significantly as a function of pH over pH ranging from 6.5 to 9.0, suggesting that H^+ does not alter either functional coupling or cooperativity in Ca^{2+}-dependent gating. In addition, channel openings were not observed in the nominal absence of Ca^{2+} at pH up to 9.0. However, increasing pH decreased the EC_{50} for Ca^{2+} activation by ~4.7-fold per 10-fold increase in $[H^+]$, displaying a linear relation between $\log(EC_{50})$ and pH over the entire range of pH studied (6.5 to 9.0). Together, these results suggest that H^+-binding does not directly modulate either the allosteric coupling between Ca^{2+}-binding and channel opening or the channel's closed-open equibrium. We may account for the pH modulation by assuming that increasing pH yields a relative energetic stabilization of the Ca^{2+}-bound states over unliganded states of the channel.

659-Pos

Identification of Divalent Cation Coordinating Residues in a K^+ Channel RCK Domain by NMR Spectroscopy

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TvoK is a prokaryotic K^+ channel whose gating is modulated by divalent cation-binding to a carboxy-terminal RCK domain. To gain insight toward mechanisms underlying divalent cation binding and subsequent conformational changes, we measured chemical shift perturbations upon ligand binding in the soluble cytoplasmic RCK domain of TvoK using heteronuclear NMR spectroscopy.Uniformly ^{15}N-labeled, highly deuterated TvoK RCK domain was overexpressed in *E.coli* and purified by affinity and gel-filtration chromatography. ^{15}N-HSQC spectra showed well-dispersed crosspeaks corresponding to >85% of the 238 predicted backbone NH groups. Five-point titration experiments using 0 to 100 $\in$ 1/4M Mn^{2+} identified 12 residues that surrounded a putative divalent cation binding site, as indicated by spectral line-broadening due to the paramagnetic relaxation enhancement effect of Mn^{2+} (Mn-PRE). Partial resonance assignments, made through a combination of HNCA experiments and residue-specific ^{15}N-labeling, identify D192 and E226 as key residues in divalent cation coordination, as indicated by high sensitivity to Mn-PRE ($K_{app} < 10 \in 1/4M$). Further resonance assignments will identify remaining residues that lie within ~15 Å of the binding site. These experiments may reveal differences between the structural and chemical properties of the TvoK binding site and the Ca^{2+}-selective binding site of the MthK RCK domain, which may underlie differential selectivities of MthK and TvoK RCK domains for divalent cations.

660-Pos

Voltage-Dependent Motions Reported from the N-Terminal Region in Human Slo1 BK_{Ca} Channels: S0 and Voltage Sensor Operation

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The transmembrane region of large-conductance, voltage- and Ca^{2+}-activated K^+ (BK_{Ca}) channel α subunits (Slo1) possesses a unique topological feature when compared with those of other voltage-activated proteins: S0, an additional transmembrane segment that renders their short N-terminus extracellular (Wallner *et al.*, 1996, *PNAS*). S0 mediates the interaction between pore-forming α and auxilliary β subunits, which facilitate channel activation and, in the case of β2 and β3, induce fast inactivation. Recent findings have proposed that S0 is flanked by transmembrane voltage-sensing domains S2-S4 (Liu *et al.*, 2008, *JGP*). We used cut-open oocyte voltage-clamp fluorometry to explore the role of S0 in the function of the BK voltage sensor. By substituting unique Cysteines at positions 17-19 (20 thought to be at the extracellular tip of S0) of hSlo1 M4, and labeling them with the environment-sensitive fluorophore TMRM, we have resolved voltage-dependent conformational rearrangements, with $V_{half} = -79 \pm 2.9$ mV and $z = 0.84 \pm 0.046\ e^0$. Intriguingly, channel activation in these Cysteine mutants exhibited a facilitated voltage dependence of ionic conductance ($\in'' V_{half} = -30$ mV) compared to pseudo-WT channels. We provide direct evidence that the N-terminus influences the operation of the voltage-sensing S2 and S4 transmembrane domains: mutation R20A induced pronounced shifts in the activations of voltage-sensing segments S2 and S4 ($\in'' V_{half} = 46$ mV for S2, 66mV for S4) and a 35% reduction in the effective charge (z) of both. These results strongly indicate that the N-terminal region plays a significant role in the voltage sensor operation of human BK_{Ca} channels.

