pore structure in different recording conditions and in various channel states. Here we describe experiments in which external Ba\(^{2+}\) was applied to Kv4.2 channels to determine the properties and state-dependence of Ba\(^{2+}\) block. In the standard protocol, [K\(^{+}\)], was 3.5 mM and 10 ms pulses to 80 mV were applied at 1 Hz from a V\(_{m}\) of −80 to −100 mV. \(v_{\text{block}}\), derived from a monoexponential fit of a diary plot of the peak current amplitudes, decreased as the [Ba\(^{2+}\)] increased, however the value for \(v_{\text{block}}\) saturated with higher (20-40 mM) concentrations. This suggests that, as in Kv1 channels, there are serial binding sites in the resting conformation of the channel. Increasing [K\(^{+}\)] from 3.5 to 40 mM decreased the steady-state level of Ba\(^{2+}\) binding sites in the resting conformation of the channel. Increased [K\(^{+}\)] revealed roughly 3-fold greater block of inactivated channels (\(v_{b}=80\) mV) compared to the block of resting channels (\(v_{b}=80\) mV). This observation supports the widely-held notion that the outer pore mouth of inactivated Kv4 channels is not constricted, i.e., not P/C-type inactivated, but nonetheless implies that the outer pore does undergo a substantial structural change in the transition from the resting to the inactivated state.

1540-Pos Board B450
Closed-State Inactivation of a Voltage-Gated Potassium Channel Involves Inter-Subunit Interactions
Jessica Wollberg, Robert Bähring
We study the structural determinants of inactivation in voltage-gated potassium (Kv) channels of the Kv4 subfamily, which exhibit preferential closed-state inactivation (CSI). There is strong evidence that dynamic coupling between the S4S5 linker (S4S5) and the main S6 activation gate (S6) plays a central role in CSI (Barghaan and Bähring, J Gen Physiol 133: 205-224, 2009). In particular, absence or loss of contact between S4S5 and S6 is thought to uncouple the activation gate from the voltage sensor.

Here we examined the role of dynamic coupling between the S4S5 residue Phe 326 and the S6 residue Val 404 in CSI. We expressed Kv4.2 wild-type (wt), the single mutants Kv4.2 F326A and Kv4.2 V404A, and the double mutant Kv4.2 F326A:V404A in Xenopus oocytes and measured CSI proper. Our data support the hypothesis that dynamic coupling occurs between the S4S5 residue Phe 326 and the S6 residue Val 404 in CSI. We expressed Kv4.2 wild-type (wt), the single mutants Kv4.2 F326A and Kv4.2 V404A, and the double mutant Kv4.2 F326A:V404A in Xenopus oocytes and measured CSI proper.

1541-Pos Board B451
Diversity in Interaction and Gating Modification of Kv4 Channels by Hptx2
Christopher V. Desmone, Stephanie C. Santoro, Chang Xie, Edward C. Holner, Poojai Mahikaji, Vladimir E. Bondarenko, Michael J. Morales
Hptx2 is an inhibitor cysteine knot (ICK) toxin that selectively modifies Kv4 gating. Two bulky, hydrophobic amino acids at positions 275 and 276 in the S3b transmembrane segment are required for gating modification by Hptx2; a LV dyad in Kv4.3 and LF in Kv4.1. However, substitutions of other hydrophobic amino acids in Kv4.3 at the same position also allow Hptx2 gating modification, showing that the gross hydrophobic character of the binding site is key for toxin interaction. An S3b mutation, Kv4.3[N280A], reduced the Kd value 10-fold. Surprisingly, Kv4.3[L275N820A] was as sensitive to Hptx2 as WT Kv4.3. Other hydrophobic amino acid substitutions for N280 showed similar effects. This suggests that these mutations created an additional Hptx2 binding determinant. Gating modification of Kv4.3 is highly voltage-dependent, while that of Kv4.1 is much less so. Swapping four non-conserved S3b amino acids between Kv4.1 and Kv4.3 switched the voltage-dependence of Hptx2 gating modification. To understand this disparity, we modeled the activation gating of Kv4.3 and Kv4.1 and incorporated the effects of Hptx2 into the kinetic models. The models feature four voltage-dependent transitions between closed states followed by a voltage-independent transition to the open state. The disparity in gating modification lies in the relative influence of toxin on the voltage-dependent transitions in the two channels. In Kv4.3, they are more strongly affected by toxin. In contrast the Kv4.1 voltage-independent transition from the closed-pre-open to open state is most affected by Hptx2. Therefore, a higher proportion of toxin-bound Kv4.1 channels are in the closed-pre-open state; this decreases the voltage dependence of toxin-bound Kv4.1 opening. These data show that amino acids near the Hptx2 binding site play a role in the kinetics of Kv4 channel activation gating.

