paired with the native short A359. In consequence, we found that the paired arginine to serine mutations at the subsequent positions 362,365 and 365,368 also produce omega current. This was taken as evidence that S4 slides in 3 steps through a pore towards the activated state. Each pair of 2 short serines in between the thread of long arginines appears as a gap which allows a leak when in the omega pore.

Here, we present further studies of the above mutants over a wider voltage range, in addition with the alpha pore open (434W) to detect the open state with alpha current. We found that S4 in all 3 omega constructs can reach the final open state where omega current has disappeared and alpha current appears. This provides clear evidence that all 3 S4 constructs - despite the arginine-to-serine gap - still proceed to the open state, although at different voltages. We also analyzed in detail the voltage range over which the omega pore remains open, i.e. is occupied by the gap. In the cases where the pore could be closed, the voltage dependence for the closing process was studied. While all constructs close in outward direction, a clear inward directed closing of the omega-pore could so far only be demonstrated for the RR/365,368/SS construct.

2699-Pos

Pore Dilation in C-Type Inactivation of Potassium Channels

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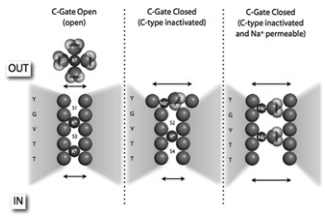
It is commonly thought that C-type inactivation is the result of constriction or "collapse" of the outer mouth of a voltage-gated K^+ channel. It is well established that conduction requires that the selectivity filter be a good fit for a dehydrated K^+ ion. In theory, failure to conduct could thus result from dilation of the filter, making it too large to effectively complex a K^+ ion. Our modeling and simulations as well as a review of the literature give support to this idea. Lowering or removing K^+ from the external (and/or the internal) solution is known to promote C-type inactivation. Our simulations show that external K^+ removal causes dilation of the outermost site in the selectivity filter, an expected consequence of the mutual repulsion between the oriented dipoles of the filter.

Dilation makes the outer site capable of accepting a partially hydrated Na^+ ion, but inactivates the channel because the Na^+ cannot move into Sites 2-4, which are K^+ selective. Removing internal K^+ causes the inner sites to dilate, making the pore Na^+ selective. The chain of events that follow channel activation and allow pore dilation are under investigation.



2700-Pos

Time and Voltage-Dependent Slowing in the Off Gating Currents from Kv3.1

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Kv3.1 channels are characterized by fast activation and deactivation kinetics with a quite positive threshold of activation. In the central nervous system they are involved in the high-frequency firing pattern of neurons. Given some controversy on Kv3.1 channel gating, we performed an in-depth analysis of WT Kv3.1 gating currents elicited in mammalian Ltk^- cells. The Q/V curves determined from integrating either Q_{ON} or Q_{OFF} were comparable and were best fitted with a double Boltzmann distribution. The Q/V curves were shifted by approximately 15 mV towards more hyperpolarized potentials compared to the ionic G/V curve, indicating that a substantial charge was moved during closed-state transitions. The double component in the Q/V curve and a bi-exponential decay of I_{gON} indicated that the charge was carried by at least two distinct transition steps, contrary to earlier reports for Kv3.2b, but more consistent with *Shaker*, Kv1.5 and Kv2.1 channels. Since the integrals of Q_{ON} and Q_{OFF} were identical, there was no charge immobilization within a 125 ms depolarization. The time course of I_{gOFF} was fast after short depolarizations, but displayed *Shaker*-like slowing with increasing step duration for depolarizations positive to the threshold for ionic current activation. Since 4-AP blocks the concerted opening step in *Shaker*, we used it to separate the two components of I_{gOFF}. In the presence of 3 mM 4-AP only the fast I_{gOFF} remained, indicating that the slowing is linked to a final concerted step in channel opening which appears to be a general gating feature of Kv channels.

2701-Pos

Molecular Determinants of the Slow Off-Gating Component in Shaker K^+ Channels

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The electromechanical coupling occurs between the ion conducting pore and the peripheral voltage sensors in voltage-gated ion channels. It has been established that the voltage sensor undergoes a complex conformational change upon depolarization of the membrane potential, which eventually leads to opening of the helical bundle crossing. The S4-S5 linker, the covalent link between voltage sensor and pore, has been shown to anneal to the C-terminus of the S6. This interaction is essential for electromechanical coupling. Still the molecular mechanism of the electromechanical coupling remains unclear. Here, we used voltage clamp fluorometry in a cut-open oocyte configuration in order to further elucidate the residues responsible for the energy transfer from the sensor to the pore. We identified an intersubunit interaction, coupling the S4-S5 linker to the neighboring S6. This interaction is responsible for the slow component of the gating current upon return to negative membrane potential. The slow off gating component has been described early on but its molecular determinants were not identified previously. Alteration of any of the three interaction partners involved variably leads to shift of the QV, uncoupling of pore from voltage sensor movement and abolishment / slowing (increase of binding energy) of the slow off gating component. We also demonstrated that the interaction does not require pore opening, although it is increased in the open pore state. The data suggest that this interaction stabilizes the activated state and thereby contributes to electromechanical coupling.

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2702-Pos

Biochemical and Functional Conversion of KChIP4a into KChIP1 for Modulation of Kv4 Inactivation

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Inactivation in Kv channels plays a key role in dynamic regulation of neuronal excitability. Multifunctional auxiliary subunit KChIPs1-4 co-assemble with pore-forming rapidly inactivating Kv4 α subunits to form a native complex that encodes somatodentritic A-type K^+ current in neurons. KChIP1 increases Kv4 surface expression, speeds up steady-state inactivation with a moderate effect on fast inactivation. In contrast, KChIP4 variant KChIP4a functions as a suppressor of inactivation to eliminate the fast inactivation of Kv4 channels, but neither promotes surface expression nor has any effect on recovery time constant. We have recently determined the crystal structure of KChIP4a that reveals a distinct hydrophobic N-terminus as well as a core structure showing overlap with known KChIP1. In this study, we transfected HEK 293 cells that were solubilized andFast Protein Liquid Chromatography (FPLC) analysis was performed with Size Exclusion Chromatography (SEC) using a Superdex 200 column. The SEC-FPLC assay reveals that KChIP4a proteins self-associate to form multimers with three peaks, compared with a single peak of KChIP1 dominated by monomer. Mutations in N-terminal key residues of KChIP4a can turn multimeric proteins into monomers with a single peak on SEC. Two-electrode voltage clamp in oocytes confirms that disruption of multimeric state of KChIP4a proteins can convert its non-inactivating function into fast inactivating like KChIP1. Taken together, our findings of key residues in N-terminal KChIP4a critical for conversion between fast and slow inactivation may be a site of action for chemical compounds that can alter channel inactivation for a potential therapy of membrane excitability-related disorders.

2703-Pos

Structural Determinants of Closed-State Inactivation Studied with Channel Chimeras

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Shal-gene-related voltage-gated potassium (Kv4) channels exhibit a prominent low-voltage-induced closed-state inactivation. Recent experimental results show that the S4 voltage sensor drives closed-state inactivation (Dougherty et al., J Gen Physiol 131: 257-273, 2008), and that the S4S5 linker and the main S6 activation gate are instrumental in the installment of closed-state inactivation (Barghaan and Bähring, J Gen Physiol 133: 205-224, 2009). In particular, an inactivated voltage sensor conformation correlates with a temporary