Mg$^{2+}$ at this site ("F330Y") decreased PoC by 0.37 down to 0.13. We conclude that opening at low voltages. PoC for G327F and G327Y were 0.19 and 0.01, respectively. Substitution at that site revealed that hydrophobicity is most important for channel activation. Under this condition, 10 mM Mg$^{2+}$ activates (down) or activated (up). We therefore recorded single-channel activity and increases mean open duration. These effects are attenuated at 100 mV, consistent with the hypothesis that Mg$^{2+}$ can bind to the closed channel and shorten the latency. However, it is not clear whether the closed-channel binding occurs when the voltage sensors are deactivated (down) or activated (up). We therefore recorded single-channel activity in macro-patches held at constant --50 mV, where voltage sensors occasionally activate. Under this condition, 10 mM Mg$^{2+}$ decreases mean closed duration and increases mean open duration. These effects are attenuated at --100 mV, and become negligible at --150 mV, where the voltage sensors are mainly deactivated. For BK channels modified to have deactivated voltage-sensors (R167E), 10 mM Mg$^{2+}$ has little effect on mean closed and open durations at --50 mV. In contrast, for BK channels modified to have constitutively activated voltage sensors (R210C), 10 mM Mg$^{2+}$ shortens the mean closed durations and lengthens the mean open durations at --200 mV. The above observations are consistent with a model in which voltage sensor deactivation inhibits BK channel opening by Mg$^{2+}$. Supported by NIH grant AR32805 and AHA 10POST4490012.

**3150-Pos Board B258**

Voltage Sensor Deactivation Inhibits BK Channel Opening by Mg$^{2+}$

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Functional and structural studies suggest that intracellular Mg$^{2+}$ activates BK channels through interaction with the voltage-sensing domain (Yang et al. 2007, 2008; Horrigan & Ma 2008; Yuan et al. 2010; Wu et al. 2010). To further explore the mechanism of activation of BK channels by Mg$^{2+}$ through the low affinity E374/E399 Mg$^{2+}$ sites located beneath the voltage sensors, we use single-channel and two-channel experiments on BK channels mutated to remove the high affinity Ca$^{2+}$ sites. We find that 10 mM Mg$^{2+}$ shortens the latency to first channel opening after a voltage jump to +100 mV from --100 mV, consistent with the hypothesis that Mg$^{2+}$ can bind to the closed channel and shorten the latency. However, it is not clear whether the closed-channel binding occurs when the voltage sensors are deactivated (down) or activated (up). We therefore recorded single-channel activity in macro-patches held at constant --50 mV, where voltage sensors occasionally activate. Under this condition, 10 mM Mg$^{2+}$ decreases mean closed duration and increases mean open duration. These effects are attenuated at --100 mV, and become negligible at --150 mV, where the voltage sensors are mainly deactivated. For BK channels modified to have deactivated voltage-sensors (R167E), 10 mM Mg$^{2+}$ has little effect on mean closed and open durations at --50 mV. In contrast, for BK channels modified to have constitutively activated voltage sensors (R210C), 10 mM Mg$^{2+}$ shortens the mean closed durations and lengthens the mean open durations at --200 mV. The above observations are consistent with a model in which voltage sensor deactivation inhibits BK channel opening by Mg$^{2+}$. Supported by NIH grant AR32805 and AHA 10POST4499012.

**3151-Pos Board B256**

Influence of Hydrophobic Residues on BK Channel Gating

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Large-conductance calcium- and voltage-activated potassium (Slo1 BK) channels participate in the control of vascular tone and neurotransmitter release. A neuronal splice variant of BK channels with altered sequence in the S6/RCK1 linker exhibits increased open probability in 100 μM calcium at --150 mV (PoC) compared to “wild-type” BK channels (Soon et al. 2008, Channels 2:278-282). To identify amino-acid residues underlying this notable change in gating behavior, we expressed wild-type and mutant human Slo1 BK channels in HEK 293 cells and analyzed their calcium- and voltage-dependent gating in the inside-out configuration of the patch-clamp technique. Mutation G327L, located in the linker connecting the S6 helix to RCK1, most strongly increased PoC from 0.02 to 0.24. Systematic substitution at that site revealed that hydrophobicity is most important for channel opening. At low voltages, PoC for G327F and G327V were 0.19 and 0.01, respectively; addition of a single hydroxy group (“F327Y”) decreased PoC by 0.18. The same change in hydrophobicity by mutation F315Y within S6 has qualitatively opposite effects (Lippait et al. 2000, J Physiol 529:131-138). Phenylalanine scanning mutagenesis of the S6/RCK1-linker region revealed that the mutation K330F most strongly increased PoC around 0.5. Introduction of an additional hydroxy group at this site (“F330Y”) decreased PoC by 0.37 down to 0.13. We conclude that hydrophobicity within the S6/RCK1-linker region is a critical determinant of the calcium-dependent gating.

