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Mechanisms Of Ion Channel Function And Regulation

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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, 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, thereby decreasing membrane excitability. GIRK channels, which are activated via G protein-coupled neurotransmitter receptors (GPCRs) are found in neurons and 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 1). 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 only pertussis toxin-sensitive GPCRs. The activation of these channels is mediated via direct binding of the free Gβγ 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, Gβγ

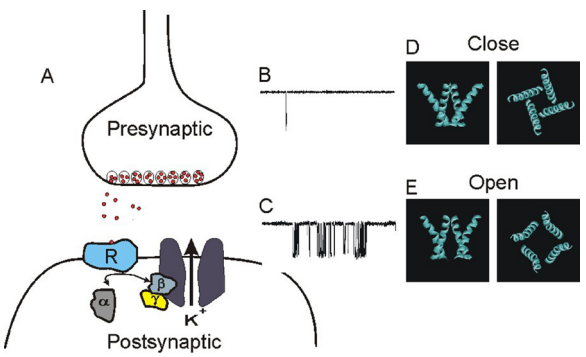

Fig. 1 *Following receptor stimulation Gβγ is free to gate the channel, A. Gating of GIRK single channel is mainly characterized by an increased channel bursting, B vs. C. A model depicting the second transmembrane domain rearrangement during channel gating, D.*

binding, gating specificity, and the transduction of these events to promote ion flux. These issues of coupling also pertain 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.

To answer some of the questions raised above. We are currently using yeast-based screens to

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. We also employ various molecular techniques to understand channel function at the single molecule level mainly by using patch clamp single channel recordings and single molecule fluorescence. Conformational dynamics and, signaling specificity of channel gating are being investigated using fluorescence resonance energy transfer (FRET) combined with advanced microscopy techniques (Fig 2). We are currently developing non conventional patch clamp recording techniques to enable the dual measurements of structural changes associated with channel function in combination with atomic force microscopy (AFM). Metabolic regulation and subcellular localization of the channels are being investigated at two levels, one using conventional biochemical electrophysiological approaches and second by the mass production of channel domains for structural and proteomic studies. The involvement of GIRK channel in pancreatic beta cell function is studies using both cell line and transgenic mice.

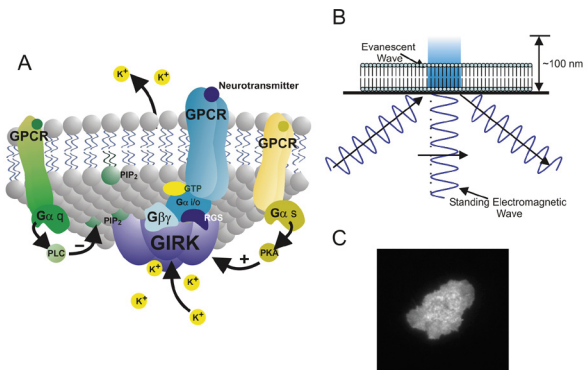

Fig. 2 *A, view of the membrane associated signaling complex that modulate GIRK channels. B, principles of total internal reflection (TIRF) microscopy and their used to selectively image cell membrane associated signaling proteins. C, TIRF image of a cell expressing fluorescently-labeled GIRK channels.*

Selected Publications

Kubo, Y., Reuveny, E. Slesinger, P.A., Jan, Y.N. and Jan, L.Y. (1993) Primary structure and functional expression of a rat G protein coupled muscarinic potassium channel. Nature 364, 802-806.

Reuveny, E., Slesinger, P.A., Inglese, J., Morales, J.M., Iniguez-Lluhi, J., Lefkowitz, R.J., Bourne, H.R., Jan, Y.N. and Jan L.Y. (1994) Activation of the cloned muscarinic potassium channels by G protein bg subunits. Nature 370, 143-146.

Slesinger, P.A., Reuveny, E., Jan, Y.N. and Jan, L.Y. (1995) Identification of cytoplasmic structures involved in G-protein gating of the muscarinic potassium channel, GIRK1. Neuron 15, 1145-1156.

Ford, C.E., Skiba, N.P. Bae, H., Daaka, Y., Reuveny, E., Shekter, L.R., Rossal, R., Weng, W., Yang, C.S., Iyengar, R., Miller, R.J., Jan, L.Y., Lefkowitz, R.J. and Hamm, H.E. (1998) Molecular basis for interactions of G protein bg subunits with effectors. Science, 280, 1271-1274.

Sadja R., Smadja, K. Alagem, N. and Reuveny, E. (2001) Coupling Gβγ-dependent activation to channel opening via pore elements in inwardly rectifying potassium channels. Neuron 29, 669-680.

Alagem, N., Devir, M. and Reuveny, E. (2001) Mechanism of $Ba2^{+}$ block of inwardly rectifying K^{+} channel: Differential contribution by two discrete residues. J. Physiol. 534.2 381-393.

Zeidner, G., Sadja, R. and Reuveny, E. (2001) Redox-dependent gating of G protein coupled inwardly rectifying potassium channels. J. Biol. Chem. 276, 35564-35570.

Sadja, R, Alagem,N. and Reuveny, E. (2002) Graded contribution of the Gβγ binding domains to GIRK channel activation. Proc. Natl. Acad. Sci. USA 99, 10783-10788.

Reuveny, E. Trapping the sensor. (2002) Neuron, 35, 814-815.

Riven, I., Kalmanzon, E., Segev, L. and Reuveny, E. (2003) Conformational rearrangements associated with the gating of the G protein coupled potassium revealed by FRET microscopy. Neuron, 38, 225-35.

Alagem, N., Yesylevskyy, S. and Reuveny, E. (2003) Control of Open State Stability by the Pore Helix in inwardly rectifying K^{+} Channels. Biophys. J. 85, 300-312.

Sadja, R. Alagem, N. and Reuveny, E. (2003) Gating of GIRK channels: Details of intricate, membrane delimited signaling complex. Neuron 39, 9-12.

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