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Dynamic Coupling of Voltage Sensor and Gate Involved in Closed-State Inactivation of Kv4.2 Channels

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Voltage-gated potassium channels related to the *Shal*-gene of *Drosophila* (Kv4 channels) mediate a subthreshold-activating current (I_{SA}) which controls dendritic excitation and the backpropagation of action potentials in neurons. Kv4 channels also exhibit a prominent low-voltage-induced closed-state inactivation, but the underlying mechanism is poorly understood. We examined a structural model, in which uncoupling of the voltage sensor from the cytoplasmic gate mediates inactivation in Kv4.2 channels. Support for such a model comes from our finding that chimeric swapping of S4-S5 linker and distal S6 sequences between N-terminally truncated Kv4.2Δ2-40 and *Shaker*IR channels slowed inactivation of the former and induced a form of fast macroscopic inactivation in the latter under two-electrode voltage-clamp in *Xenopus* oocytes. We performed a Kv4.2 alanine scanning-mutagenesis in the S4-S5 linker, the initial part of S5, and the distal part of S6 and functionally characterized these mutants. In a large fraction of the mutants (> 80%) normal channel function was preserved, but the mutations influenced the likelihood of the channel to enter the closed-inactivated state. Depending on the site of mutation, the onset kinetics of low-voltage inactivation and/or the kinetics of recovery from inactivation were accelerated or slowed and the voltage dependence of steady-state inactivation was shifted positive or negative. In some mutants these inactivation parameters remained unaffected. Double-mutant cycle analysis based on kinetic and steady-state parameters of low-voltage inactivation revealed that residues known to be critical for voltage-dependent gate-opening, including Glu 323 and Val 404, are also critical for Kv4.2 closed-state inactivation. Selective redox modulation of corresponding double-cysteine mutants by dithiothreitol (DTT) tert-butyl hydroperoxide (*tb*HO_2) supported the idea that these residues are involved in a dynamic coupling, which mediates both transient activation and closed-state inactivation in Kv4.2 channels.

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The Role of ILT Mutations in Individual Subunits

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The concurrent mutation of three hydrophobic residues (V369I, I372L, and S376T) in the N-terminus of the S4 segment of *Shaker* K^+ channel slows down the kinetic of channel activation by a ~6-fold factor and shifts the conductance-voltage (G-V) curve to 115 mV (Smith-Maxwell *et al.*, 1998). We sought to determine the effect of the ILT mutations in heterotetrameric concatemerized channels with different stoichiometries to gain insights into the mechanisms of action of the ILT mutations. The channels were expressed in *Xenopus* oocytes and studied using cut-open oocyte voltage clamp and patch clamp techniques. The G-V curve of a concatemer with the ILT mutations in only one subunit (wtILT/3wt) is centered at ~8 mV compared with ~115 mV for the homotetramers *Shaker* ILT, while the homotetramer concatemerized *Shaker* zH4 Δ(6-46) (4wt) as a $V_{1/2}$ of −20 mV. The slope of the G-V curve is reduced. The kinetics of activation and deactivation are similar between 4wt and wtILT/3wt. The ILT mutations were introduced on the single wild type voltage sensor of a heterotetramer having three subunits with the four gating charges neutralized (wtILT/3mut; mut = R362Q/R365Q/R368N/R371Q). The G-V of the wtILT/3mut heterotetramer is right shifted ($V_{1/2}$ ~43 mV) compared with the wtILT/3wt and its slope is even more reduced. The number of closed states estimated from the Cole-Moore shift with an exponential function raised to a power n was slightly higher for wtILT/3wt than that of 4wt [$9.5 \pm 2(9)$ vs. $7.5 \pm 1.5(8)$, P=0.05]. However, wt/3mut and wtILT/3mut have the same estimates [n=$1.58 \pm 0.4(5)$ vs. $1.8 \pm 0.7(7)$, P=0.5]. wtILT/3wt has the same single channel conductance than *Shaker* ILT. We conclude that a single ILT wild type subunit is not sufficient for fully modifying the concerted step as in homotetramers. (Support: NIHGM30376 and NSERC fellowship to DGG)

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Gating currents from neuronal K_v7 channels