**3152-Pos Board B257**

Voltage-Dependent Inactivation Gating at the Selectivity Filter of the MthK K+ channel

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Voltage-dependent K+ channels can undergo a gating process known as C-type inactivation, which involves entry into a non-conducting state through conformational changes near the channel’s selectivity filter. Here we report on a form of voltage-dependent inactivation gating observed in MthK, a prokaryotic Ca2+- and Ba2+-gated K+ channel. In single-channel recordings, we observe that Po decreases with depolarization, with a half-maximal voltage of 96 ± 3 mV. This gating is kinetically distinct from blockade by internal Ca2+ or Ba2+, suggesting it may arise from a different intrinsic mechanism. Inactivation gating toward more positive voltages by increasing external [K+] (47 mV per 10-fold increase in [K+]i), suggesting that K+ binding to the extracellular side of the channel stabilizes the open-conductive state. The open-conductive state was stabilized by other external cations, and selectivity of the stabilizing site followed the sequence: K+ ≈ Rb+ > Cs+ > Na+ > Li+ ≈ NMG+. Selectivity of the stabilizing site is weaker than that of sites that determine permeability of these ions, suggesting that the site may lie toward the external end of the MthK selectivity filter. We could describe MthK gating over a wide range of positive voltages and external [K+] using kinetic schemes in which the open-conductive state is stabilized by K+ binding to a site that is not deep within the electric field, with the voltage-dependence of inactivation arising from both voltage-dependent K+ dissociation and transitions between non-conducting (inactivated) states. These results provide a quantitative working hypothesis for voltage-dependent, K+-sensitive inactivation gating, a property that may be common to other K+ channels.

**3153-Pos Board B258**

C. elegans Slo-2b uses its RCK1 Domain as a Ca2+ Sensor and does not Exhibit CT Dependence

Zhe Zhang, Qiong-Yao Tang, Diomedes E. Logothetis.

Slo-2 channels play an important role in the adaption of neuronal firing rates and have been implicated in protection against ischemia. Slo-2 channels belong to the family of high-conductance potassium channels but their gating mechanism is unique and has been reported to exhibit species differences. The rat Slo2 (Slack) channel is activated by Na+ and CT, whereas the C elegans Slo-2a has been reported to be sensitive to Ca2+ and CT. Here, we report isolation of a novel isoform of the C. elegans channel Slo-2b (F08b12.3c) that was cloned from ESTs (YK15221e1, YK1193) of C.elegans, which has a distinct N-terminal region (by 18 amino acids) compared to the previously reported Slo-2a (F08b12.3b) (Yuan et al. 2000). This new clone shows voltage- and Ca2+-activated macroscopic currents when expressed in Xenopus oocytes. We find that the C. elegans Slo-2b channel isoform exhibits a unitary conductance consistent with Slo-2a but it is not activated by CT. Furthermore, the current characteristics of Slo-2b can be described well by the Horrigan-Aldrich model, which had been developed to describe Slo1 current properties. Mutagenesis screening revealed that the Slo-2b channel with mutation of a critical Glu residue in the RCK1 domain largely controls Ca2+ sensitivity. In contrast, mutations of negatively charged residues around the region corresponding to the Na+ sensitive site of Slack channels in the RCK2 domain do not affect Ca2+ sensitivity of the Slo-2b channel. Thus, we conclude that the Ca2+ sensor of the Slo-2b in the RCK1 domain is largely sufficient to confer Ca2+-sensitivity to the Slo-2b channel isoform.