a new Markov model for the human sodium channel that includes three closed states (C_1, C_2, and C_3), three closed-inactivated states (IC_1, IC_2, and IC_3), one open state (O), one fast open-inactivated state (IF), and one slow open-inactivated state (IS). This final model was chosen from the several Markov models that had been analyzed. The aim was to develop a Markov model with minimum number of states which gives the best description of the several sets of experimental data. We started from the model with three closed states, one open state, and one open-inactivated state (connected only to open state). This minimal model reproduced the current-voltage relationship, the voltage dependence of normalized channel conductance, the time constant of deactivation, and gave reasonable approximation to the time-to-peak current. However, the model did not simulate well the steady-state inactivation relationship, voltage dependence of the inactivation kinetics and recovery from inactivation. We have modified this model by adding three closed-inactivated states parallel to the existing activation pathway. This model gave good description for the kinetics and steady-state properties of the fast inactivation of the channel. As the experimental data shows bi-exponential inactivation and recovery, we included additional slow inactivated state connected to the open state. The resulting model reproduced well both the inactivation-recovery and the activation-deactivation data.

2693-Pos Board B463

Simulated Block of Brain Na^+ Channels by Ranolazine Predicts Selective Binding to Inactivated Conformations

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The antianginal drug ranolazine inhibits the increased persistent Na^+ current associated with several $Na_V1.1$ congenital mutations causing epilepsy and migraine syndromes. This inhibition is consistent with fast-inactivated state binding. In contrast, recently published studies of ranolazine block of the cardiac $Na_V1.5$ and skeletal muscle $Na_V1.4$ have found evidence for pre-open and/or open state binding. In the present study, computer modeling to identify the channel states necessary and sufficient to reproduce the experimentally-observed block of brain Na^+ channels by ranolazine. A minimal Markov model of a brain Na^+ channel was developed that reproduces all voltage-dependent gating behaviors including activation ($V_{½}$= −21.8mV), fast-inactivation ($V_{½}$= −62.6mV), slow-inactivation ($V_{½}$= −67.4mV), persistent current (0.23% at −10mV) and single channel mean open time (2.6msec at 0mV). In addition to three closed states, this model includes one state each for pre-open (***PO***), open (***O***), fast-inactivated (***FI***) and slow-inactivated (***SI***) states. The binding rates of ranolazine to HEK293 cells stably expressing the $hNa_V1.2$ isoform were measured (K_{ON}= $1M^{-1}msec^{-1}$ and K_{OFF}= $5e^{-5}msec^{-1}$) and were used to guide simulated binding to ***PO***, ***O***, ***FI*** or ***SI*** channel conformations. Only simulated binding to the ***FI*** state delayed the recovery from fast inactivation (second phase tau: control 656msec, 30uM ranolazine 729msec), which is a common feature of ranolazine block of all Na^+ channel isoforms. Simulations of evoked neuronal action potential firing with 10uM ranolazine binding either to the ***FI*** or ***SI*** state demonstrated equal effectiveness for the reduction of excitability (#evoked APs during 4sec: control 174, ***FI*** binding 54, ***SI*** binding 53). No effect on AP# or frequency was observed with simulated binding to either ***PO*** or ***O*** states. Our data suggest that ranolazine binds to the inactivated states of brain Na^+ channels.

Voltage-gated K Channels: Gating II

2694-Pos Board B464

Role of Structural Water in the Stabilization of the KcsA C-Type Inactivated Selectivity Filter: Evidence from High-Resolution Structures

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A first glimpse into the structural changes underlying the interconversion between conductive and non-conductive C-type inactivated forms of the K+ channel selectivity filter (SF) at modest resolution was obtained from crystal structure of open-inactivated KcsA (O/I) [1]. On the basis of these structures, we developed a set of double cysteine crosslinking pairs that sharply increase the resolution of KcsA crystals with its gate trapped in the open configuration. Here, we present the crystal structures of KcsA locked-open (O) together with mutations that stabilize either the C-type inactivated filter, Y82A (O/I); or the fully conductive filter, E71A (O/O) @ 2.3 and 2.1 Å, respectively. Comparison of these structures helps identify a novel water network (H2O_1, H2O_2 and H2O_3) behind the channel SF that stabilizes the O/I conformation of the channel. H2O_1 interacts with the backbone carbonyl groups of V76 and E71 from a neighboring subunit and with H2O_2+H2O_3 within the same subunit. In KcsA, the presence of the E71 residue on the channel pore helix is essential to drive the channel to the O/I state while providing a high-dielectric media suitable for the formation of this water-molecule network behind the SF. The intersubunit nature of this interaction, which is present only in the O/I state, provides a cogent structural explanation for the cooperative character of the C-type inactivation process in K+ channels. Additionally, we propose that the formation of a similar water-molecules mediated H-Bond network behind the SF of other K+ channels is essential to stabilize the O/I state.

