Ion Channels: From Single Molecule Studies to Function *in-vivo*

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Ion channels are elementary excitable units integrated in the cell membranes of all 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, such 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.

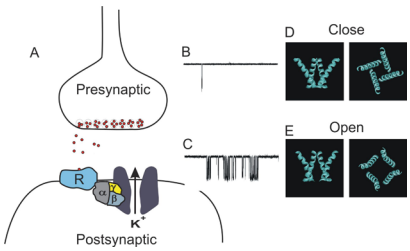

Fig. 1 *Following receptor stimulation Gbg is free to gate the channel, A. Gating of GIRK single channel is mainly characterized by an increased channel bursting, B vs. C. A model depicting the second transmembrane domain rearrangement during channel gating, D.*

One subset of K^+ selective channels, the G protein coupled inwardly rectifying K^+ channels (GIRK), are the main focus of the laboratory. These channels are involved in many physiological responses that include, regulation of heart beat rate, nociceptive pain, hormone secretion and control of seizures. Neurotransmitters such as dopamine, serotonin, adenosine and GABA exert their inhibitory actions, in part, by activating GIRK channels at the post synaptic membrane. 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, heart and endocrine 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 binding, gating specificity, and the transduction of these events to promote ion flux (Figure 2). 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.

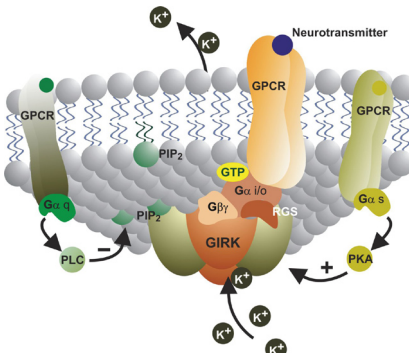

Fig. 2. *View of the membrane associated signaling complex that modulate GIRK channels.*

To answer some of the questions raised above. We are currently using yeast-based screens to identify elements involved in gating specificity 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 (Fig. 3). Conformational dynamics and, signaling specificity of channel gating are being investigated using fluorescence resonance energy transfer (FRET) combined with advanced microscopy techniques. To monitor both signaling protein dynamics as well as intramolecular protein rearrangements.

We are currently developing mouse lines to study, both neurological manifestation of Down's syndrome, and normal ion channel functional plasticity using fluorescence techniques (Fig. 4). Metabolic regulation and subcellular localization of the channels are being investigated at two levels, one using conventional biochemical and electrophysiological approaches and second by the mass production of channel domains for structural and proteomic studies. Finally, a more general fluorescent based approach is being developed to detect G protein associated signaling in living cells for the generation of both nanosensors for GPCR activity and for orphan GPCRs drug discovery approaches.

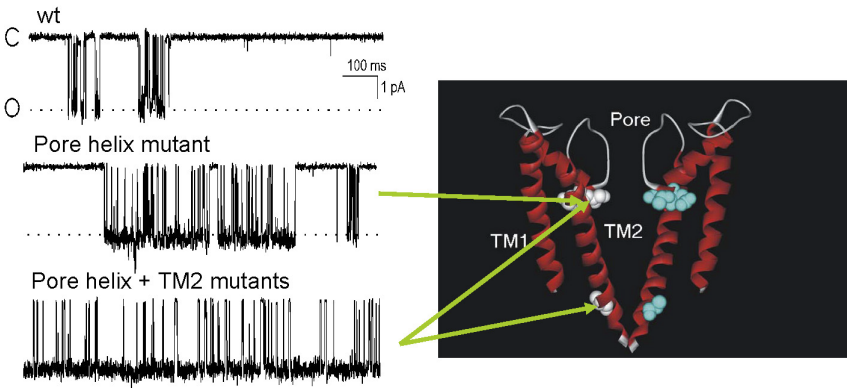

Fig 3. *Single channel recordings traces (right) of mutants shown in the structure model of KcsA (left).*

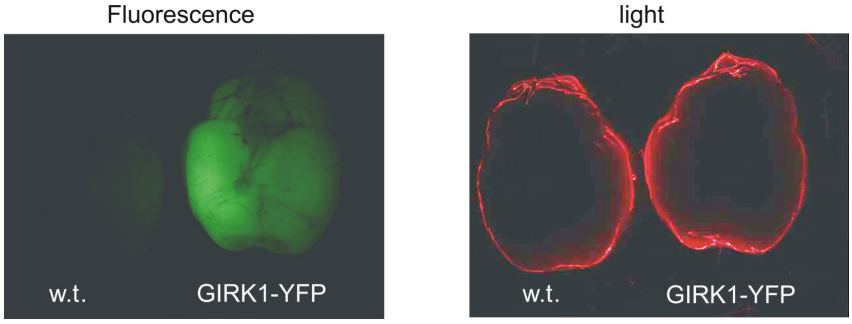

Fig 4. *Brain from a wt and knockin mice where the GIRK1 gene was replaced with a wt gene fused with yellow fluorescent protein (YFP). Left panel, a fluorescent image (only the brain from the knockin mouse is fluorescing- right brain). Right panel, the same brains under normal illumination from the bottom.*

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