924-Pos Board B803

Isoform-specific Dominant Negative Effects of LQT2 Mutations in hERGa and hERGuso Variants

Matthew R. Stump, Qiuming Gong, Zhengfeng Zhou.
Oregon Health & Science University, Portland, OR, USA.

Mutations in the human ether-a-go-go-related gene (hERG) cause type 2 long QT syndrome (LQT2). Two C-terminal variants of hERG (hERGa and hERGuso) have been identified in the heart. hERGa represents the full-length hERG protein and hERGuso is a truncated form that lacks most of the C-terminal region. More than 70% of LQT2 mutations are located in both hERGa and hERGuso variants. Previous studies, however, have focused primarily on the effect of LQT2 mutations in the hERGa isoform. In the present study, we compared the dominant negative effects of two LQT2 mutations A561V and G628S in the hERGa and hERGuso isoforms. When coexpressed with wild-type (WT) hERGa, hERGa-A561V interrupted the trafficking of WT hERGa to the plasma membrane, while hERGa-G628S functionally suppressed WT hERGa channel current. When hERGuso-A561V was coexpressed with WT hERGa, fully glycosylated WT hERGa protein was observed, indicating that the trafficking of WT hERGa channels to the plasma membrane is not interrupted by hERGuso-A561V. We also found that hERGuso-G628S failed to cause a dominant negative suppression of WT hERGa channel current. In co-immunoprecipiation experiments, the association of HA-tagged WT hERGa and Flag-tagged hERGa-A561V was readily observed, while the association of HA-tagged WT hERGa and Flag-tagged hERGuso-A561V was observed only after overexposure. These results suggest that the dominant negative effects of A561V and G628S are lost in the context of the hERGuso isoform. The lack of dominant negative suppression of WT hERGa trafficking and function observed in hERGuso LQT2 mutants suggests minimal association and co-assembly of hERGa and hERGuso.

925-Pos Board B804

Probing the KcsA Permeation Pathway using Barium Block

Kene N. Piasta, Christopher Miller.
Brandeis University, Waltham, MA, USA.

A single mutation in KcsA, E71A, was previously shown to suppress C-type inactivation leading to a constitutively open channel. Addition of micromolar Ba^{2+} to the system blocks the permeation of K^+ and follows a bimolecular kinetic scheme, where the open dwell time is Ba^{2+} dependent and the blocked dwell time is not. We show that both internal and external Ba^{2+} block this mutant with two distinct modes, a fast and slow block time of tens of milliseconds and hundreds of milliseconds respectively, with similar voltage dependence. External K^+ in the millimolar range causes a lock-in of the Ba^{2+} and allows the dissociation constant for K^+ at the S1 K^+ site. Using other ions such as Na^+, Li^+, and Rb^+ the selectivity of the lock-in site will be determined.

926-Pos Board B805

Asymmetrical Ligands in the Potassium Channel Cavity

Michael J. Lenaeus, Adrian Gross.
Northwestern University Medical School, Chicago, IL, USA.

Quaternary ammonium (QA) compounds are the most extensively studied class of potassium channel blockers. Molecules such as tetraethylammonium (TEA) and tetrabutylammonium (TBA) have long been used in functional experiments designed to elucidate the drug-binding, ion-binding and gating behaviors of a wide variety of potassium channels. More recently, the development of potassium channel crystallization techniques has allowed co-crystal structures of KcsA in complex with QAs to be solved. The four-fold symmetry of potassium channels, however, has limited such experiments to symmetrical QA molecules, meaning that the binding sites for pharmacological important molecules remain unknown. Molecules such as octyltriethylammonium (C8), local anesthetics and anti-arrhythmics likely bind at or near the known QA binding site, but the hydrophobic interactions that stabilize these molecules have yet to be resolved due to a symmetry mismatch between the channel and its blockers. Here we present several strategies for overcoming this symmetry mismatch and solving the structure of KcsA in complex with asymmetrical molecules. We have used these strategies to solve the structure of KcsA in complex with a number of asymmetrical ligands - including C8, C10, several arsenic-containing derivatives of asymmetrical QA molecules and amiodarone. Our structures demonstrate the existence of a hydrophobic binding site located between the inner helices of two KcsA monomers and suggest this region of the channel may be important for the stabilization of ''drug-like'' ligands in the potassium channel cavity.

927-Pos Board B806

The Water-ion Coupling Ratio for Ion Permeation through the KcsA Potassium Channel: Dependencies on Concentration and Species of Permeating Ions

Masayuki Iwamoto, Hirofumi Shimizu, Shigetoshi Oiki.
Univ. Fukui, Fukui, Japan.

