in the conformers, which we suggest represent a progression of changes during the closed to open transition. These global rearrangements, encompass systematic changes in the ion selectivity filter right through to the distal cytoplasmic domains. The study reveals that gating of KirBac channels proceeds *via* an asymmetric intermediate. Our results indicate one structure technically represents an open pore (conducting) state, despite a relatively narrow aperture at the cytoplasmic face. Further widening of the aperture is prohibited due to constraints imposed by the lattice packing. To confirm the parameters of a conducting channel, a wide-open conformer must also be determined and conform to the same pattern of molecular changes. Only in this way can we establish firm criteria that define the gating states. Our group is now working at crystallising an unambiguously open channel, and a closed channel of KirBac3.1. We are producing inward rectifier K^+ channels as recombinant proteins in *E. coli*, and extracting them from the membrane fraction. Whereas the KirBac3.1 structures were obtained from soaking additives into crystals of one particular form, the underlying crystal lattice is, as mentioned, unfortunately incompatible with obtaining a conformer with a wider pore aperture. We have, however, recently obtained crystals under completely different precipitant conditions. Our next step is to test these for diffraction and put them through a series of iterative improvement steps.

This study will provide a starting point for deciphering whether a common mechanism for gating the pore is maintained in all K^+ channels, evidenced by the nature of conformational changes in the pore and adjoining regions, or if the family genres markedly differ.

Kuo A, Gulbis JM & Doyle DA.(2003) *Science*, **300:** 1922-6.

A link to two-pore domain potassium channel regulation

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The two-pore domain potassium (K2P) channel family play an important role in the regulation of the resting membrane potential and excitability of cells. So named as each α-subunit of these channels contains two pore domains, the K2P channels also contain an unusually large extracellular pre-pore linker called the M1P1 loop.

There are currently 15 members of this family, which can be divided into 6 sub-families on the basis of structural and functional properties. Among these subfamilies is the Tandem pore Acid Sensitive K subfamily (TASK1 (K2P 3.1), TASK3 (K2P9.1) & TASK5 (K2P 15.1)). As their name suggests, the TASK channels are sensitive to extracellular acidification, with a histidine at position 98 shown to be crucial for the pH sensitivity of TASK1 and TASK3 channels (see Kim, 2005). We have shown previously that zinc is a selective blocker of TASK3 channels with little effect on TASK1 in physiological conditions (Clarke *et al.*, 2004). This selective block involves both H98 and a glutamate residue (E70) within the M1P1 loop, suggesting that the M1P1 loop may lie close to the pore and play an important role in channel regulation (Clarke *et al.*, 2004). The long extracellular M1P1 loop is not conserved in potassium channels with known crystal structures. However, a structural homology model of TASK1 based on known potassium channel structures was recently published (Yuill *et al.*, 2007) where the M1P1 loops are assumed to lie in close association at position N53, a position homologous to TWIK1 C69, an M1P1 cysteine responsible for channel dimerization (Lesage *et al.*, 1996).

In this study we create cysteine mutants and M1P1 chimaeric channels using standard PCR techniques. The cDNA of these mutants was injected into *Xenopus laevis* oocytes and 1-3 days later used for two-electrode voltage clamp recordings exactly as previously described (Clarke *et al.*, 2004). All experiments were approved by the Animal Ethics Committee of the University of Sydney. We demonstrate that while engineered cysteines at TASK3 N53 on opposing M1P1 loops do not appear to form disulphide bonds, cysteines at positions 70 and 98 are able to form both inter- and intra-subunit spontaneous disulphide bonds therefore the M1P1 loops of TASK3 channels lie in close apposition to the pore. Through NMR Spectroscopy studies we show that the M1P1 loops are likely to form alpha helices and chimaeric exchange of the M1P1 loops between TASK channels demonstrates that this unusual linker plays a role in the TASK channel pH sensing mechanism.

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Mapping the insectophore of κ-atracotoxins: insect-selective BK_{Ca} channel blockers that reveal a novel insecticide target

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Arthropod pests vector numerous pathogens of human and veterinary health importance, and they destroy ~10% of the world's food supply (Oerke & Dehne, 2004). Despite the introduction of transgenic crops and other biological control methods, chemical insecticides remain the dominant approach for combating these arthropods. Unfortunately, the vast majority of these insecticides act on one of just five nervous system targets and reliance on just a handful of targets has promoted the evolution of insecticide resistance. Along with human and environmental health concerns, the problem of insecticide resistance has intensified the search for new insect-control methods.

The Janus-faced atracotoxins (J-ACTXs) are a family of insect-selective excitatory neurotoxins from Australian funnel-web spiders that are lethal to both agronomically and clinically relevant insects (Wang *et al.*, 2000; Maggio & King, 2002b) but display no activity in vertebrates (Wang *et al.*, 2000). These toxins possess a rare vicinal disulfide bond between two adjacent cysteine residues that is critical for activity (Wang *et al.*, 2000). An alanine scan of the representative family member J-ACTX-Hv1c has delineated the key functional residues of the toxin (Maggio & King, 2002 a&b). This epitope is restricted to five key residues (Arg^8, Pro^9, Cys^{13}-Cys^{14} and Tyr^{31}) that form a bipartite surface patch on a single face of the toxin structure. Despite this wealth of structural data, their molecular target has proved elusive.

We previously speculated (Maggio & King, 2002b) that the J-ACTXs might target voltage-gated potassium (K_V) channels since the pharmacophore residues Arg^8 and Tyr^{31} overlay well with the conserved LysTyr/Phe dyad of vertebrate K_V channel blockers (Dauplais *et al.*, 1997). In the present study, we tested this hypothesis by whole-cell patch-clamp analysis of cockroach dorsal unpaired median (DUM) neurons. Based on this finding we have renamed this toxin family κ-ACTX-1 based on the previously established nomenclature for atracotoxins.

κ-ACTX-Hv1c selectively blocked cockroach calcium-activated K^+ (K_{Ca}) channels with an IC_{50} of 2 nM, but not other insect voltage-gated K_V, Na_V or Ca_V channels. κ-ACTX-Hv1c also blocked heterologously expressed cockroach BK_{Ca} (*pSlo*) channels without a significant shift in the voltage-dependence of activation. Moreover, the block was voltage-dependent, indicating that κ-ACTX-Hv1c is likely to be a pore blocker rather than a gating modifier. The molecular basis of the insect selectivity of κ-ACTX-Hv1c was established by its failure to significantly inhibit mouse *mSlo* currents (IC_{50} ~10 μM) and its lack of activity on rat dorsal root ganglion neuron $I_{K(Ca)}$. We used a panel of point mutants to identify the molecular epitope (insectophore) on the toxin that mediates its interaction with K_{Ca} channels, and we show that this insectophore is strikingly different to that of vertebrate K_V channel toxins.

κ-ACTX-Hv1c is the first insect-specific K^+ channel blocker identified from spider venom and its lethal block of insect BK_{Ca} channels validates these ion channels as a potential insecticide target. Moreover, the channel-binding epitope of the toxin mapped in the current study provides a template for the rational design of novel chemical insecticides that act specifically on insect BK_{Ca} channels.

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