1. Cuello, L.G., et al. Nature, 2010. 466(7303): p. 203-8.

2695-Pos Board B465

A Random Mutagenesis Approach to Probing Electromechanical Coupling in the Hyperpolarization-Activated Channel MVP

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MVP, the hyperpolarization-activated potassium channel from the archaeon M. jannaschii, poses an interesting case study for understanding how the voltage-sensing domain (VSD) couples to the pore domain in Kv channels. Although the VSD of MVP adopts the same orientation in the membrane and senses changes in the polarity of the membrane potential in the same manner as canonical Kv channels, the pore of MVP opens in response to hyperpolarization; thus, like the eukaryotic hyperpolarization-activated cyclic-nucleotide-gated (HCN) channels, MVP opens when the S4 helix moves inwards and closes when the S4 moves outwards. Because hyperpolarization-activated channels use the same VSD for inverse gating, they offer a unique opportunity for understanding the molecular basis of coupling the VSD and pore domain. Since structural evidence in canonical Kv channels suggests that the movement of the S4 is transmitted to the pore domain via the S4-S5 linker, we have set out to identify key residues of MVP necessary for electromechanical coupling. We probed the S4-S5 linker and S6 helix with a random mutagenesis screen using the potassium uptake deficient strain LB2003 in conjunction with a MVP library consisting of single amino acid substitutions along the linker with full codon coverage. By screening this library under different potassium concentrations, we have identified mutations in the linker and S6 that abolish WT complementation on potassium-depleted media (Loss of Function). This approach has allowed high-throughput screening of all possible amino acid identities along the linker that are incompatible with channel function. Here we present preliminary assessments of the expression profile, stability and functional properties of these mutants.

2696-Pos Board B466

Initial Opening Steps of K^+ Channels Probed by Extracellular Multivalent Cations

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The structural model of the open state of the Kv1.2-2.1 chimeric channel (Long et al., PDB 2R9R) has 4 negative residues distributed in S1, S2 and S3, all bound to positive residues in S4. Extrapolating the model to the resting state, the two outermost negative residues, E183 in S1 and E226 in S2, are exposed to the extracellular fluid and, in theory, not bound to S4 residues; E183 and E226 should thus be available to bind external cations. We have examined the effects on opening kinetics of multivalent cations using Shaker channels expressed in HEK cells. Addition of La^{3+} (50 μM) to the extracellular solution (in mM: 145 NaCl, 2 KCl, 10 $CaCl_2$, pH 7.0) markedly prolonged the sigmoidal delay and slowed the rising phase of K^+ current (I_K) at all voltages tested. The closing kinetics of I_K at negative voltages remained unaltered. Gating currents (I_g) recorded from a non-conducting mutant (W434F) showed that La^{3+} (50 μM) reduced the initial amplitude of I_g nearly twofold. We postulate that La^{3+} binds to the unoccupied negative side chains of E183 and E226, hindering outward S4 motion, thus increasing the lag and slowing the rise of I_K. We further postulate that La^{3+}, in the activated ''up'' state of S4, is not bound to E183 or E266, which are occupied by S4 positive charges; La^{3+} therefore has no effect on closing. Similar measurements using Ca^{2+} showed that at high concentrations it has an effect similar to that of La^{3+} on the activation time course. This Ca^{2+}-dependent prolongation of the activation lag and slowing of the rising phase of I_K is noticeably enhanced by complete removal of extracellular K^+, perhaps because K^+ and Ca^{2+} compete for E183 and E226 in the deactivated state.

2697-Pos Board B467

Molecular Determinants in K^+ Channel hERG Inactivation Gating

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Voltage-gated K+ (Kv) channels play a crucial role in the generation of cellular action potentials. Kv channels open and close in response to changes in