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Gating and permeation of G protein coupled potassium channels

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Ion channels are elementary excitable units integrated in the cell membrane of nerve, muscle and other tissues. They produce and transduce electrical signals in living cells. Their physiological roles are diverse from being responsible for the generation and propagation of nerve impulses, synaptic transmission, muscle contraction, salt balance and hormone release. Thus, due to their diverse physiological role, they have been targeted pharmacologically, and many drugs have been developed as local and general anesthetics, muscle relaxants, cardiac anti- arrhythmic and oral hypoglycemics. Ion channels have also been found to be involved in many genetic diseases such as cystic fibrosis, cardiac arrhythmia, Liddle syndrome (hypertension) and ataxia. Thus, understanding structural and functional aspects of ion channels is of great importance.

One subset of K^+ selective channels, the G protein coupled inwardly rectifying K^+ channels (GIRK), are the main focus of the laboratory. Neurotransmitters such as dopamine and GABA exert their inhibitory actions, in part, by activating GIRK channels. These channels permit K^+ ion flux at membrane potentials near the cell's resting potential, but not at more depolarized potentials, thereby decreasing membrane excitability. GIRK channels, which are activated via G protein-coupled neurotransmitter receptors are found in neurons, in heart and pancreatic tissues. In the central nervous system, for example in the hippocampus, GIRK channels were found to increase K^+ conductance at the postsynaptic, but not at the presynaptic cleft, to mediate inhibitory neurotransmission (Fig. 1A). In the autonomic nervous system, the best example for the involvement of GIRK channels is the regulation of the heartbeat by the parasympathetic system via the vagus nerve. Overall, common in all systems examined, GIRK channels are activated via stimulation of pertussis toxin-sensitive G protein coupled receptors. The activation of these channels is mediated via direct binding of the free $G\beta\gamma$ subunits of the G protein, released from the G protein trimer following receptor stimulation. Despite the available information regarding the elements involved in this gating action, there is still a gap in our understanding of the coupling between stimulus detection, $G\beta\gamma$ binding, and the transduction of these events to promote ion flux through the channel pore. This issue of coupling also pertains to a rather universal open question related to all ion channels having regulated openings. Since the pore region of GIRK channels has a high sequence homology to the pore region of all K^+ channels superfamily, and has a general architecture of ion channels found in many species from archea to human, they can also serve as classical prototype of ion channels designed to translate intracellular chemical transmission to electrical signaling. Our laboratory is specifically involved in studies designed to identify molecular determinants involved in channel gating, proteins involved in channel sub-cellular localization and the dynamics of channel gating. Molecular biology, advanced electrophysiology and fluorescent techniques are being employed in the various projects.

In an initial attempt designed to answer some of the questions

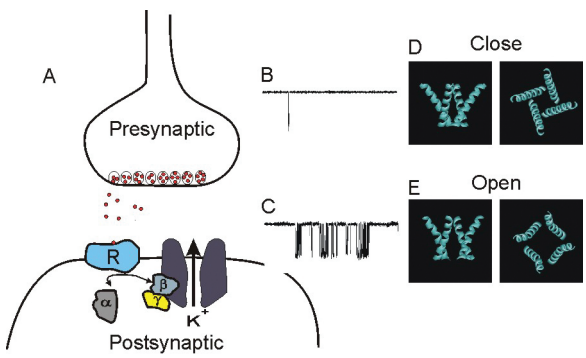

Fig. 1 *Following receptor stimulation $G\beta\gamma$ is free to gate the channel, A. Gating of GIRK single channel is mainly characterized by an increased channel bursting, B vs. C. A model depicting the second transmembrane domain rearrangement during channel gating, D and E, respectively.*

raised above. We have developed a yeast-based novel screen that enables the identification of elements involved in late coupling events just preceding channel pore openings, which were otherwise impossible to detect by conventional biochemical or structure-functional approaches. The screen was based on functional complementation of a yeast strain that lacks both high and low K^+ transport systems. Mutant channels with impaired gating, e.g., constitutively active G protein independent, were

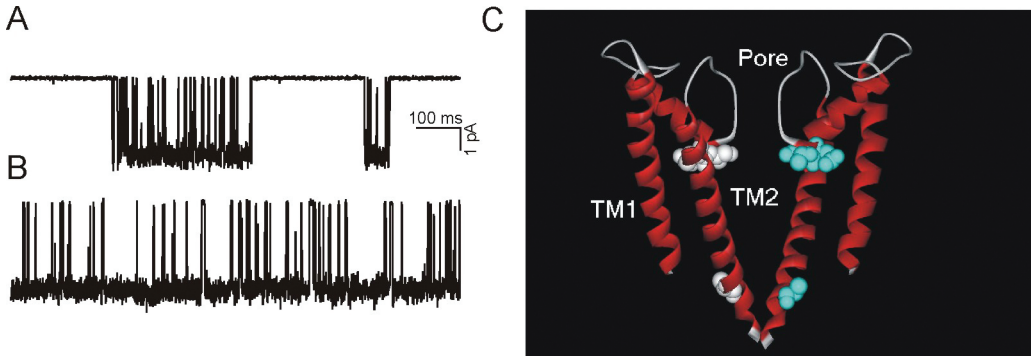

Fig. 2 *Stabilization of the channel opening state (A vs. B) by mutation in the pore and the second transmembrane domain (side chains of mutant residues in CPK),C.*

able to rescue the yeast phenotype. This screen then allowed us to randomly mutate the full-length channel molecule with no bias toward any particular molecular entity. Using this approach we were able to screen tens of thousands of mutations simultaneously. Using these strategies, several important observations related to channel function were obtained, such as, we were able to demonstrate that the link between binding of the G protein subunits to the cytoplasmic domains and opening of the channel pore is mediated through a specific transmembrane element. We were also able to show, at the single molecule level, that the open gating state of the channel is mainly determined by the stabilization of channel bursting, a cluster of channel openings and closings separated by long closure times. Thus, at the single channel level, gating control is mediated by both the second transmembrane domain of the channel, and elements in the channel pore, the permeation pathway (Fig. 2).

Selected Publications

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