661-Pos

Charge Substitution of a Deep-Pore Residue Suggests Structural Rearrangements During BK Channel Activation

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During scanning mutagenesis of the S6 region of BK channels, we found a deep pore residue (M314 in hSlo) with interesting gating phenotypes. At pH7, the histidine substitution at this position (M314H) gave rise to currents with significantly slower deactivation kinetics than the wild-type BK. Deactivation became even slower with intracellular pH6 and pH5. To test whether this observation is an effect due to side-chain charges, we made three other charge-substituting mutants, M314K, M314E and M314D. For M314K, deactivation kinetics of the currents at pH7 was similar to that of M314H at pH5. This is consistent with the fact that the lysine (K) side-chain has a more basic pKa than that of histidine (H). For both M314H and M314K, increasing proton concentration (lowering pH) made the deactivation slower while reducing proton concentration (increasing pH) made the deactivation faster. For M314E, deactivation kinetics of the currents was fast at pH5 and became slower as the intracellular proton concentration was reduced to pH6, 7, 8, 9 and 10. The M314D channels could stay open at negative potentials in 0 Ca2+, at pH7, 8, 9 and 10. The most effective way to close the M314D channels was to increase the intracellular proton concentration to pH5. The results from all the charge-substituted mutants (M314H, M314K, M314E and M314D) support the idea that neutralizing the side-chain at the 314 position helped the channels close while charging the side-chain made it difficult for the channels to close. We propose that the charged form of the substituted residues may prefer the polar environment of the pore and stabilize the open state. The 314 residue may become more exposed to the pore as the channels transition from the closed to the open conformation.

662-Pos

Contribution of S3-S4 Extracellular Loop Residues to Block of Kca2 Channels by Apamin

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Small conductance Ca^{2+}-activated K^+ ($K_{Ca}2$) channels are widely distributed within the CNS and peripheral tissues. The cloning of these channels revealed three subtypes, each displaying a different sensitivity to block by the $K_{Ca}2$ selective toxin apamin. Mutation studies of $hK_{Ca}2.1$ and $rK_{Ca}2.2$ have indicated the importance of particular residues in both the channel pore and S3-S4 extracellular loop for block by apamin.

It has been reported that mutation of threonine (T)216 within the S3-S4 loop of $hK_{Ca}2.1$ to the corresponding serine (S) in $rK_{Ca}2.2$ resulted in a current that was more sensitive to block by apamin (Nolting et al, 2007; JBC 282, 3478). We have further investigated the residues in this extracellular loop region that contribute to block by apamin, d-tubocurarine (dTC) and tetraethylammonium (TEA). Block of expressed $K_{Ca}2$ channel current was assessed using outside-out macropatches, with current activated by 1 μM intracellular Ca^{2+}. Mutation S245 to T, to mirror the previously reported mutation of $hK_{Ca}2.1$(T216S), resulted in a reduction in the sensitivity to apamin and no change in the sensitivity to TEA and dTC. Double point mutation of the loop YA246/7 of $rK_{Ca}2.2$ to the corresponding LV of the apamin-insensitive $rK_{Ca}2.1$, abolished block by apamin, reduced sensitivity to dTC sensitivity, but did not affect sensitivity to TEA. In contrast, generation of $K_{Ca}2.2$(Y246L) reduced sensitivity to block by apamin, but did not change sensitivity to dTC. These data suggest that additional residues within the S3-S4 extracellular loop contribute to the high sensitivity to block by apamin exhibited by $K_{Ca}2.2$.

