

1642-Pos**Identification of Regulatory Phosphorylation Sites in Slack and Slick Sodium Activated Potassium (K_{Na}) Channels**

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Na^+ -activated potassium channels (K_{Na} channels), which are encoded by the *Slack* and *Slick* genes contribute to neuronal adaptation during sustained stimulation, and, in auditory brainstem neurons, may regulate the accuracy of timing of action potentials. These channels have been found to be modulated very potently by activation of protein kinase C (PKC) and by receptors linked to activation of this kinase. Activators of PKC increase the amplitude of Slack-B currents and slow their rate of activation, and in contrast, activation of PKC decreases the amplitude of Slick currents. Heteromeric Slack/Slick channels are regulated by PKC to a greater extent than either Slack-B or Slick heteromers (90% decrease in amplitude). Previous experiments using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) have identified three serine residues in Slack-B that are phosphorylated under basal conditions, but are not within consensus sites for PKC. In order to study the mechanisms of regulation of Slack and Slick channels by phosphorylation, we have begun to identify the specific residues that undergo phosphorylation by protein kinase C. Consensus sequence analysis predicts that there are 13 potential sites of possible PKC phosphorylation in the Slack-B sequence. We have constructed individual site mutants for each of these sites in which the serine/threonines have been mutated to alanines to prevent phosphorylation at these residues. These mutants were expressed in *Xenopus* oocytes and their response to a PKC-activating phorbol ester (TPA) was characterized by two-electrode whole cell clamp electrophysiology. Of the 13 consensus site mutants, only one generated currents that matched wild-type Slack-B currents in their amplitude and kinetic behavior, but completely failed to respond to application of TPA, suggesting that the phosphorylation state of a single residue regulates Slack-B current amplitude and rate of activation.

1643-Pos**Voltage Sensor Activation Facilitates Magnesium-Gated Channel Opening in BK Channels**

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Under physiological conditions, Mg^{2+} is an intracellular activator of Ca^{2+} - and voltage-activated potassium (BK) channels. To investigate gating by Mg^{2+} acting through its low affinity site located under each S4 voltage sensor (Yang et al. 2007, 2008; Horrigan & Ma 2008), we studied a BK channel in which the high affinity Ca^{2+} sites in both the RCK1 domain and the calcium bowl were disabled by mutation. Using single-channel recording from inside-out patches to measure channel activation after voltage steps from -100 mV to $+100$ mV, we found that 10 mM Mg^{2+} reduces the latency to first opening after the voltage step and increases channel activation through an increase in the number of openings per burst and mean open duration. This suggests that Mg^{2+} can bind to both closed and open states of the channel, but it is not clear whether the closed-state binding occurs at the negative or positive potential. Therefore we recorded single-channel activity in macro-patches held at a constant -50 or -100 mV. At -50 mV, when the voltage sensors are occasionally activated, 10 mM Mg^{2+} decreases the duration of the closed intervals between bursts of activity and increases burst duration through an increase in the number of openings per burst and mean open duration. At -100 mV, when the voltage sensors are mainly deactivated, 10 mM Mg^{2+} has little effect on the closed intervals between bursts or mean open duration, and most of the bursts are unitary, consisting of a single brief opening. The above data are consistent with a model in which Mg^{2+} can bind to the BK channel in the closed conformation when the voltage sensors are activated. The bound Mg^{2+} then facilitates opening. Supported by NIH grant AR32805.

1644-Pos **Zn^{2+} Activates Human Large-Conductance Ca^{2+} -Activated K^+ Channel**

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Zinc plays a crucial role as an integral structural and catalytic factor in many transcription factors and enzymes and this metal ion (Zn^{2+}) is also increasingly recognized as a potential intracellular signaling molecule. However, the direct Zn^{2+} sensors especially those membrane localized are not fully established. Using inside-out patch clamp recording, we observed that heterologously expressed human large-conductance and voltage- and Ca^{2+} -activated

