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.

Supported by FONDECYT No1070049, AnilloCientifico ACT/24 and NIHGM030376

664-Pos

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

Patricia Morales[1], Line Garneau[2], Hé lène Klein[2], Lucie Parent[3], Rémy Sauvé[3].
[1]Université de Montréal, GREPROM, Department of Physics, Montreal, QC, Canada, [2]Université de Montréal, GREPROM, Montreal, QC, Canada, [3]Université de Montréal, GREPROM, Department of Physiology, Montreal, QC, Canada.

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

Guoxia Liu, **Roland Wu**, Xin Jin, Xiaowei Niu, Neelesh Chudasama, Yongneng Yao, Richard Weinberg, Arthur Karlin, Steven Marx.
Columbia University, New York, NY, USA.

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

Jiusheng Yan, Richard Aldrich.
University of Texas at Austin, Austin, TX, USA.

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

Owen Jeffries.
University of Edinburgh, edinburgh, United Kingdom.

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.