a mutation located between S6 and the putative ATP binding site is found to alter the coupling between ATP binding and channel opening by reducing ATP sensitivity and shifting the G-V relationship to more positive voltages at a given [ATP]; however, increasing [ATP] shifts the G-V back toward negative voltages. The effects of this mutation can be explained by an allosteric model for the coupling between ATP binding and channel opening in which the mutation reduces the allosteric factor for the coupling.

1829-Plat
PIP2 is Required to Couple the Voltage-Sensing Domain to the Pore for the Activation of KCNQ1 by Voltage
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Voltage-gated ion channels are vital for the generation of action potentials that orchestrate various physiological processes. Phosphatidylinositol 4,5-bisphophate (PIP2) is a membrane lipid that modulates several voltage-gated channels (TRP, HCN, CaV, Kv, KCNQ). KCNQ channels are unique in that they cannot be activated by voltage in the absence of PIP2 and the loss of KCNQ channel activity due to PIP2 depletion is known to be physiologically relevant. Although residues governing PIP2 sensitivity have been identified for several KCNQ channels, it is unclear where PIP2 binds and what it does to channel gating. Voltage-dependent gating involves coupled conformational changes in the voltage-sensing domain (VSD) and the pore domain (PD) providing three possible mechanisms of PIP2 action: VSD activation, PD opening, or coupling between VSD activation and PD opening. We combine voltage-clamp fluorometry wih expression of a voltage-sensitive lipid-phosphatase in order to measure VSD activation and PD opening simultaneously while manipulating PIP2 abundance. We find that PIP2 is not necessary for VSD activation; however, PIP2 is required for VSD-PD coupling. Deriving a simple mathematical model, we find that PIP2 regulation of coupling is sufficient to recapitulate the experimental results without any direct effects on PD opening. These findings suggest PIP2 may bind at the VSD-PD interface that, in KCNQ1, is densely populated with basic residues near the intracellular face of the membrane. By mutating these residues and measuring the effects on channel function, PIP2 sensitivity and VSD-PD coupling, we propose a PIP2 binding site. In our resulting model, the negatively charged PIP2 headgroup resolves the electrostatic repulsion between basic residues on the VSD and the PD, thus holding the two domains together and allowing the transfer of conformational energy between them.

1830-Plat
Long QT Mutations and KCNE1 β-Subunit Modulate S4 Movement in KCNQ1 Channel
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The KCNQ1 potassium channel is expressed in many different cell types and plays widely different physiological roles. For example, in the heart KCNQ1 forms part of the voltage-gated IKs channels that contributes to limiting the duration of the cardiac action potential. The different functions of the KCNQ1 channel are mainly due to the co-assembly of the KCNQ1 channel with different beta subunits from the KCNE family. The IKS channel consists of 4 α-subunits (KCNQ1) which assemble with 2 to 4 β subunits (KCNE1). Mutations in either KCNQ1 or KCNE1 subunit cause multiple cardiac arrhythmia syndromes such as long QT syndrome, short QT syndrome, and familial atrial fibrillation. Here we use Voltage clamp fluorometry (VCF) to directly study the effects of wild type, mutant KCNQ1 and KCNE1 subunits on the voltage sensor movement in KCNQ1 channel. We assess the voltage sensor movement (fluorescence) and channel opening (current), in order to understand the coupling between the KCNQ1 voltage sensor and channel gate in the presence of KCNE1. Our data show that KCNE1 splits the voltage sensor movement in two separates phases. An early phase (1) involving voltage sensor movements to its active state upon changes in the membrane potential occurs at hyperpolarized potentials. A second phase (2) that happens at much more depolarized potentials involves an additional voltage sensor movement which is tightly coupled to channel opening. Mutations in KCNE1 that cause arrhythmia shift the voltage dependence of the voltage sensor movements of either phases 1 or 2 (or both), revealing some of the molecular mechanisms underlying the pathophysiology of these arrhythmia-causing mutations. Our data suggests a putative mechanism for how KCNE1 exerts its effects on the voltage sensor movement during IKs channel activation.

