

conformation, in the presence of explicit water-membrane environment. [Supported by NIH grant GM062342 and GM067887]

1549-Pos Board B459

Gate Closure in Kv1.5 Channels is not Dependent on the Status of the Selectivity-Filter

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Voltage-gated potassium (Kv) channels are tetramers of 6-transmembrane domain (S1-S6) α -subunits. The activation gate that seals off the ion conducting pore is under control of the voltage-sensing domain and locates at the bundle crossing of the S6 segments. After channel opening most Kv channels display slow inactivation, a process that involves rearrangements of the selectivity filter (SF) resulting in a non-conducting channel although the S6-gate is open. Recent evidence argued for a strong coupling between inactivation and activation: after gate opening the S6 segment undergoes structural rearrangements that would be transmitted up to the level of the SF destabilizing its conformation. Substituting in Kv1.5 residue T480 that locates at the bottom of the SF by an alanine generated mutant channels which instead of inactivating displayed a second open state, characterised by slowly increasing current. Several molecular dynamics (MD) simulations showed a MD trajectory that displays a hydrophobic collapse of the central cavity with S6 dynamics decreasing the pore radius of the S6-gate. The simulations further showed that the SF did constrict in WT channels but not in the mutant. Ionic current measurements of the mutant T480A channels showed that after prolonged depolarizations - pushing the channels into the second conducting state - the activation gate could close and reopen with the channel remaining in the second conducting state. To convert the channels back to their original conducting state longer repolarizing times were needed. Thus, using the T480A pore mutant we could directly determine from ionic currents that gate closure in Kv1.5 channels does not depend on the status of the SF which, as suggested by Deutsch et al. implies the existence of a channel state with both a closed gate and inactivated selectivity filter. (Support: FWO-G025608)

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Genetic Algorithm Based Computer Simulation Reveals a New Property of Kv1.3 Inactivation

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Kv1.3, a voltage-gated potassium channel found in human T cells, enters a non-conducting state during prolonged depolarization by the slow P/C type inactivation, whose exact mechanism is yet to be determined. We have previously shown that acidic extracellular pH (pH_e) slows the inactivation process in the presence of low extracellular potassium concentration ($[\text{K}^+]_e$), whereas speeds it in the presence of high $[\text{K}^+]_e$. Our aim was to generate a gating scheme, based on a Markov model, which is able to explain the experimental results as a continuous function of pH_e and $[\text{K}^+]_e$.

We developed a new computer program based on the Gillespie algorithm that is able to generate simulated records from given gating schemes and compare the resulting curve to the original recording, i.e. it provides an indicator for the goodness of a given scheme. Optimizing of the fitted parameters was achieved using a genetic algorithm. The algorithm was implemented in parallel and run in multi-core and multi-processor environments.

As the program enabled us to optimize the gating scheme for more than one measurement record at a time, we could conclude that our simple model having two open microstates and one inactivation pathway from both of them was unable to explain the effects of pH_e and $[\text{K}^+]_e$. Introduction of a new binary parameter and therefore the duplication of inactivation pathways was needed to fully explain the experimental results. We therefore assume that not only the binding of a single potassium ion influences the speed of inactivation, but the conducting pore can have two conformations with different conduction and inactivation properties depending on the $[\text{K}^+]_e$.

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Molecular Rearrangements During Slow Inactivation of the Shaker-IR Potassium Channel

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Crosstalk between the activation and slow inactivation gates in Shaker potassium channels is now well-established. The activation gate perceives the conformation of the inactivation gate (Panyi and Deutsch, 2006, 2007). Closure of the inactivation gate speeds opening and slows closing of the activation gate, i.e., stabilizing the gate in the open configuration. If this coupling in-

