and causes N-type inactivation. We explored the contacts between alpha and beta2 subunits by determining the extent of endogenous disulfide bond formation between cysteines substituted in the extracellular flanks of the two beta2 transmembrane (TM) helices, TM1 and TM2, and in the extracellular flanks of each of the seven alpha TM helices, S0-S6. We found that the extracellular ends of beta2 TM2 and alpha S0 are close and that beta2 TM1 is close to both S1 and S2. At their extracellular ends, TM1 and TM2 are not close to S3, S4, S5 or S6. Beta2 TM1 and TM2 are like pincers on either side of the alpha voltage-sensor domain, S0-S4. In all tested pairs of cysteine-substituted alpha and beta2, we found that disulfide crosslinks favored the closed state, shifting the conductance-voltage curves toward more positive potentials and slowing the kinetics of activation. N-type inactivation, involving three specific beta2 residues in its cytoplasmic, N-terminal segment preceding TM1, was not affected by any of the crosslinking. This is consistent with the above locations of TM1 and TM2 because a minimum of 12 residues, spanning up to 40 Å, allows the three N-terminal inactivating residues to reach the pore (Xia et al., 2003 J. Gen. Physiol. 121:125). The positions of the beta2 TM helices are similar to the locations that we previously reported for beta1 TM1 and TM2 (Liu et al., 2008 PNAS 105:10727). Supported by NIH NS054946.

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The β2 subunit modulation of BK channels is determined by membrane-spanning and cytoplasmic domains In Slo1

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Ca^{2+} and voltage activated BK channels are composed of pore forming Slo1 subunits. These channels are modulated by various tissue-specific accessory β subunits, which render BK channels the phenotypes necessary for different physiological functions. Here we study Ca^{2+} sensitivity increase in BK channel activation by the β2 subunit, and elucidate the structural domains in Slo1 that determine this modulation. We found that β2ND (β2 with $\underline{NH_2}$-terminus $\underline{d}$eleted to remove inactivation) (Wallner et al., *PNAS* **96**(7):4137-42, 1999) increased Ca^{2+} sensitivity in mouse Slo1 (mSlo1) but not in drosophila Slo1 (dSlo1). Taking advantage of these differential effects, chimeras of mSlo1 and dSlo1 were studied. When chimeras in the mSlo1 background contained the S0 transmembrane segment and the N-terminal region of RCK1 (regulator of K^+ conductance) termed the AC region (Krishnamoorthy et al., *JGP* **126**(3): 227-41, 2005) from dSlo1, β2ND failed to increase Ca^{2+} sensitivity. When these same regions from mSlo1 were in dSlo1, the channels showed increased Ca^{2+} sensitivity in association with β2ND. Thus, the mouse AC region and S0 segment are necessary and sufficient for the β2 subunit to increase Ca^{2+} sensitivity. Previous studies suggested that each Slo1 subunit contains two different Ca2+ binding sites (Xia et al., *Nature* **418**(6900): 880-4, 2002). To further investigate the β2 subunit modulation, we studied the effect of β2ND with mutations of the binding sites. We found that the effect of β2ND was nearly intact when either site was ablated and was completely destroyed when both sites were mutated. These results suggest that the β2 subunit may affect an allosteric activation pathway that is common to both binding sites, and S0 or the AC region is part of such pathway.

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The Locations of the Beta4 Transmembrane Helices in the BK Channel

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The large-conductance, Ca^{2+}- and voltage-activated potassium channel (BK) alpha subunit is modulated by one of four types of beta subunits, each imparting unique electrophysiological properties. BK beta4 is expressed in brain. It slows both activation and deactivation, with only small shifts in V_{50}, and confers resistance to block by charybdotoxin and iberiotoxin. In mice, deletion of beta4 causes temporal lobe epilepsy. We explored the contacts between alpha and beta4 subunits by determining the extent of endogenous disulfide bond formation between cysteines substituted in the extracellular flanks of the two beta4 transmembrane (TM) helices, TM1 and TM2, and in the extracellular flanks of each of the seven alphaTM helices, S0-S6. We found that the extracellular ends of beta4 TM2 and alpha S0 are close and that beta4 TM1 is close to both S1 and S2. At their extracellular ends, TM1 and TM2 are not close to S3, S4, S5 or S6. Beta4 TM1 and TM2 are like pincers on either side of the alpha voltage-sensor domain, S0-S4. Crosslinking of beta4 TM2 to S0 further slowed activation and deactivation kinetics, with either no effect on V_{50}, or causing a small hyperpolarizing shift. Thus, crosslinking enhances the predominant effect of beta4 on the transition rates between the activated and deactivated states, with little effect on the free energy differences between these states. Supported by NS054946.

