channel function. Our results show that Mg^{2+} and Ba^{2+} binding to the high affinity Ca^{2+} binding site (Calcium Bowl) also induce structural rearrangements of the gating ring, although to a lesser extent than those observed after Ca^{2+} binding. All the same, these rearrangements are not strictly coupled to the opening of the pore. Structural changes of the gating ring induced by Ca^{2+}, Mg^{2+} and Ba^{2+} show different magnitude and voltage-dependence. These results indicate the existence of a complex movement of the gating ring induced by the binding of divalent cations to the calcium bowl.

References
1. Latorre and Brauchi. Biol Res (2006) vol. 39 (3) pp. 385-401.
2. Yuan *et al*. Science (2010) vol. 329 (5988) pp. 182-6.
3. Miranda *et al*. PNAS (2013) 110(13) pp.5217-22.
4. Zhou *et al*. PNAS (2012) 109 (28) pp11413-8.
5. Zeng *et al*. J Gen Physiol (2005) vol.125 pp 273-286.

2208-Plat

Substitutions at F380 in S6 by Small Hydrophobic Amino Acids Makes the Opening Transition in BK Channels Rate Limiting

Willy Carrasquel-Ursulaez[1,2], Gustavo F. Contreras[1,2], Romina Sepúlveda[3,4], Daniel Aguayo[3], Fernando D. Gonzalez-Nilo[3,1], Carlos González[1], Ramón Latorre[1].
[1]Centro Interdisciplinario de Neurociencias de Valparaíso, Universidad de Valparaíso, Chile, [2]Doctorado en Ciencias mención Neurociencias, Universidad de Valparaíso, Chile, [3]Center for Bioinformatics and Integrative Biology, Universidad Andrés Bello, Chile, [4]Doctorado en Biotecnología, Universidad Andrés Bello, Chile.

Large conductance Ca^{2+} and voltage-activated K^+ channels (BK) opening is mediated by a cross-talk of modular voltage and Ca^{2+} sensors. BK open probability is enhanced by increasing cytosolic Ca^{2+} concentration and/or depolarization. These stimuli activate sensors that are coupled by allosteric interactions to channel gating. The physical basis underlying the coupling between sensors activation and pore opening remains elusive. We found that replacement of the F380 residue in the transmembrane segment S6 by small hydrophobic amino acids, promotes a large and positive voltage shift of the open probability-voltage curve with minor changes in the gating charge-voltage curve. Using the Horrigan and Aldrich allosteric model, we show that these gating modifications are a consequence of large changes in the open-closed equilibrium and in the coupling between voltage and Ca^{2+} sensors with channel opening. In the presence of saturating internal Ca^{2+}, the F380A mutant increases the energy barrier that separates closed from open states by about 16 kJ/mol compared to the wtBK. The allosteric factor describing interaction between channel opening and voltage sensor activation decreases from 19 in the wt BK channel to 6 in the F380A mutant, while the deactivation rate interpolated to 0 mV is 4.8 times faster in mutant. Molecular modeling suggest the existence of a hydrophobic ring formed by residues F380 and L377 of contiguous subunits that could function as a lever support site for coupling between both sensors and channel opening. Interestingly, the mutation (F380A) allows observing gating currents with minor contaminants of ionic currents, so it could be used for detailed voltage sensor studies in the presence of permeant cations.

This work was supported by Fondecyt grant 1110430 to RL and Fondecyt 1131003 to FGN.

2209-Plat

Dynamic Ca^{2+} Sensitivity of BK Channels

Panpan Hou[1], Feng Xiao[2], Ming Yuchi[2], Guohui Zhang[3], Ying Wu[1], Wei Wang[1], Wenping Zeng[1], Mingyue Ding[2], Zhengxing Wu[1], Jianming Cui[3,4], **Jiuping Ding**[1].
[1]Key Laboratory of Molecular Biophysics, Huazhong University of Science and Technology, Ministry of Education, College of Life Science and Technology, Wuhan, Hubei, China., Wuhan, China, [2]Key Laboratory of Image Processing and Intelligent Control, Huazhong University of Science and Technology, Ministry of Education, Department of Biomedical Engineering, College of Life Science and Technology, Wuhan, Hubei, China., Wuhan, China, [3]Department of Biomedical Engineering, Center for the Investigation of Membrane Excitability Disorders, Cardiac Bioelectricity and Arrhythmia Center, Washington University, St Louis, MO 63130, USA, St Louis, MO, USA, [4]Department of pharmacology, Soochow University college of pharmaceutical Sciences, Suzhou, 215123, China, Suzhou, China.