volves movement of the S6 transmembrane segment, then we predict state-dependent changes in accessibility of residues lining the channel cavity. We engineered cysteines, one at a time, at positions 470, 471, 472, 473, and 474 in a T449A Shaker-IR background and determined modification rates for the cysteine modifying reagents, MTSET and MTSEA, in the open, closed, and inactivated state of the channel. Neither reagent, applied from the intracellular side, modifies cysteines at 470-474 in the closed state. Both 470C and 474C are rapidly modified in the open state and at approximately one-tenth this rate in the inactivated state. In contrast, 471C is not modified in the open state but can be modified by MTSEA but not MTSET in the inactivated state. Residue 472C cannot be modified in any of the three states. Mutant 473C did not express current. Our findings are consistent with a rotation of S6 in the inactivated state, which increases the accessibility of residue 471 while simultaneously decreasing accessibility of residues 470 and 474. Any model of C-type inactivation in the Shaker Kv channel must conform to these experimental observations.

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Shakerir and Kv1.5 Mutant Channels with Enhanced Slow Inactivation also Exhibit $[\text{K}^+]_o$ -Dependent Resting Inactivation

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Previous studies have shown that in N-type-inactivation removed *Shaker* (*ShakerIR*) channels, the T449K and T449A mutations enhance slow inactivation. Additionally, these mutant channels show a loss of conductance in 0 K^+_o that was attributed to an inactivation process occurring from the closed, resting state and which we refer to as resting inactivation. Similar behaviour is also observed in Kv1.5 channels with a mutation in the turret (H463G). Although the time courses for the onset of and recovery from resting inactivation are unknown, a comparison of the kinetics for resting inactivation at -80 mV and slow inactivation at 50 mV may provide information on whether resting and depolarization-induced inactivation are mechanistically related. Thus, we performed an analysis of the kinetics for the onset of and recovery from resting and depolarization-induced inactivation of these mutant channels. Although the time constant for slow inactivation at 50 mV (τ_{inact}) was reduced by the *ShakerIR* T449K, T449A and Kv1.5 H463G mutations, τ_{inact} was insensitive to changes in $[\text{K}^+]_o$, suggesting that the loss of conductance in 0 K^+_o was not due to accelerated slow inactivation. The time constant for resting inactivation (τ_{R1}), estimated by monitoring the decrease in the peak current evoked by a brief test pulse to 50 mV after a variable exposure time to 0 K^+_o solution at -80 mV , was at least an order of magnitude larger than τ_{inact} . Although the time courses for the onset of inactivation varied between mutants, for each mutant the time course of recovery from 0 K^+_o -induced resting inactivation was the same as that for recovery from slow inactivation. These results suggest that the 0 K^+_o -induced resting inactivation of these mutant *ShakerIR* and Kv1.5 channels is mechanistically related to slow inactivation.

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Voltage Sensor Immobilization in Kv1.2 Channels

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The voltage gated potassium channel family contains several functionally distinct isoforms which serve to shape the duration, frequency and timing of action potential firing in electrically excitable cells. Several mechanistic elements that contribute to the function of the voltage sensing apparatus are not yet fully described, especially in channels other than the prototypical *Shaker* potassium channel. We have studied voltage sensor rearrangements of Kv1.2 channels using two-electrode voltage clamp fluorometry and gating current recordings in mammalian cells. Fluorescence measurements reporting on voltage sensor movement revealed a transition into a relaxed state upon prolonged depolarization, causing a left shift in the fluorescence-voltage relationship. Gating current measurements of wild type Kv1.2 channels recorded in permeant ion free solutions ($\text{NMDG}^+_{\text{int}}/\text{TEA}^+_{\text{ext}}$) also displayed a left shifted Q-V after depolarizing pre-pulses, reflecting a stabilization of the activated state of the voltage sensor. To enable examination of the effects of different cations and processes of inactivation in Kv1.2 a non-conducting double mutant channel mimicking the permanently slow inactivated and non-conducting *Shaker* W434F, Kv1.2 (W366F, V381T) was created. Off-gating currents recorded from this channel in the presence of permeant ions displayed less voltage sensor stabilization in the activated state than the wild type channel. These data suggest that cations play a specific role in regulating voltage sensor dynamics in the Kv1.2 channel.