Myristic and/or palmitic acid incorporation to proteins is a mean by which cells tether proteins to the intracellular leaflet of plasma membranes. Two types of protein myristoylation have been reported; one occurs co-translationally at the N-terminus (e.g. c-Src) and the other post-translationally at an internal amino-acid residue. Here, we tested whether Slo1 might undergo post-translational myristoylation as it lacks an N-terminal consensus site for myristoylation. HEK-293T cells expressing Slo1 or c-Src (positive control) were metabolically radiolabeled with [^{3}H]-myristic acid and subjected to immunoprecipitation; radiolabeled proteins were detected by autoradiography. Our data show that Slo1 incorporates [^{3}H]-myristic acid (n=5) via a post-translational mechanism as assessed by the lack of effect upon inhibition of protein synthesis with cyclohexamide. As control, cyclohexamide treatment reduced c-Src myristoylation confirming its co-translational incorporation (n=3). Next, we sought to determine what type of chemical bond is involved in Slo1 protein myristoylation. Hydroxylamine (NH_2OH) at pH10 but not Tris-HCl at pH10 (negative control) or NH_2OH at pH 7, cleaves hydroxyester bonds. Treatment of **[^{3}H]-**myristoyl-Slo1 with NH_2OH, pH10 but neither treatment with Tris-HCl at pH10 nor NH_2OH at pH7, completely removed incorporated myristic acid from Slo1 (n=3). Possible palmitoylation of Slo1 via a thioester bond was excluded because treatment of labeled Slo1 with NH_2OH at pH7 which cleaves thioester bonds or 1.4 M β-mercaptoethanol, a reducing agent, did not alter the signal. Further, we did not observe Slo1 labeling using [^{3}H]-palmitate (n=2). These data strongly support an involvement of a hydroxyester chemical bond between myristic acid and Slo1 S/T/Y residue(s). In conclusion, we show for the first time that Slo1 protein is post-translationally myristoylated at an internal site. This myristoylation might play a role in controlling Slo1 channel structure, function or trafficking. Supported by NIH.

2439-Pos Board B409

Palmitoylation Controls BK Channel Regulation By Phosphorylation

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Large conductance calcium- and voltage- gated potassium (BK) channels are important regulators of physiological homeostasis and their function is potently modulated by protein kinase A (PKA) phosphorylation. PKA regulates the channel through phosphorylation of residues within the intracellular C-terminus of the pore-forming α-subunits. However, how PKA phosphorylation of the α-subunit effects changes in channel activity are unknown. The STREX variant of BK channels is inhibited by PKA as a result of phosphorylation of a serine residue within the evolutionary conserved STREX insert. As this inhibition is dependent upon phosphorylation of only a single α-subunit in the channel tetramer we hypothesised that phosphorylation results in major conformational rearrangements of the C-terminus. Using a combined imaging, biochemical and electrophysiological strategy we have defined the mechanism of PKA-inhibition of BK channels. We demonstrate that the cytosolic C-terminus of the STREX BK channel uniquely interacts with the plasma membrane via palmitoylation of evolutionary conserved cysteine residues. PKA-phosphorylation of STREX dissociates the C-terminus from the plasma membrane resulting in channel inhibition. Abolition of channel palmitoylation by site-directed mutagenesis or pharmacological inhibition of palmitoyl-transferases prevents PKA-mediated inhibition. Thus PKA inhibition of BK channels is conditional upon the palmitoylation status of the channel. Palmitoylation and phosphorylation are both dynamically regulated thus cross-talk between these two major post-translational signalling cascades provides a novel mechanism for conditional regulation of BK channels. Interplay of these distinct signalling cascades has important implications for the dynamic regulation of BK channels and the control of physiological homeostasis.

