the model of Horrigan and Aldrich (J Gen Physiol. 120, 267, 2002). In contrast, at such negative voltages, H+ at pH = 6.2 was much less effective in increasing open probability, leading to the estimated C value = ~1.3, placing H+ between Ca2+ and Mg2+ in the ability to promote opening in the absence of voltage sensor activation. Likewise, at the respective saturation concentrations, H+ was less effective than Ca2+ at regulating channel kinetics. Our electrophysiological measurements and simulations collectively suggest that H+ is a partial agonist of the RCK1 high-affinity Ca2+ sensor and that a small change in the relative position of His and Asp residues in the sensor, ~0.1 nm, may underlie the activation of the channel by H+. Supported by NIH.

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Pharmacological Evidence For Deep Pore Gating In SK Channels

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SK channels ($K_{Ca}2.1$-$K_{Ca}2.3$) are gated by Ca^{2+} through calmodulin bound to a domain (CaMBD) in the cytoplasmic C-terminus. The $[Ca^{2+}]_i$ response curve is identical for all SK subtypes and a similar gating applies for the related IK channel ($K_{Ca}3.1$). Pharmacological intervention with SK/IK channels is achievable by peptides and synthetic pore blockers but also by modulators of the gating process. Positive modulators of IK and SK channels, such as 1-EBIO and NS309, induce a shift of the $[Ca^{2+}]_i$ response curve towards lower $[Ca^{2+}]_i$ and have been known for several years.

Recently the ''opposite'' principle, negative gating modulation, was described for NS8593 (Strøbæk et al., 2006). NS8593 selectively inhibits SK channels and in whole-cell patch-clamp experiments, using HEK293 cells transiently transfected with hSK3, a K_d value of 108 ± 33 nM (n=12) was obtained, whereas hIK was insensitive to 10 μM NS8593. The positive modulation by 1-EBIO is mediated via the C-terminus (Pedarzani et al., 2005), but we have found that SK3 channels in which the C-terminus was substituted with the corresponding IK channel tail retained their NS8593-sensitivity (Biophys. J. 2008 94: 2183). Now we show, that amino acids in the deep pore are important for NS8593-induced inhibition: IK channels where T250, a residue just below the selectivity filter, or V275, in TM6, was substituted with the corresponding amino acids from SK3 became sensitive to NS8593 with K_d values of 513 ± 274 nM (n=6) and 4181 ± 1530 nM (n=9), respectively. Interestingly, these exact amino acids are essential for block of IK channels by TRAM-34 and clotrimazole (Wulff et al., 2001). Additional experiments will be conducted to determine whether NS8593 acts as a negative gating modifier on these IK mutants and whether these amino acids play a role in the normal gating process of SK channels.

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In Vivo Measurements Of a Ca^{2+}- And Voltage-Activated K^+ Channel Intramolecular Distances Using Genetically Encoded Reporters

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The large-conductance Ca^{2+} and voltage-activated K^+ (BK_{Ca}) channel possesses an Alfa pore-forming subunit and 4 different tissue specific regulatory Beta subunits. The Alfa subunit of BK_{Ca} channels contains the S4-based voltage sensor of Kv channels, but is an exception inside the S4 superfamily of ion channels because it is made up of seven (instead of 6) transmembrane segments (S0-S6) with the N terminus facing the extracellular side. Our knowledge is limited of how this extra transmembrane segment affects the architecture of BK_{Ca} channels. Equally mysterious is the actual structure of the Alfa-Beta subunit complex. Here we use the genetically encoded Lanthanide Binding Tag (LBT) that binds Tb^{3+} as LRET donor and the Charibdotoxin (CTX) labeled with Tetramethylrhodamine (TMR) for in vivo spectroscopic studies of intramolecular distances and interactions between Alfa and Beta1 subunit of BK_{Ca} channel. We have measured the distance between the extracellular end of S0, S1, S2, and S3 in the Alfa subunit to the TMR in the CTX blocking the pore with and without Beta1 subunit. We have also measured the distance from TM1, TM2 and loop of Beta1 subunit to the TMR in the toxin. We found that segment S0 is further away than the other segments with respect to the center of the pore in the absence of Beta1 subunit, locating it in the periphery of the molecule. However, it becomes closer to the center when Alfa is co expressed with Beta1 subunit. Beta1 co-expression also changes the position of S2. We found that some transmembrane domains of Alfa are further away than Beta1's transmembranes domains, indicating that the Beta subunit is embedded in the protein. Support: NIHGM30376 and FONDECYT 1070049.

