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,

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.

2449-Pos Board B419

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 deleted 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.

2450-Pos Board B420

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.

2451-Pos Board B421

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.

2452-Pos Board B422

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.

2453-Pos Board B423

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.