coupling. Interestingly, phloretin was more efficacious in 0 Ca^{2+} than at higher Ca^{2+} concentrations, suggesting phloretin also affects Ca^{2+}-dependent gating. Mechanisms of action similar to phloretin may prove useful in selectively activating BK channels at hyperpolarized membrane potentials. Thus, compounds that are structurally similar (phloridzin, naringenin, and 2-PT) or dissimilar (NS1619 and niflumic acid) were screened at −80 mV in 0 Ca^{2+} for phloretin-like activity. Although most openers increased nP_o two- to three-fold, only NS1619 (100 μM) was as efficacious as phloretin, suggesting these compounds may share similar mechanisms of action.

3468-Pos Board B329
The Interaction of Voltage-Sensor and Gate in BK Channels
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To study the molecular basis and mechanism of voltage sensor/gate interaction in BK channels we performed an alanine-scan of the S4-S5 linker and the C-terminal end of S6 in mSlo1. Potassium conductance was measured over a wide range of voltage and calcium and fit to the Horrigan-Aldrich (HA) gating model. Open probability was measured at extreme negative voltages to determine if mutations altered the stability of the gate when voltage sensors are not activated (L in the HA model). Mutations in both linker and S6 were identified that alter L and therefore could be involved in voltage-sensor/gate interaction. The largest inhibitory effects were observed at the ends of S4-S5 linker (Q222A, I233A and K234A) and P319A in S6, each of which decreased L by 10- to 100-fold and shifted V0.5 to more positive voltages. I233A, unlike Q222A or K234A, also produced a decrease in voltage-dependence that is consistent with a decrease in voltage-sensor/gate coupling (D in the HA model). A structural model, based on Kv1.2, suggests inter- or intra-subunit interactions could potentially exist between these sites. Similarly, mutations near the end of S6 (R329A, K330A) that produce an approximate 3 fold increase in L without changing V0.5, may decrease voltage-sensor/gate coupling and could potentially interact with the N-terminal end of the linker where mutations F223A, L227A also produced a moderate increase in L. On the other hand, the largest increases in L were observed with mutations in S6 (P320A, E321A, E324A) that have no obvious interaction partner in S4-S5 and potentially alter the stability of the gate directly. In summary, we have identified several candidates that may be involved in voltage-sensor/gate interaction and will provide a basis for future experiments involving double mutant cycle analysis.

3469-Pos Board B330
An Extracellular Domain of BK Channel β1 Accessory Subunit Modulates the Activities of Voltage Sensor and Gate
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A family of tissue-specific auxiliary β subunits modulate large conductance, voltage- and calcium- activated potassium (BK) channel gating properties to suit their diverse function. Paradoxically, β subunits both promote BK channel activation through a stabilization of voltage-sensor activation, and reduce BK channel openings through an increased energetic barrier of the closed-to-open transition. The molecular determinants underlying β-subunit function, including the dual gating effects remain unknown. Here, we report the first identification of a β1 functional domain consisting of Y74, S104, Y105 and I106 residues located in the extracellular loop of β1. These amino acids reside within two regions of highest conservation among related β1, β2 and β4 subunits. Analysis in the context of the Horrigan-Aldrich gating model revealed that this domain functions to both promote voltage-sensor activation, but also reduce intrinsic gating. Free energy calculations suggest that the dual effects of the β1 Y74, S104-I106 domain can be largely accounted for by a relative destabilization of channels in open states that have few voltage sensors activated. These results suggest a unique and novel mechanism for β subunit modulation of voltage-gated potassium channels wherein interactions between extracellular β subunit residues with the external portions of the gate and voltage sensor regulate channel opening.