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Arterial Smooth Muscle BK Channel Beta1 Subunits Determine Ethanol-Induced Cerebrovascular Constriction

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Ethanol at concentrations obtained in circulation after binge drinking (≤50 mM) causes cerebrovascular constriction, which primarily results from ethanol-induced inhibition of arterial smooth muscle voltage- and calcium-gated potassium (BK) channels (Liu et al., 2004). Cerebrovascular myocyte BK channels are made of channel-forming α (encoded by *KCNMA1*) and smooth muscle-abundant β1 subunits (encoded by *KCNMB1*). After cloning α (''cbv1'', AY330293) and β1 (FJ154955) subunits from rat cerebral artery myocytes, we set to identify the molecular effector of ethanol-induced inhibition of channel activity and cerebrovascular constriction. Cbv1 and cbv1+β1 channels were expressed in *Xenopus* oocytes and channel steady-state activity (NPo) was recorded in inside-out (I/O) macropatches at a wide $Ca^{2+}{}_i$ range (0.3-100 μM). Ethanol potentiated current at $Ca^{2+}{}_i$<20 μM while inhibiting current at $Ca^{2+}{}_i$>30 μM. Beta1 subunits shifted the crossover for ethanol-induced macroscopic current potentiation to inhibition towards lower $Ca^{2+}{}_i$ (≤3 μM). This shift was paralleled by a similar shift in NPo. To evaluate β1-modulation of ethanol action in native channels, we probed ethanol on BK channels in cerebral artery myocytes isolated from *wt* (C57BL/6) and *KCNMB1* K/O mice. Recordings were performed in I/O patches, at Vm=-20, -40mV and $Ca^{2+}{}_i$=10 μM. In myocytes from *wt* mice (having BK made of α and β1 subunits) 50 mM ethanol significantly decreased NPo (-23.4 ± 9%). In contrast, ethanol reversibly increased NPo in *KCNMB1* K/O myocytes. Finally, we pressurized isolated mouse cerebral arteries and evaluated the impact of β1 subunit modulation of ethanol action on organ function. In vessels from *wt* animals ethanol caused a robust decrease in diameter (-14%). In contrast, *KCNMB1* K/O vessels were resistant to this ethanol action. Our data indicate that BK β1 subunits are the functional targets mediating ethanol-induced cerebrovascular constriction. NIH Grant AA11560 (AMD).

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Disulfide Crosslinking Between BK Channel Alpha And Beta1 Subunits In The Membrane Domain

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The large-conductance, Ca2+- and voltage-activated potassium channel (BK) alpha subunit is modulated by one of four types of beta subunits, each imparting unique electrophysiological properties. The beta1 subunit is expressed in smooth muscle, where it renders the BK channel sensitive to cytoplasmic calcium in a voltage range near the smooth-muscle resting potential and slows activation and deactivation. Previously, we inferred from the extents of endogenous disulfide bond formation between cysteines substituted in the extracellular flanks of the transmembrane (TM) helices of alpha and of beta1, that the extracellular flank of S0, the unique seventh TM helix of BK alpha, is surrounded on three sides by the extracellular flanks of S1 and S2 and the four-residue, extracellular loop between S3 and S4. We also found that the extracellular flanks of beta1 TM2 and alpha S0 are close and that that the extracellular end of beta1 TM1 is close to the extracellular flanks of both alpha S1 and S2. Within the membrane domain, where presumably there is less flexibility than in the extracellular flanks, we also see endogenous (no added reagents) crosslinking one to two helical turns into the membrane domain. Within the same alpha subunit, Cys-substituted residues in S0 readily form disulfides with Cys-substituted residues in S4, and to a lesser extent with Cys-substituted residues in S3. Also within the membrane domain, we find that cysteines in alpha S0 readily form disulfides with cysteines in beta TM2. Thus, the positions of alpha S0 and of beta1 TM2 relative to alpha S0 are similar in the membrane domain and in the extracellular flanks of these TM helices.
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Locations Of The Beta2 Transmembrane Helices In The BK Potassium Channel

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The large-conductance, Ca2+- and voltage-activated potassium channel (BK) alpha subunit is modulated by one of four types of beta subunits, each imparting unique electrophysiological properties. Beta2, which is expressed in the brain, adrenal chromaffin cells, pancreas, and ovaries, increases Ca2+ sensitivity of BK alpha, by shifting voltage-dependent activation to more negative voltages,