2440-Pos Board B410

Bovine and Mouse SLO3 K^+ Channels: Many Functional Differences Map to the Same Region

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Genes pertaining to male reproduction, especially those involved in sperm production, morphologically and functionally evolve much faster than their non-sexual counterparts. *SLO3* is an especially intriguing example of such a rapidly evolving gene. The *SLO3* gene encodes a K^+ channel which is expressed only in mammalian sperm and is evolving much faster than its close paralogue *SLO1* which is expressed in brain and other organs. We cloned the bovine orthologue of *SLO3* (*bSLO3*) and compared its primary sequence and functional properties to its mouse orthologue (*mSLO3*) which we previously cloned. A comparison of bSLO3 and mSLO3 primary sequences showed far less conservation than for SLO1 proteins in mouse and bovine species. Functionally, bSLO3 and mSLO3 also differ markedly with respect to their voltage range of activation, their ion selectivity, and their activation kinetics. Remarkably, although there are many regions of low conservation between bSLO3 and mSLO3 proteins, we found that all of the different functional properties that we measured map to a small region of low conservation in the RCK1 domain. One or more of these different functional properties may reflect differences in the resting membrane potentials of sperm in bovine and mouse species. This work was supported by National Institute of Health grants 1R21HD056444-01A1 to C.M.S. and R24 RR017342-01 and R01 GM067154-01A1 to L.S.

2441-Pos Board B411

Multiple Components of Ca-activated K currents in mouse pancreatic beta cells

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Multiple Components of Ca-activated K currents in mouse pancreatic beta cells
In beta cells, two types of Ca activated K channels have been described. One component, K_{slow}, is believed to be mediated by small-conductance, voltage-independent (SK) channels; it is thought to regulate the duration of intervals between successive action-potential bursts observed when beta cells are exposed to moderately elevated glucose. In contrast, the functional role of the second type of Ca activated K channels, the large conductance, Ca- and voltage-activated, BK channels, is not well understood. BK channel subunit genes have been detected in insulin-secreting cell lines, and BK channels have been observed functionally in rodent beta cells; however, early studies with BK channel blocking drugs have failed to identify a role for these channels in the electrical excitability or the stimulus-secretion coupling of beta cells.
Using patch clamp recording under quasi-physiological conditions, we show that the BK channel current can contribute up to a half of the outward current activated by depolarizing pulses whose amplitude resembles the voltage excursion of beta cell action potentials. Kinetic and pharmacological experiments reveal that the beta cell BK current consists of several pharmacologically, kinetically, and possibly spatially, distinct components. Our results suggest that the BK current could play a significant role in regulating beta cell electrical excitability of stimulus secretion coupling.

2442-Pos Board B412

Calcium Binding Causes A Conformational Change in The RCK1 Domain of The BK(Ca) Channel

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Calcium plays a major role in controlling the opening and closing of the large conductance BK_{Ca} channels. Two high affinity binding sites have been identified in the channel structure and one of these sites is the DRDD loop in the N-terminus of the RCK1 domain. Mutation of the first aspartate in this conserved DRDD motif significantly reduces Ca^{2+} sensitivity and hence this residue has been implicated as a coordinating group in the binding site. Here we present results on the prediction of the Ca^{2+} binding site based on a series of detailed computational studies. The basic protocol involves multiple iterations of random ion placement, implicit solvent molecular dynamics simulations and statistical analysis. Our resulting model matches very well with existing mutagenesis data, and subsequent explicit solvent molecular dynamics simulations have been performed using this Ca^{2+} bound structure. Comparison of the dynamics and conformations of the Ca^{2+} bound and unbound simulations reveal a concerted conformational change in the structure and suggest a potential mechanism for calcium dependent activation of these channels.

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Comparative Mechanisms Of Activation Of The Slo1 BK Channel By Ca2+ And H+ Mediated By The RCK1 Domain

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Large-conductance Ca2 ± and voltage-gated K+ (Slo1 BK) channels are allosterically activated by depolarization and intracellular Ca2+. High-affinity activation of the channel by Ca2+ involves two sites, the Ca2+ bowl sensor and the RCK1 sensor, the latter of which is also required for the stimulatory action of intracellular H+ (Hou et al., Nat Struct Mol Biol. 15, 403, 2008). We investigated the comparative effects of Ca2+ and H+ on activation of the Slo1 BK channel mediated by the RCK1 sensor using a Ca2+ bowl-defective mutant expressed in HEK cells. Decreasing pHi from 7.5 to 6.2 shifted the voltage-conductance (GV) curve to the left by ~50 mV. The shift in GV by H+ was, however, only ~40% of that caused by a saturating concentration of Ca2+ in the mutant. Single-channel measurements at negative voltages where voltage sensor activation is negligible verified that 200 uM Ca2+ drastically increased open probability, corresponding to the allosteric coupling factor C = ~4 in

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,