Large-conductance calcium-activated K^+ (BK-type, mSlo1 or mutations) channels, abundantly distributed in the excitable cells, response to the Ca^{2+}-influx to regulate membrane potentials. However, it remains unknown how they react to the fast Ca^{2+}-influx. Flash photolysis provides the most rapidly calcium release for detecting actions of Ca^{2+}-binding proteins. In this study, our results revealed that the uncaged Ca^{2+} activated a voltage- and calcium-independent biphasic BK current with τ_f~0.2 ms and τ_s~10 ms, of which the fast component ratio R_f is voltage-dependent only. We further demonstrated that this occurred as the uncaged Ca^{2+} was not a constant. Moreover, a novel method was developed to calculate its time course, and finally to obtain the Ca^{2+}-binding rate constant k_b ~$2.7*10^8$ $M^{-1}s^{-1}$ suitable for all of BK-type models, suggesting that BK channels as a calcium sensor may be capable to detect local Ca^{2+} in many calcium events occurring in nervous activities.

2210-Plat

NMR Structural Study of the Domains of the KCNH Channels and its Insight into Channel Gating

congbao kang.
Agency for Science, Technology and Research, Singapore, Singapore.

The voltage-gated potassium channels (KCNH) include EAG (Ether-à-go-go), ERG (EAG-related gene) and ELK (EAG-like) channels. The gating of KCNH channels is regulated by membrane potentials and these channels play important roles in cardiac repolarizaton, neuronal excitability, cellular proliferation and tumor cells. KCNH channels contain an N-terminal region formed mainly by the Per-Arnt-Sim (PAS) domain and N-cap region, six transmembrane segments (S1-S6) and a C-terminal region containing a cyclic-nucleotide-binding homology domain (CNBHD). The first four transmembrane segments (S1-S4) form the voltage-sensor domain (VSD) that is important for sensing the changes of membrane potential across the membrane a pore domain. The S5 and S6 segments form the pore domain that is responsible for the ion transport across the membrane. We are using NMR spectroscopy to explore the structures of different domains of KCNH channels such as hERG. The structures of the PAS domains of hERG and KCNH channel from Zebrafish were determined using NMR spectroscopy. To investigate the interaction between the PAS domain and the CNBHD, the solution structure of the CNBHD was also invested by NMR. Solution structure of the CNBHD of Zebrafish showed its similarity to X-ray structure. Further binding study suggested that the C-linker region of the channel might be important for interaction with PAS domain. We also carried out some NMR study on the transmembrane segments of the hERG channel, which might provide insight into gating of the KCNH channels.

2211-Plat

Structural Basis of Lipid-Driven Conformational Changes in the Hyperpolarization-Activated Potassium Channel MVP

Amelia M. Randich[1], Ernesto Vargas[2], Sherry S. Wanderling[1], Eduardo Perozo[1].
[1]Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA, [2]Biophysical Sciences, University of Chicago, Chicago, IL, USA.

DOTAP is a bilayer-forming lipid containing a positively charged trimethylammonium group instead of a negatively charged phosphodiester. When reconstituted in pure DOTAP membranes, KvAP is unable to open due to large positive voltage shifts in its mid-point of activation. Based on these results, it has been hypothesized that non-phosphate lipids stabilize the down (resting) conformation of the voltage sensor. We have tested this idea by studying the influence of non-phospholipids on the gating properties of channels with inverse electromechanical coupling. In contrast to most Kv channels, the pore domain of hyperpolarization-activated channels opens with the downward movement of S4 (at hyperpolarizing potentials). Therefore, for any hyperpolarization-activated channel, reconstitution in DOTAP should stabilize the down state of the VSD, favoring the open state and not the closed state. We carried out a CW-EPR analysis of the local structure and dynamics of the prokaryotic hyperpolarization activated potassium channel MVP, after reconstitution in either PC:PG (3:1) or DOTAP liposomes, in the absence of a resting potential. We focused on residues in the S4, S4-S5 linker, and lower S6 of MVP as a way to monitor the key regions that participate in voltage sensing and gate coupling. In PC:PG phospholipids, the inner bundle gate of MVP is closed, as seen from strong spin-spin coupling between residues lining the S6. In contrast, our findings suggest that DOTAP biases the conformation of the VSD in MVP towards the down state, which leads to a structural reorganization of the lower S5 region and conformational changes in the pore domain. The activation gate displays key hallmarks associated with the open conformation. These lipid-driven structural changes point to a working model for the inverse electromechanical coupling in hyperpolarization-activated channels.

Platform: Protein Design and Folding

2212-Plat

Smooth Functional Transition Along a Mutational Pathway with an Abrupt Protein Fold Switch

Christian Holzgräfe, Stefan Wallin.
Lund University, Lund, Sweden.

Maynard-Smith (Maynard Smith. 1970. 10.1038/225563a0) conjectured that protein evolution occurs through the accumulation of single mutations,