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The Kv7 family of voltage-gated K+ channels consists of five members (Kv7.1- Kv7.5), each showing specific tissue distribution and physiological role. Mutations in Kv7.1 cause the long QT syndrome, whereas Kv7.4 mutations underlie a rare form of slowly progressive deafness (DFNA2); finally, mutations in Kv7.2 and Kv7.3 genes have been identified in families affected by Benign Familial Neonatal Seizures. Disease-causing mutations often affect residues in the voltage-sensing domain of Kv7 subunits. In the present study, we have characterized the ionic and gating current of homomeric neuronally-expressed Kv7 channels (Kv7.2-Kv7.5) using the cut-open oocyte voltage clamp. The ionic and gating current were recorded at 18°C and also at 28°C to speed up gating charge movement. Increasing the recording temperature from 18°C to 28°C caused an accelerated activation/deactivation kinetics of the ionic currents in all homomeric Kv7 channels (the Q10 for the activation kinetics at 0 mV was 3.8, 4.1, 8.3, and 2.8 for Kv7.2, Kv7.3, Kv7.4 and Kv7.5). Moreover, currents carried by Kv7.4 channels (and less so Kv7.2) also showed a significant increase in their maximal value. Gating currents were only resolved in Kv7.4 and Kv7.5 channels, possibly because of an higher membrane channel density; ON gating charges at saturated potential (+40mV) amounted to 1.34 ± 0.34 (Kv7.4) and 0.79 ± 0.20 nC (Kv7.5). At 28°C, Kv7.4 gating currents had the following salient properties: 1) similar time integral of ON and OFF, indicating charge conservation; 2) a left-shift in the V1/2 of the ON gating charge when compared to ionic currents; 3) a rising phase in the OFF gating charge after depolarizations to values >0 mV. These results represent the first description of Kv7.4 and Kv7.5 gating currents and may help to clarify the molecular consequence of disease-causing mutations affecting channel gating. Supported by NIH GM30376 and Telethon GGP07125.

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Contributions of the central hydrophobic residue in the PXP motif of Voltage-Dependent K+ Channels to S6 flexibility and Gating Properties

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Voltage-gated K+ channels are composed of four subunits each of which contains a voltage-sensing domain (S1-S4) and a pore domain (S5-P loop-S6). The exact molecular mechanisms underlying the opening and closing of the channel pore are still unclear, although evidence suggests that this process involves pivoting bending of the inner pore-lining S6 segments at the ''helix-bundle crossing''. Shaker-like (Kv1.1) channels contain a highly conserved Pro-Val-Pro (PVP) motif at the base of S6 that produces a kink in the S6 helices and provides a flexible element thought to be essential for channel gating. The role of proline-induced kinks in transmembrane helices is well known, but the role of the small hydrophobic valine between these prolines, is unknown, and interestingly, Shab-like (Kv2.1) channels possess a an isoleucine at this position (PIP). Here we show that the exact nature of this central hydrophobic residue within the PXP motif confers unique functional properties to Kv1 channels, including changes in activation and deactivation kinetics, voltage-dependent properties and open probabilities, but that single-channel conductance and cell expression levels are not affected. In support of these functional changes, molecular dynamic simulations demonstrate that valine and isoleucine contribute differently to S6 flexibility within this motif. These results therefore indicate that the nature of the central hydrophobic residue in the PXP motif is an important functional determininat of Kv channel gating by contributing, at least in part, to the relative flexibility of this motif.

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A P-helix Mutant In A *Shaker*-type Kv Channel Converts The Inactivated State Into A Conducting One

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Voltage-gated potassium (Kv) channels are tetramers of α-subunits, each composed out of six membrane-spanning helices (S1-S6) with a pore loop between S5 and S6 that forms the channel's selectivity filter. The activation gate that seals off the ion conducting pore in a closed channel is controlled by the transmembrane potential. After channel activation most Kv channels display C-type inactivation, a process that is believed to involve reorientations of the selectivity filter and results in a non-conducting channel although the channel gate is open. hKv1.5 (a *Shaker*-type Kv channel) displays such C-type inactivation. Here we report that an alanine substitution for residue T480 that is located at the end of the pore-helix prevents hKv1.5 channels from entering the inactivated state. The mutant T480A had an isochronal activation curve similar to Wild Type hKv1.5 when determined with 250ms depolarizing steps. Longer depolarizations (~5 seconds) caused WT channels to inactivate (~58%) displaying an inactivation curve with a midpoint of -23.2 ± 1.2 mV and a slope factor of about 4. However, T480A did not inactivate and such long depolarizations caused an additional (slow) activation at more negative potentials thus generating an isochronal activation curve with properties that were similar to the