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inactivation process plays a critical role in controlling the length and frequency of cardiac action potentials, as well as the firing patterns in neurons. The molecular process underlying the C-type inactivation mechanism remains unexplained despite the accumulation of experimental evidences showing the key role played by the channels' selectivity filter and some neighboring residues. It's been recently shown that the prokaryotic KcsA channel undergoes C-type inactivation like its eukaryotic counterparts (Gao et al., PNAS, 102:17630 (2005)), establishing KcsA as a perfect prototypic model to study the structural basis of the inactivation mechanism. An X-ray structure of the KcsA channel obtained in presence of low K+ concentration (Zhou et al., Nature 414:43 (2001), pdb code 1K4D) has since then been postulated to correspond to the C-type inactivated state of the channel. While the structural analysis of this static conformation suggests that pore lining amide hydrogens would prevent the permeation of ions, uncertainties remain about its stability under physiological conditions and its ion occupancy state. These questions are of primary importance to better understand the relevance of this structure to the physiological regulation of ion permeation in K+ channels. Using molecular dynamics simulations and free energy calculations, we investigated on the stability, selectivity, and conductance of the selectivity filter of KcsA in this putative inactivated state.

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Ancillary subunits and S3b amino acid substitutions alter the affinity of HpTx2 for Kv4.3

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HpTx2 is an ICK gating modifier toxin that selectively inhibits Kv4 channels, making it an excellent probe for understanding Kv4 structure and function. To characterize the molecular determinants of interaction, we performed alanine scanning of Kv4.3 S3b linker. HpTx2-Kv4.3 interaction had a K_d = 2.4 μM. Two alanine mutants in Kv4.3 caused a dramatic increase in K_d: 6.4 μM for V276A and 25 μM for L275A. LV275AA nearly eliminated toxin interaction. Unlike HaTx and other well-characterized ICK toxins, HpTx2 binding does not require a charged amino acid. To determine if the identity of the amino acids altered HpTx2 specificity, we constructed LV275IF. This mutation decreased the K_d to 0.54 μM, suggesting that the hydrophobic character of the binding site is the most important property for interaction with HpTx2. Two of the alanine mutants, N280A and D282A caused stronger interaction of HpTx2 with Kv4.3; the K_d for Kv4.3 [N280A] was 0.26 μM. We propose that these mutations either remove a steric barrier to HpTx2 occupation of S3b, or that removal of these polar side chains increases toxin affinity by increasing the hydrophobic character of the binding site. To understand Kv4.3-based transient outward currents in native tissues, we tested the affinity of HpTx2 for Kv4.3 co-expressed with KChIP2b. The toxin's K_d for Kv4.3+KChIP2b was 0.95 μM. HpTx2 binds to the closed state of Kv4.3, which is stabilized by KChIP2b. We propose the increased affinity is due to the increased stabilization of the closed state. These data show that HpTx2 binding to Kv4.3 has aspects in common with other ICK gating modifier toxins, but that the interventions that increase toxin affinity suggests flexibility toward channel binding that belies its unusual specificity for Kv4 channels.

3398-Pos Board B445
HpTx2 Interaction With Kv4.3 and Kv4.1 Reveals Differences in Gating Modification

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HpTx2 is an ICK gating modifier toxin specific for Kv4 channels. The K_d of HpTx2 for two similar channels, Kv4.3 and Kv4.1, was 2.4 μM and 7.1 μM, respectively. HpTx2 inhibition of Kv4.3 is highly voltage dependent, shifting the current-voltage relationship to more depolarized potentials; consistent with the classic behavior of gating modifier toxins. However, modification of Kv4.1 gating is much less voltage dependent. Site directed mutagenesis of the S3b interaction site of Kv4.3 and Kv4.1 shows that the same two conserved bulky hydrophobic amino acids are required for HpTx2 interaction with each channel. While the interior of the binding site is conserved between Kv4.3 and Kv4.1, three amino acids adjacent to the binding site are not conserved. Swapping these amino acids between Kv4.3 and Kv4.1 swaps the phenotypic response to toxin, while having minimal effect on gating properties of the channels. We modeled the activation gating of Kv4.3 and Kv4.1 and incorporated the effects of HpTx2 into the kinetic parameters of activation. The model is similar for both channels; it has four voltage-dependent transitions between the closed states followed by voltage-independent transition to an open state. Voltage-dependent transitions in Kv4.3 are more strongly affected by toxin. In Kv4.1, the 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, compared to toxin-bound Kv4.3 channels. This decreases the voltage dependence of toxin-bound Kv4.1 opening. The model closely recapitulates our experimental data. These data show that amino acids near the HpTx2 binding site play a role in the kinetics of Kv4 channel activation gating.