1542-Pos Board B452
Identification of a Kv4 Channel Hydrophobic Binding Pocket for the Inhibitory Effects of Polysaturated Fatty Acids
Robert Heler, Jessica A. Bell, Linda M. Boland
Polysaturated fatty acids such as arachidonic acid (AA) inhibit Kv4 potassium channel function. Finding the binding sites of fatty acids to the channel can provide useful insights into the structural basis of ion channel modulation and the molecular mechanisms of lipid regulation of neuronal excitability. We tested the hypothesis that the inhibitory effects of fatty acids on Kv4 channels require access to a hydrophobic binding pocket within the membrane-embedded region of the channel. We prepared a homology model of Kv4.2 and used a molecular docking approach to identify residues that may interact with AA. Candidate residues identified by AutoDock Vina were individually point mutated in the rat Kv4.2 cDNA. Wild-type and mutant RNAs were then prepared by in vitro transcription and injected into Xenopus oocytes to generate Kv4.2-Chla. The capacity for available binding pocket was tested by measuring the inhibitory effects of AA using two-microelectrode voltage clamp electrophysiology. Wild type Kv4.2 peak currents were 30\% inhibited by a 5 min exposure to 10 \mu M AA, applied externally. We identified three mutations that significantly impaired this effect; each showed less than 5\% inhibition by 10 \mu M AA. We assessed the molecular disruption of the AA binding pocket by simulating the structural change in the molecular model. In contrast, mutations introduced to other residues located near but not within the putative binding pocket did not disrupt the fatty acid inhibition of Kv4.2 current, indicating the specificity of the residues which comprise the binding pocket. Our results suggest that AA may inhibit Kv4 channel function by interfering with the function of structural domains which are not in the permeation pathway.

1543-Pos Board B453
Protein Kinase C Influence on Kv4.3 Closed State Inactivation
Chang Xie, Stephanie C. Santoro, Harold C. Strauss, Michael J. Morales
Kv4.3 is expressed as a short and a long form that differ by a 19 amino acid insertion downstream from the S6 transmembrane segment. It is typically expressed with ancillary subunits, the most prominent of which are cytoplasmic proteins called KClnPs. Activation of PKC by phorbol esters modulates closed-state inactivation (CSI) in Kv4.3. PKC reduces CSI in the short form of Kv4.3 (Kv4.3S), however, the 19 amino acid insertion in Kv4.3L changes the effect to increasing the magnitude of CSI through phosphorylation of a threonine unique to the long form. KClnPb blocks the effect of PKC on CSI in both Kv4.3S and Kv4.3L. In contrast, 70-amino acid KClnPd was permissive for PKC modulation of CSI characteristic seen in both forms of Kv4.3. These data suggested the presence of additional PKC phosphorylation sites in Kv4.3. Bioinformatic analysis suggested that the most likely PKC phosphorylation sites were in the N-terminal cytoplasmic domain of Kv4.3 at S47, T53, T100 (all in the T1-D domain), and S165 (in the T1-S1 linker). Mutagenesis of any of the four amino acids reduced CSI; T53, T100, or S165 were influenced by PKC to a much lesser extent than WT Kv4.3S, while S47A dramatically reduced CSI. In all cases, there was little difference between mutations in the short and long Kv4.3 isoforms. Mutations in S6 at amino acids V399 and V401 have been shown to have dramatic effects on CSI. In the presence of these mutations, the Kv4.3L inactivation age-dependence of Hptx2 gating modification. To understand this disparity, we modeled the activation gating of Kv4.3 and Kv4.1 and incorporated the effects of Hptx2 into the kinetic models. The models feature four voltage-dependent transitions between closed states followed by a voltage-independent transition to the open state. The disparity in gating modification lies in the relative influence of toxin on the voltage-dependent transitions in the two channels. In Kv4.3, they are more strongly affected by toxin. In contrast the Kv4.1 voltage-independent transition from the closed-pre-open to open state is most affected by Hptx2. Therefore, a higher proportion of toxin-bound Kv4.1 channels are in the closed-pre-open state; this decreases the voltage dependence of toxin-bound Kv4.1 opening. These data show that amino acids near the Hptx2 binding site play a role in the kinetics of Kv4 channel activation gating.