specific steps in voltage-activation path of an ion channel are altered by a potent allosteric inhibitor. The K^+ channel peptide inhibitor guangxitoxin-1E (GxTX) is partial inverse agonist of the voltage gated potassium channel subtype Kv2.1 from rat. At saturating concentrations of GxTX, Kv2.1 requires higher voltages to activate. The GxTX modulated conductance requires more voltage to activate. It has more sigmoidal activation kinetics, activates slower, and deactivates more rapidly. With GxTX bound, all gating charge requires more voltage to translocate, indicating that GxTX stabilizes resting voltage sensors by 20,000-fold, or 9 k_BT. Remarkably, with GxTX bound, the rate of fast outward gating charge movement lost its positive voltage dependence. This suggests that GxTX can bind to channels in all conformations, but when gating charge is most intracellular, the toxin-channel complex adopts a stable conformation from which escape is time-dependent, but not voltage-sensitive. Channel kinetics were analyzed with a model that included a single conducting state for the channel, preceded by two voltage-dependent gating steps in each subunit, one independent and one concerted between subunits. When fit to the sigmoidicity and activation kinetics of Kv2.1 ionic currents, this model was predictive of the measured gating currents. However, with GxTX bound, this model was no longer able to predict gating current kinetics, indicating that a new conformational transition was rate limiting toxin-channel activation. We conclude that GxTX modulates Kv2.1 ion channels by ''trapping'' each voltage sensor in a conformation which is insensitive to voltage, yet where all gating charge remains on the intracellular side of the transmembrane electric field. Following escape from the GxTX-''trapped'' state, gating charges can translocate outward, allowing channels to open with GxTX bound.

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KCNE1-Dependent Sumoylation of $K_V7.1$ Subunits Determines the Voltage-Dependence of Cardiac I_{Ks} Channels

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$K_V7.1$ and KCNE1 subunits assemble to form I_{Ks} channels, which are fundamental to cardiac repolarization. Here, we identify SUMOylation of I_{Ks} channels as a fundamental regulatory pathway in the heart. A report (Qi et al. Neuron. 2014) that partial deficiency of SENP2 deSUMOylase in mice produced seizures, bradycardia and sudden death in association with hyperSUMOylation of M-current channels ($K_V7.2/K_V7.3$) led us to study I_{Ks} currents in neonatal mice. We found intracellular application of SENP2 to increase I_{Ks} current due to a −20 mV shift in the voltage-dependence of activation ($V_{1/2}$); in contrast, SUMO2 application decreased the current due to a +20 mV shift in $V_{1/2}$. A 40 mV excursion in $V_{1/2}$ between the deSUMOylating and SUMOylating conditions was seen also when mouse or human $K_V7.1$ and KCNE1 subunits were expressed in Chinese hamster ovary (CHO) cells. Förster resonance energy transfer (FRET) confirmed co-assembly of YFP-SUMO2 and I_{Ks} channels formed with $K_V7.1$-CFP. Consistent with SUMOylation, mutation of a single target residue in $K_V7.1$ abolished FRET and the effects of SENP2 or SUMO2 on I_{Ks} current density. To count the number of SUMO2 subunits in I_{Ks} complexes, total internal reflection fluorescence (TIRF) microscopy with simultaneous two-color photobleaching was used. I_{Ks} channels carry a maximum of four SUMO2s, one on each Kv7.1 subunit. Unexpectedly, Kv7.1 channels studied in the absence of KCNE1 carry at most two SUMO2s despite having four available Kv7.1 SUMO-sites. Modification of both Kv7.1 channels and I_{Ks} channels by two SUMO2s produced a 20 mV shift in $V_{1/2}$ while four SUMO2s produced a 40 mV shift in I_{Ks} channels. We propose that KCNE1-dependent SUMOylation of $K_V7.1$ is required to yield the native biophysical attributes observed for I_{Ks} channels in mammalian cardiac myocytes.