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Molecular and Functional Expression of the Best2 Ca2±activated Cl- Channel in Mouse Submandibular Salivary Gland

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Activation of Cl^- channels in salivary acinar and duct cells is essential for saliva production. Anion efflux through an apical Ca^{2+}-dependent Cl^- channel (CaCC) is the rate limiting step for fluid secretion by acinar cells. The ionic composition of the primary saliva is then modified by salivary ducts. CaCC may support electrolyte reabsorption by duct cells of several types that constitute the duct system. The molecular identity of salivary CaCC is currently under vigorous examination. Here we explored the function of Best2, a member of the Bestrophin family of CaCCs, in the mouse submandibular salivary gland. Heterologous expression of the Best2 transcript in HEK293 cells produced Ca^{2+}-activated Cl^- current with the biophysical and pharmacologic properties that closely resembled the current found in native salivary cells. A recently developed $Best2^{-/-}$ mouse where the gene was disrupted by insertion of Lac Z was used to further characterize the role of this channel in the exocrine salivary gland. Even though Best2 expression was abolished, the amplitude and properties of the Ca^{2+}-activated Cl^- current in the acinar cells obtained from Best2-deficient mice were the same as the Ca^{2+}-activated Cl^- current in wildtype cells. Consistent with the observation the fluid secretion rate was not significantly different in *Best2* null mice. *Best2* gene was highly expressed in the duct cells of submandibular glands as revealed by X-gal staining. While, the ionic composition and osmolality of the saliva was not significantly altered in mice lacking Best2, the possibility of the functional compensation has been investigated in duct cells. Granular duct cells failed to present Ca^{2+}-sensitive component of anion conductance. The properties of Cl^- channels in intercalated, striated and excretory duct cells are currently under investigation.

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Angiotensin Ii Activates Calcium-Dependent Cl- Channels In Human Cardiac Fibroblasts

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This study reports for the first time the presence of chloride channels on the plasma membrane of human cardiac fibroblasts in culture, by means of the iodide efflux and the patch clamp methods. The angiotensin II and the calcium ionophore A23187 activate a chloride conductance that shares pharmacological similarities with calcium-dependent chloride channels already described in other cell types. Using the iodide efflux technique it was shown that Ag II could induce an anionic efflux after binding to AT1 receptors (with an EC50 = 13.8 ± 1.3 nM). Blockade of chloride efflux by calphostin C and KN 62 indicates that this activation is dependent on PKC and/or CaMKII. This calcium-dependent chloride current which is characterized in human cardiac fibroblasts is potentially involved in the secretion by cardiac fibroblasts of growth factors; collagen and pro-inflammatory mediators released in particular pathological conditions.

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SKA-31, A New Activator of KCa2 And KCa3.1 Potassium Channels, Potentiates the EDHF Response and Lowers Blood Pressure

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Small-conductance (KCa2.1-2.3) and intermediate-conductance (KCa3.1) calcium-activated K^+ channels are critically involved in modulating calcium-signaling cascades and membrane potential in both excitable and non-excitable cells. Activators of these channels constitute useful pharmacological tools as well as potential new drugs for the treatment of ataxia, epilepsy, and hypertension. We here used the neuroprotectant riluzole as a template for the design of KCa2/3 channel activators that are potent enough for *in vivo* studies. Out of a library of 55 benzothiazoles we identified two compounds, SKA-20 (anthra[2,1-*d*]thiazol-2-amine) and SKA-31 (naphtho[1,2-*d*]thiazol-2-amine), which are 10-20 times more potent than riluzole and activated KCa2.1 with EC_{50}s of 430 nM and 2.9 μM, KCa2.2 with EC_{50}s of 1.9 μM, KCa2.3 with EC_{50}s of 1.2 μM and 2.9 μM, and KCa3.1 with EC_{50}s of 115 nM and 260 nM. Likewise, SKA-20 and SKA-31 activated native KCa2.3 and KCa3.1 channels in murine endothelial cells and the more ‘‘drug-like’’ SKA-31 (half-life 12 hours) potentiated endothelium-derived hyperpolarizing factor-mediated dilations of carotid arteries from $KCa3.1^{+/+}$ mice but not from $KCa3.1^{-/-}$ mice.