3470-Pos Board B331
Accessory Beta Subunits Alter the Interaction Between the Membrane-Spanning and Cytosolic Domains in BK Channels
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Large conductance, Ca^{2+} and voltage-dependent K^+ (BK) channels modulate physiology processes such as smooth muscle contraction and neurotransmission. BK channels are composed of the pore-forming α subunit (slo1) and four types of auxiliary β subunits (β1-4). While these β subunits show structural homology, they regulate gating properties of BK channels with distinct characteristics. For instance, the β1 subunit targets the membrane-spanning voltage sensor domain (VSD), while the β2 subunit targets the cytosolic domain (CTD) of slo1 to enhance Ca^{2+} sensitivity. Here we study how these β subunits perform a similar function by affecting two different structural domains of slo1. Using patch clamp techniques to study BK channels expressed in *Xenopus* oocytes, we found that both β1 and β2ND (the N-terminal residues 2-20 deleted to eliminate inactivation) alter channel activation by Mg^{2+} that is bound at the interface between VSD and CTD, suggesting that both β subunits alter the interaction between the two domains. Our data also show that a disulfide bond between residues 99C (in VSD) and 397C (in CTD) forms in the absence but not in the presence of β subunits, indicating that the alignment between the VSD and CTD is changed by both β subunits. Conversely, two mutations, N172R and N172RE399R, which have been found to alter the alignment between the VSD and CTD by introducing electrostatic forces between the two structural domains, change the effects of both β subunits on BK channel activation. Taken together, these results suggest that both β1 and β2 subunits alter the interactions between the VSD and the CTD by targeting one of the domains respectively to activate BK channels. This mechanism might be common for different β subunits modulating BK channel gating.

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Voltage- and Ca^{2+}-Activation of BK Channels Studied with Highly Constrained Allosteric Gating Mechanisms
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BK channels are activated synergistically by depolarization and micromolar intracellular Ca^{2+}. An allosteric gating scheme for a simplified BK channel with one voltage sensor and one Ca^{2+} site on each of the four subunits would lead to a minimal 50 state two-tiered model with 25 closed states (upper tier) and 25 open states (lower tier). It has been previously shown that allosteric models of this type with few constrained rate constants can approximate the gating of BK channels. We now explore to what extent such models with idealized gating (imposed by highly constrained rate constants) can account for the single-channel gating. Single-channel data were collected over wide ranges of voltage and Ca^{2+}, and successive interval durations were measured and binned into 2-D dwell-time distributions. The idealized models were then globally fitted to the distributions using maximum likelihood methods to estimate the rate constants and allosteric gating parameters. An idealized model with independent voltage and Ca^{2+} sensors modulating the opening and closing rates could approximate the gating, but with some obvious differences between predicted and experimental data. The most likely parameters in this model indicated that each of the activated voltage and Ca^{2+} sensors increased the opening rates an additional ~10-40 fold, with little effect on the closing rates. Adding a tier of flicker closed states and/or allowing specified cooperativity among and between the voltage and Ca^{2+} sensors improved the description of the data. Such highly constrained models provide a means to include the large numbers of states entered during gating of BK channels with multiple sensors per subunit, while limiting the number of gating parameters sufficiently to allow insight into gating mechanism. Supported by AHA grant 10POST4490012 and NIH grant AR32805.

3472-Pos Board B333
Purification of the Voltage Sensor Domain of KCNQ1 and its Interactions with Cholesterol and Sphingomyelin
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Human KCNQ1 (Kv7.1 or KvLQT1), the transmembrane domain of which is composed of a voltage sensor domain and a pore domain, plays a crucial role in cardiovascular diseases by playing a central role in cell repolarization as part of the action potential. It is well known that KCNQ1 is co-expressed with KCNE1 to form the Iks channel, for which mutations in either protein can cause cardiac arrhythmias and hearing loss in humans. KCNQ1 is also expressed in different epithelia, where it is involved in water and salt transport. The plasma membrane environment of KCNQ1 and KCNE1 contains both cholesterol and sphingolipids that may be involved in the formation of microdomains often referred to as "lipid rafts". Lipid rafts are important for cardiovascular function and may serve as platforms where Kv channels exhibit distinct functional properties relative to conditions in which they are in the bulk