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The Transitions between Two Open States of the KCNQ1 Potassium Channel Produce Inactivation-Like Phenotype

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In response to membrane depolarization the KCNQ1 channel undergoes activation as well as ''partial inactivation'', which is indicated by a ''hook'' in the tail current upon repolarization, fitting to the classic ''foot-in-the-door'' mechanism. KCNQ1 associates with the auxiliary subunit KCNE1 to form the I_{Ks} channel in the heart that is important for controlling heart rhythm. The KCNE1 association eliminates the hook current, which is one of the hallmarks of the radical modulation of channel function by the subunit. The mechanisms underlying this unique inactivation and its modulation by KCNE1 remain elusive. Recently, we have shown that the KCNQ1 channel opens during voltage sensor movements to both the intermediate and fully activated states, resulting in intermediate open (IO) and activated open (AO) states. In this study, we found that the previously called the open and inactivated states of KCNQ1 were actually the IO and AO states, respectively, and the hook currents came from the transitions between IO and AO. The appearance of hook currents and the AO state shows similar time course and voltage dependence and can be altered similarly by mutations. The association of KCNE1 suppresses the IO state, and the elimination of the transitions between IO and AO eliminates the hook current. These results indicate that the inactivation-like properties originate from the transitions between the IO and AO states. A kinetic model based on this mechanism provided an excellent fit to the voltage dependent activation and inactivation-like behavior of KCNQ1 channels.

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Kv1.2 Channels at the Interface of Redox and Electrical Excitability

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Kv1.2 is a prominent neuronal potassium channel subtype linked to severe epilepsies and movement disorders. We have demonstrated that neuronal and heterologously-expressed Kv1.2 channels exhibit use-dependent activation, a behavior characterized by progressive potentiation of channel activity during trains of repetitive stimuli. Use-dependent activation arises from a gating mode shift from a 'reluctant' slowly-activating mode to a 'willing' rapidly-activating mode with hyperpolarized voltage-dependence of activation. Moreover, Kv1.2 subunits recruit this use-dependent phenotype to heteromeric channel complexes. In this study, we demonstrate that use-dependent activation of Kv1.2 channel complexes is strongly regulated by a variety of exogenous and physiological redox species. Under ambient redox conditions, Kv1.2 channels exhibit marked cell-to-cell variability of use-dependent activation. However, exposure to mild reducing conditions normalizes this response, such that a pronounced use-dependent phenotype is consistently observed, together with a dramatic depolarizing shift of voltage-dependent activation with a V1/2 of 51+/- 6 mV. Mutagenesis of candidate cysteine residues in Kv1.2 did not affect redox sensitivity, therefore we hypothesize a role for an extrinsic redox-sensitive interacting partner. Using a variety of redox buffers, we demonstrate that use-dependent activation is steeply regulated by redox potentials between −50 and −100 mV, within the typical extracellular range. Furthermore, effects of membrane-impermeable reducing agents demonstrate that use-dependent activation is regulated by the extracellular redox state. Taken together, these findings suggest that Kv1.2 is a unique transducer of the extracellular environment, and may translate altered extracellular redox conditions to changes in cellular electrical function.

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Molecular Simulations of Ion Permeation in Potassium Channels

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Ion permeation in potassium channels is important in many physiological functions. The exact mechanism of potassium ions crossing their channels remains unknown and has to be further investigated. Currently, two main mechanisms are discussed: i) a 'knock-on' model, in which the selectivity filter of the channel is simultaneously occupied by two potassium ions, separated by water molecules, or ii) a 'direct knock-on' model, which predicts a higher, on average, number of ions in the filter, interacting on short distances via direct ion-ion contacts. Consequently, in the latter model, no water co-transport is expected to occur during ion permeation. Nowadays, atomistic computer simulations can reach timescales corresponding to thousands of ion permeation events, offering a direct visualization of the permeation mechanism, together with its underlying energetic profile. However, the fine details and microscopic steps of such permeation mechanism are expected to be extremely sensitive to the quality of approximations used to describe molecular interactions in a given molecular system. Therefore, a realistic mechanism can be derived from such simulations only when ion-protein interactions are described in an accurate manner. Herein, we use several simulation techniques, spanning different levels of theory, to study ion permeation in potassium channels. We aim to obtain a comprehensive understanding of the permeation process and to unravel the mechanism consistent with available experimental and theoretical data.

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Does Proton Conduction in the Voltage-Gated Proton Channel hH_V1 Involve Grotthus Hopping via Acidic Residues?

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H_v1 are ubiquitous highly selective voltage-gated proton channels involved in male fertility, immunology and the invasiveness of certain forms of breast