663-Pos

A Molecular Model for the Bkca Channel and the Location of B1 in the B1/A Subunit Complex

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We describe two approaches for the construction of a 3D molecular model for BK. First, we used a LRET technique in which an encoded **L**anthanide **B**inding **T**ag (**LBT**) that binds Tb^{3+} plays the role of the donors and SulphoRodhamine Methanothiosulfonate (TMSR) attached to Charibdotoxin (ChTX)) plays the role of the acceptor. The data obtained allowed us to determine the distances from the center of symmetry of the channel to the external aspect of S0, S1, S2, S3-S4 linker in the α subunit. The distances from the channel center of symmetry to TM1, TM2 and three positions in the β1 loop were obtained using the same methodology, by inserting LBT's in the β1 subunit. Second, molecular

simulations of ChTX-TMSR complex bound to the BK pore were used to obtain the conformational space of TMSR bound to ChTX. This simulation was used to evaluate the population of the different conformations that TMSR can adopt. The conformational sampling of TMSR was used to recalculate all the distances and estimate the position of the donors in 3D coordinates. The new distance values were used as distance restrains to build a final model of BK. The initial homology model of BK was built using as reference structure the Kv chimeric crystal of Kv1.2-Kv2.1 mammalian channel. Our model refined with LRET experiments shows a structure of α subunit alone with the extra-transmembrane segment S0 located in a pocket in the voltage sensor domain, and a concave shape for BK extracellular face. The β1 external loop, on the other hand, lies very near to the BK pore and forms a structure similar to an alpha greek letter.

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664-Pos

Stabilizing the Interactions Between the Calmodulin N-Lobe and KCa3.1 in C-Terminus Increases Channel Activity

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The Ca2+ sensitivity of the voltage-insensitive calcium activated potassium channel of intermediate conductance KCa3.1 is conferred by calmodulin (CaM) constitutively bound to the membrane-proximal region of the channel intracellular C-terminus. A study was undertaken to investigate how the interactions between the CaM N-lobe and the KCa3.1 channel CaM binding domain (CaMBD) in C-terminus correlates with the channel opening process. A 3D-structure of the KCa3.1/CaM complex was first generated by homology modeling with MODELLERv9.0 using as template the crystal structure of the KCa2.2/CaM complex (PDB: 1G4Y). The resulting structural model of KCa3.1 plus CaM predicts that the segment L361-S372 in KCa3.1 should be responsible for the Ca2+ dependent binding of the channel to the CaM-N lobe, with residues L361 and Q364 facing residues E45, E47 and D50 of CaM. To test this model residues in L361-S372 segment were substituted by Cys and the action of MTSET(+) and MTSACE (neutral charge) measured on channel activity. Inside-out patch clamp recordings showed that the binding of the charged MTSET+ reagent to the Q364C mutant resulted in a strong current increase, an effect not seen with the neutral MTSACE. The mutations E45A and E47A in CaM prevented the current increase initiated by MTSET+ on the Q364C mutant. A single channel analysis confirmed that the binding of MTSET+ to Q364C caused an increase in the channel open probability by a destabilization of the channel closed state. Altogether, our results are compatible with the formation of ionic bonds between the positively charged Cys-MTSET+ complex at position 364 in KCa3.1 and the negatively charged E45 and E47 residues in CaM, and confirm that an electrostatic stabilization of the CaM/KCa3.1 interactions can lead to an increase in the channel open probability. (Supported by CIHR).