1831-Plat
Identification of a Novel PIP2 Interaction Site and its Allosteric Regulation by the RCK1 Site Associated with Ca^{2+} Coordination in Slo1 Channels
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PIP2 is necessary for activation of Slo1 channels, but the molecular determinants mediating its effects are not known. Slo1 channel activity is regulated by Ca^{2+} and membrane voltage. Increased Ca^{2+} has been reported to enhance PIP2 sensitivity but the mechanism is not understood. Here we combined electrophysiology, mutagenesis and computational modeling methods to identify critical Slo1 interaction sites with PIP2 and study their relationship to Ca^{2+} regulation. We first confirmed that Ca^{2+} indeed increases the apparent affinity for PIP2. We mutated each of the two reported Ca^{2+} coordination sites, in the RCK1 and RCK2 domains, and found that only the RCK1 site affected PIP2 sensitivity. Further mutagenesis results suggested that the RCK1 Ca^{2+} coordination site also modulates PIP2 sensitivity independently of the presence of Ca^{2+}. We have identified the positively charged residues K392/ R393 in the RCK1 domain, mutation of which greatly decreased PIP2 sensitivity of Slo1 channels. We found that RCK1 acidic residues involved in Ca^{2+} sensitivity together with a cluster of basic residues in close proximity all coupled with the PIP2 sensitive residues. We propose that Ca+ coordination by RCK1 acidic residues removes an inhibitory interaction with the PIP2 sensor, thus increasing channel activity.

1832-Plat
Free-Energy Relationships in Ion Channels Dually Activated by Voltage and Ligand
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Many ion channels are modulated by multiple stimuli which allow them to integrate a variety of cellular signals and precisely respond to physiological needs. Understanding how these different signaling pathways interact has been a challenge in part due to the complexity of underlying models. In this study, we analyzed the energetic relationships in polymodal ion channels using linkage principles. We first show that in proteins dually modulated by voltage and ligand, the net free-energy change can be obtained in a model-independent manner, by measuring the charge-voltage (QV) and the ligand binding curves, using the median transformation (Wyman, J., J. Am. Chem. Soc., 1964; Chowdhury, S., and Chanda, B., J. Gen. Phys., 2012). Next, we show that the voltage-dependent changes in ligand occupancy of the protein can be directly obtained by measuring the QV curves at multiple ligand concentrations, which also allow us to reconstruct ligand binding curves at different voltages. More significantly, we establish that the shift of the QV curve between zero and saturating ligand concentration is a direct estimate of the interaction energy between the ligand and voltage-dependent pathway. These free-energy relationships were tested by numerical simulations of a detailed gating model of the BK channel. Furthermore, as a proof of principle, we estimate the interaction energy between the ligand binding and voltage-dependent pathways for HCN2 channels whose ligand binding curves at various voltages are available. These emerging principles will be useful for high-throughput mutagenesis studies aimed at identifying interaction pathways between various regulatory domains in polymodal ion channels.

Platform: Voltage-gated Ca Channels

1833-Plat
Effects of a Novel Cav1.1-R1242G Mutation Leading to Hypokalemic Periodic Paralysis
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Hypokalemic periodic paralysis (HypoPP) is an autosomal dominant muscle disorder characterized by attacks of flaccid weakness associated with a decrease in the serum potassium level. HypoPP is caused by mutations in the skeletal muscle calcium ($Ca_v1.1$)?1 or sodium ($Na_v1.4$)? subunit. Up to date, 14 of 15 known HypoPP mutations affect the outmost two arginines in voltage-sensing S4 segments, suggesting the dysfunction of voltage sensor involved. A fresh perspective for HypoPP pathophysiology comes from the discovery of a hyperpolarization-induced inward gating pore current through the modified gating pore in $Na_v1.4$ HypoPP mutations. The spectrum of HypoPP mutations has not been defined, so it remains to be determined whether the theory of the gating pore current could account for all cases of HypoPP. For $Ca_v1.1$ HypoPP mutations, the investigation is impeded by the difficulty of heterologous expression. Here, we describe an American family with a HypoPP type