3399-Pos Board B446
A Potassium Channel Blocking Toxin Isolated From The Venom Of The Scorpion *Centruroides suffusus suffusus*

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Voltage-gated potassium channels are widespread among various cell types playing roles in numerous cellular functions by controlling the membrane potential. Pharmacological manipulation of these channels carries the potential of selective interference with such well-defined cellular functions if molecules that bind to them with high affinity and specificity are available. Scorpion venoms have long been known for containing small peptide toxins bearing these properties. We are particularly interested in peptides blocking T cell Kv1.3 channels whose normal operation is essential for T cell mediated immune responses.
We screened HPLC purified peptide fractions from *Centruroides suffusus suffusus* scorpion venom for Kv1.3 channel blocking potency. Screening resulted in one biologically active peptide component, which was purified to homogeneity and named Css20. The toxin's amino acid sequence was completed by Edman degradation and mass spectrometry analysis. It contains 38 amino acid residues with a molecular weight of 4,000.3 Da, tightly folded by three disulfide bridges.
We have found that Css20 preferentially blocked the currents of the voltage-gated K^+ channels Kv1.2 and Kv1.3. Its blocking potency was tested on six other potassium channels and a cardiac sodium channel, but the toxin proved ineffective at 10 nM concentration. Dose-response curves of Css20 yielded an IC50 of 1.3 and 7.2 nM for Kv1.2 and Kv1.3 channels, respectively. Interestingly, despite the similar affinities for the two channels the association and dissociation rates of the toxin were much slower for Kv1.2, implying that different interactions may be involved in binding to the two channel types; an implication further supported by *in silico* docking analyses. Based on the primary structure of Css20, the systematic nomenclature proposed for this toxin is α-KTx 2.13.

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Differential Effects of Isoflurane on Mutant Cardiac IKs Channels

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The slow delayed-rectifier potassium (IKs) channel mediates repolarization of the cardiac action potential, and its dysfunction can lead to the long QT syndrome (LQTS). The IKs channel consists of two subunits, the pore-forming α-subunit, KCNQ1, and the auxiliary β-subunit, KCNE1. Volatile anesthetics have significant inhibitory effects on the IKs current, but their underlying molecular mechanism remains to be determined. We have previously shown that F340 was a critical residue involved in this interaction, similar to the observation reported for other putative IKs antagonists. In order to further elucidate this mechanism, we investigated the effects of a volatile anesthetic, isoflurane, on mutant constructs of the IKs channel. Two KCNQ1 mutants, A341C and A344C, were constructed by site-directed mutagenesis. These two residues were chosen due to their vicinity to F340, and to their roles in inherited LQTS. Whole-cell current was recorded from transiently transfected HEK-293 cells in the absence and presence of isoflurane (0.52 ± 0.01 mM). Isoflurane inhibited wild-type- (WT) KCNQ1 by 62.1 ± 1.9% (mean±SEM, n=9). This inhibition was significantly attenuated when WT-KCNQ1 was cotransfected with WT-KCNE1 (40.7 ±4.5%; n=9). In contrast, isoflurane inhibited the mutant A344C current by 25.5 ± 2.0% (n=13), but this inhibition was not affected by the cotransfection with WT-KCNE1 (26.3 ± 3.2%, n=13). The A341C mutant alone did not express any current. However, when A341C was cotransfected with WT-KCNE1, an IKs-like current was elicited that was inhibited by isoflurane by 63.1 ± 2.9% (n =11). These results show that in addition to F340, the A341 and A344 residues also contribute to the anesthetic binding environment. Furthermore, the differential impact of KCNE1 on the isoflurane effects on WT-KCNQ1 and the mutant constructs suggests a complex role for the β-subunit in modulating the pharmacology of the IKs channel.