665-Pos

Beta Subunits Bridge Two Alpha Subunits Within the BK Channel Tetramer

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BK potassium channels contain four pore-forming alpha subunits and four modulatory beta subunits. Transmembrane (TM) helices S1-S6 of BK alpha are homologous to S1-S6 of other V-gated potassium channels; however, BK alpha contains a unique seventh TM helix, S0, N terminal to S1. The beta subunits contain two TM helices, TM1 and TM2. From the extent of endogenous disulfide crosslinking between Cys substituted for the four residues just flanking the extracellular ends of alpha S0-S6 and of beta1 TM1 and TM2, we previously inferred that the flank of S0 was closest to the four-residue loop between S3 and S4 and also contacted the flanks of S1 and S2. Furthermore, the flank of beta1 TM1 was closest to the flanks of S1 and S2, and the flank of TM2 was closest to the flank of S0. We have now extended this analysis to the membrane doman. We find that Cys in the first helical turn of S0 within the membrane forms disulfides with Cys subsituted in the first helical turns of S3 and S4 but not with similarly located Cys in S1 or S2. Thus, in the membrane, S0 is next to S3 and S4 but not to S1 and S2, although the flank of S0 reaches the flanks of S1 and S2. Furthermore, co-expression of the double-Cys mutant of alpha, W23C in the first helical turn of S0 and F144C in the S2 flank, and the double-Cys mutant of beta1, Y42C in the TM1 flank and L157C in the first helical turn of TM2, resulted in the crosslinking of two alphas through one beta1, S0 to TM2 and TM1 to S2. Thus, TM1 and TM2 of each beta subunit lie between the voltage-sensing domains (S0-S4) of adjacent alpha subunits.

666-Pos

A Novel Auxiliary Protein Allows BK Potassium Channel Activation at Resting Voltage Without Calcium

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Large-conductance, voltage and calcium-activated potassium (BK) channels are composed of the channel-forming α subunits (BKα), which are ubiquitously expressed in electrically excitable and non-excitable cells, either alone or together with tissue specific auxiliary β subunits (β1-β4). BK channel gating is dually regulated by membrane voltage and free cytosolic Ca^{2+} ($[Ca^{2+}]_{in}$). Activation of BK channels in electrically excitable cells typically requires coincident membrane depolarization and elevation in $[Ca^{2+}]_{in}$, which are not a physiological condition for most non-excitable cells. We present biochemical and electrophysiological evidence showing that in LNCaP prostate cancer cells, BK channels can be activated at low voltages without rises in $[Ca^{2+}]_{in}$ through direct complex with an auxiliary small Leucine-rich repeat containing protein (LRRCP). This LRRCP modulates BK channels gating by enhancing the allosteric coupling between voltage-sensor activation and the channel's C-O transition. This finding reveals a novel auxiliary protein of a voltage-gated ion channel that gives an unprecedented large negative shift (−135 to −150 mV) in voltage dependence and provides a molecular mechanism for activation of BK potassium channels at physiological voltages and calcium levels in non-excitable cells.

667-Pos

Control of Strex BK-Channel Palmitoylation Via a Polybasic Domain

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Large conductance voltage and calcium sensitive potassium channels (BK) are widely expressed throughout the body and encoded by a single gene (KCNMA1). The splice insertion of the STREX exon at splice site C2, generates a channel phenotype with increased calcium sensitivity and differing regulation by phosphorylation. In mammals, splicing of the STREX exon is dynamically controlled by cellular excitability as well as circulating stress and sex hormones. With STREX insertion, a palmitoylation site and a polybasic region are introduced to the channel. The interaction of the polybasic region with the plasma membrane and palmitoylation of cysteine residues in the STREX-linker between RCK1 and RCK2 may serve as a membrane targeting motif that alters the phenotype of the BK-STREX channel.

A GFP-tagged carboxyl terminal construct spanning from the S6 transmembrane domain to the COOH end region of the intracellular carboxyl terminus, localised at the plasma membrane. Membrane localisation was abolished when the STREX insert was excluded. To test whether the polybasic region is important for plasma membrane targeting, site directed mutagenesis was used to mutate positive residues in the polybasic domain into negative (E) or neutral (A) residues. These mutations abolished membrane targeting of S6-COOH-STX to the plasma membrane. Full-length channels with mutations in the polybasic region were studied in patch-clamp electrophysiology to determine calcium and voltage sensitivity, with disruption of the polybasic domain shifting apparent calcium sensitivity towards the zero (insertless) BK channel phenotype. The importance of the polybasic domain for palmitoylation of the BK-STREX channel was further confirmed by assaying 3H-palmitate incorporation into carboxyl terminal constructs.

These data suggest that the polybasic region generated by inclusion of the STREX insert is an important determinant of BK-STREX channel palmitoylation and thus contributes to the altered channel properties upon STREX inclusion.