The streaming potential (V_{stream}) is generated under osmotic gradient across the membrane (ΔOsm) by ion flux along with water flow through a narrow (single-file) pore. The value of V_{stream} provides a quantitative index for the number of water molecules accompanying a permeating ion (the water-ion coupling ratio, n), the knowledge of which is a prerequisite for elucidating the mechanism of ion permeation and selectivity of the pore. Here we evaluated the V_{stream} for ion permeation through the KcsA potassium channel, for which ion distributions in the pore were revealed from high-resolution crystal structure. The liposome patch-clamp technique combined with the osmotic jump method (Ando et al., 2005) was applied. V_{stream} was evaluated as difference of the reversal potentials with and without ΔOsm. A series of ramp command was applied before, during and after an osmotic jump for the precise estimation of V_{stream}. V_{stream} was measured at different ΔOsm (ΔOsm $\leq$ 3 Osm/kg H_2O). The data showed a linear relationship, from which the n value was obtained as a slope. At 200 mM of K^+ concentration, ΔOsm-dependency of V_{stream} was -0.46 mV/ΔOsm, and n was calculated to be 1.0. By replacing K^+ with Rb^+ (200 mM), V_{stream} was dramatically changed and the n value was 2.0. Concentration of K^+ also affected the V_{stream}; n was 2.1 in the symmetrical K^+ concentration of 20 mM. These results clearly indicated that species and concentration of conducting ions determined the coordination of water molecules and ions during permeation through the KcsA channel. Relationship of our results to the ion distribution in the crystal structures will be discussed.

928-Pos Board B807

Light At The End Of The Channel: Photochromic Blockers For Optical Control Of Ion Channels In Individual Cells

Alexandre Mourot, Matthew R. Banghart, Doris L. Fortin, Dirk Trauner, Richard H. Kramer.
University of California Berkeley, Berkeley, CA, USA.

The ability to turn rapidly proteins on and off with light is emerging as a way to manipulate cellular functions with unprecedented precision. Because most proteins are not naturally photosensitive, different strategies have come out to render them light-responsive. Here we present a novel approach to photosensitize proteins that does not require protein engineering or gene delivery. Instead, this method uses the photochromic blocker QAQ to control native K+ and Na+ channels in single cells using light. QAQ is a soluble molecule made of an azobenzene chromophore (A) flanked by two quaternary ammoniums channel blockers (Q). In the dark, QAQ binds to the internal quaternary ammonium binding site on Na+ and K+ channels and blocks both channels. Light can be used to toggle the azobenzene between its trans to cis configurations, altering the QAQ/channel interaction and thereby controlling ion conduction. Thus, QAQ functions as a reversibly caged blocker. QAQ is membrane impermeant and therefore has to be applied intracellularly to mediate channel block. Application of QAQ through the patch pipette affords optical control of action potential firing in individual dissociated hippocampal neurons. More importantly, light can be delivered with high precision to control channels in sub-cellular regions, potentially allowing local control of excitability and mapping of synaptic inputs.

929-Pos Board B808

Exploring Whether a Large Entrance To The Inner Vestibule Of BK Channels Is Required For Their Large Conductance

Yanyan Geng[1], Xiaowei Niu[2], Karl Magleby[1].
[1]University of Miami, Miami, FL, USA, [2]Columbia University, New York, NY, USA.

To explore whether a large entrance to the inner vestibule of BK channels is required for their large conductance, we examine if changing the size of the entrance alters the single-channel outward current, gamma. Previous studies suggest that E321/E324 in BK channels are located at the entrance to the inner vestibule. To test if E321/E324 are accessible to intracellular ions, we compare gamma of E321C and E324C before and after treatment with maleimido-proprionic-acid (MPA), a membrane impermeable negatively charged thiol reagent. Gamma for E321C-MPA and E324C-MPA increases by ~20%, presumably due to the added negative charge on MPA attracting K^+ ions to the inner vestibule. This suggests that E321/E324 are accessible to the conduction pathway. At the same time, gamma for E321C-MPA and E324C-MPA is still ~10% less than for wt channels, suggesting that the larger size of C-MPA compared to glutamic acid restricts current flow, although we cannot exclude that the location of the negative charge on MPA vs. glutamic acid attracts fewer K^+ ions to the inner vestibule. We also change the size of the entrance to the inner vestibule by substituting residues with different sized side chains at E321/E324. For hydrophobic substitutions, tryptophan with the largest volume has a ~30% smaller gamma than substitutions with alanine, valine, and leucine. For hydrophilic substitutions (serine, threonine, asparagine, and glutamine), larger side chains appear to decrease gamma, but any effects are small. Increasing $[K^+]_i$ from 0.15 to 2.5 M removes all differences in gamma associated with different sized side chains. All substitutions have little effect on inward current. These observations suggest that a large entrance to the inner vestibule of BK channels contributes to their large conductance.