K^+ (BKCa, Slo1 BK, or KCa1.1) channels were quickly and reversibly activated by intracellular Zn^{2+} . Zn^{2+} did not affect the unitary conductance but significantly increased the channel open probability. Macroscopic current measurements showed that Zn^{2+} -mediated activation of the channel was accompanied by a leftward shift of the conductance-voltage (G-V) curve by up to 75 mV. The effect of Zn^{2+} was antagonized by high concentrations of intracellular H^+ or Ca^{2+} , indicating the three factors activate BK channels via a common mechanism. Mutagenesis experiments showed that mutation of H365 to arginine, a critical residue for the channel activation by H^+ and Ca^{2+} , fully abolished the effect of Zn^{2+} . In addition, mutation of two other nearby acidic residues D367 or E399 to alanine also partially impaired the effect of Zn^{2+} . Collectively, our results suggest that a novel multifunctional structure located in the regulator of conductance for K^+ (RCK)1 domain is involved in Zn^{2+} coordination and activation of the Slo1 BK channel and indicate a potential role of Slo1 BK channel in Zn^{2+} signaling in both physiological condition and hypoxic/ischemic diseases in which $[Zn^{2+}]_i$ is significantly increased. (Supported in part by NIH)

1645-Pos**Molecular Determinants For hSlo1 Bk Channel Activation by Dihydroabietic Acid Derivatives**

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Large-conductance calcium- and voltage-activated potassium (BK) channels are key players in vasoregulation and in the control of neurotransmitter release. Use of an alternative exon9 results in a neuronal variant of BK channels (e9alt) with altered amino acid sequence extending from the middle of S6 to a cytosolic linker connecting to the RCK gating ring (C-linker of 14 residues). hSlo1 wild-type (e9), splice variant and mutant channels were heterologously expressed in HEK 293 cells and analyzed in inside-out patches. Channels were characterized with respect to activation by transmembrane voltage, intracellular calcium and dihydroabietic acid derivatives (DHAAs). diCl-DHAA, triCl-DHAA and Cym04 (10 μ M, Cui et al. (2008) Bioorg Med Chem Lett 18:6386-6389) were found to activate e9 channels via shifting their voltage dependence by up to ~ 40 mV to hyperpolarized potentials in the virtual absence of calcium. In contrast, e9alt channels did not exhibit a left-shift in activation. The most active compound, Cym04, was used to identify the molecular determinants for BK activation by DHAAs by mutagenesis. Differences between e9 and e9alt in calcium- and voltage-dependent gating are brought about by residues within S6 and in the C-linker. In contrast, molecular determinants for activation by Cym04 reside in the C-linker: of the 10 residues differing between e9 and e9alt, amino-acid exchanges G327L, K330N, S337N and S340G had a high impact on channel activation by Cym04. Mutation S340G almost completely abolished BK activation by Cym04 without markedly changing calcium- and voltage-dependent gating. The linker connecting the RCK gating ring and the pore of BK channels therefore represents a candidate region for binding DHAA-type, isoform-specific BK channel activators.

1646-Pos**An Epilepsy-Associated Mutation Enhances BK Channel Activity by Altering the Coupling of Calcium Sensing to Gate Activation**

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Voltage and Ca^{2+} activated BK channels modulate neuronal activities. Previous studies found that Ca^{2+} binding sites and the activation gate are spatially separated, but how Ca^{2+} binding couples to gate opening is not clear. We address this question by studying how a mutation in BK channels, which is associated with generalized epilepsy and paroxysmal dyskinesia (D434G in hSlo, the epilepsy mutation), enhances Ca^{2+} sensitivity. The epilepsy mutation is located in a structural domain (the AC region) that connects the S6 transmembrane segment with the cytoplasmic domain and close to a putative Ca^{2+} binding site. Mutagenesis studies show that the AC region is important in the coupling between Ca^{2+} binding and gate opening. We found that discrete locations in the AC region that are distant from the epilepsy mutation alter the functional effects of the mutation. Thus, the epilepsy mutation enhances Ca^{2+} sensitivity by an allosteric mechanism affecting the coupling between Ca^{2+} binding and gate opening. Interestingly, mutating the Asp residue to