597-Pos  Board B377
Reconstitution and Measurement of Ion Channel Ensembles in Droplet Bilayers
Vikasit Vijayvergiya, Shiv Acharya, Jacob Schmidt.
Department of Bioengineering, University of California, Los Angeles, CA, USA.
Droplet bilayers have been used recently to reconstitute ion channels from cellular membrane preparations. Frequently large currents are observed resulting from ion-channel ensembles which are blockable by the same pharmacological compounds that inhibit cellular ion channel currents measured with patch clamp. We have used droplet bilayers to reconstitute hERG, TRPV1, and Kv 7.1 channels from membrane preparations of HEK cells. We present our measurements of these preparations which yielded ion channel currents pA AGAIN nA in magnitude with ion selectivity, voltage dependent and time dependent conductance, and dose-dependent drug inhibition. We will also present preliminary results of our work measuring these membrane preparations in bilayer arrays, which have potential applications for sensing and pharmaceutical screening.

598-Pos  Board B378
The Conformation of KcsA’s Selectivity Filter Influences the Opening of its Activation Gate
Cholpon Tilegenova, D. Marien Cortes, Luis G. Cuello.
Cell Physiology and Molecular Biophysics, TTUHSC, Lubbock, TX, USA.
Our knowledge about structural-function relationships in K^+ channels has grown exponentially, mostly because of the use of the prokaryotic version of these membrane proteins. This is especially true for the KcsA K^+ channel, a two transmembrane, proton-gated K^+ channel from Streptomyces lividans, for which the structures, for different kinetic states, are known. This minimal version of a K^+ channel faithfully imitates the most important functional features of its eukaryotic and more complex relatives, the Kv channels. Previously, it has been shown that in KcsA, C-type inactivation is allosterically coupled to activation gating, as well as in other K^+ channels. This means that the conformational changes associated to the opening of the activation gate (AG) propagate to the channel’s selectivity filter (SF), triggering its collapse conformation. It has been shown before that the more open is the channel’s AG, the deeper inactives. By extension, the structural conformation of the SF should influence the AG opening. Thus to investigate this possibility, we have systematically studied the opening of the channel’s AG by spectroscopic approaches, in conditions that relieve or promote C-inactivation. Our results suggest that the conformational state of the channel’s SF indeed strongly influence the AG opening.

Reference:

Funding: NIH R01GM097159-01A1; Welch Foundation BI-1757

599-Pos  Board B379
Engineering hERG Channel Inner Cavity within KcsA Structure
Luis G. Cuello, D. Marien Cortes.
Department of Cell Physiology and Molecular Biophysics, Texas Tech University Health Science Center, Lubbock, TX, USA.
To provide some structural insights about the molecular basis of the inactivation gating and drug binding properties of the hERG channel, we have reasoned that a chimeric approach in which we recreate the cavity of the hERG channel in to the KcsA structure could potentially lends a structural framework to begin understand inactivation gating and pharmacology of the hERG channel from a structural point of view. Recently, we have made an important advance toward this goal by expressing, purifying and crystallizing a KcsA-hERG chimera that contains the entire inner cavity of the hERG channel. A comprehensive biochemical, functional and crystallographic study will be presented.

Support: AHA-11SDG544003

600-Pos  Board B380
PIP2 Modifies the Free Energy of the Kv1.2 Voltage-Sensor Activation
Marina A. Kasimova1, Lucie Delemotte1, Michael L. Klein2, Vincenzo Carnevale1, Mourir Tarek1.
1University of Lorraine, Vandoeuvre-lès-Nancy, France, 2Temple University, Philadelphia, PA, USA.
Application of phosphatidyl-4,5-bisphosphate (PIP2) to the voltage-gated potassium channel Kv1.2 causes the loss-of-function effect, manifested by positive-shifting the activation voltage dependence. This loss-of-function effect was attributed to the multiple site bridges formation between positive residues of the resting voltage sensor and a negative headgroup of PIP2. In this work, we uncover the free energy surfaces underlying the entire activation path of the Kv1.2 voltage-sensor embedded into the bare zwittrionic bilayer (palmitoyl-oleoyl-glycerol-phosphocholine, POPC) and into the zwittrionic bilayer with several PIP2 lipids (POPC/PIP2). Comparison between these free energy surfaces reveals that PIP2 modifies both, the relative stability of the Kv1.2 voltage-sensor states and the free energy barriers separating them. We posit that these modifications induce the loss-of-function effect observed experimentally.

601-Pos  Board B381
K^-Dependent Selectivity and External Calcium Block of Shab Potassium Channels
Froylan Gomez-Lagunas, Elisa Carrillo.
Phyisology, UNAM, Mexico City, Mexico.
Potassium channels allow the selective flux of K^- excluding the smaller, and more abundant in the extracellular solution, Na^+ ions. Here we show that Shab is a typical K^- channel that excludes Na^- under bi-ionic, Nao/Ki or Nao/Rbi, conditions. However, when internal K^- is replaced by Cs^- (Nao/Csi), stable inward Na^- and outward Cs^- currents are observed. These currents show that Shab selectivity is not accounted for by protein structural elements alone, as stated in the snug-fit model of selectivity. Additionally, here we report the block of Shab channels by external Ca^{2+} ions, and compare the effect that internal K^- replacement exerts on both Ca^{2+} and TEA blockage. Our observations indicate that Ca^{2+} blocks the channels in a site located near to the external TEA binding site, and that this pore region changes conformation under conditions that allow Na^- permeation. Based on our observations and the structural information derived from the NaK bacterial channel, we hypothesize that Ca^{2+} is probably coordinated by main chain carbonyls of the first K^- binding site of the pore.

602-Pos  Board B382
Time-Dependent Voltage Sensor Relaxation in hERG Channels
Samrat Thouta, Yu Patrick Shi, Stanislav Sokolov, Yen May Cheng, Tom W. Claydon.
Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada.
Upon membrane depolarization the voltage sensors (S4) of K^+ channels undergo conformational changes that lead to pore opening. Recent studies in Shaker have shown that prolonged depolarization reconfigures S4 into a stable relaxed state that results in a hyperpolarizing shift of the voltage dependence of S4 return and subsequent pore closure compared to that of S4 activation and pore opening. A similar mode-shift has been observed in hERG channels. The voltage-dependence of hERG ionic current deactivation is shifted by ~30 mV relative to that of activation which parallels the shifts of voltage-dependent S4 movement measured from voltage clamp fluorometry (VCF) and gating current recordings. In this way, S4 relaxation may contribute to the characteristic slow deactivation gating of the channel. Here we report VCF recordings of S4 movement in I663P channels, in which the pore gate is trapped open and therefore functionally isolated from the voltage sensor, which show a similar ~25 mV mode-shift between S4 activation and deactivation. These data demonstrate that the mode-shift of hERG ionic current is due to voltage sensor relaxation, which is an intrinsic property of the voltage sensor. The time-dependence of hERG S4 relaxation has not yet been fully characterized. We measured the time-dependence of relaxation by applying depolarizing steps of increasing duration and observing the progressive slowing of S4 return. Initial VCF data from ~60 mV steps up to 2 s in duration suggest that entry of S4 into the relaxed state in hERG channels occurs with a tau = 271 ± 106 ms (n=4). This suggests that voltage sensor relaxation in hERG channels occurs within a physiologically relevant time course that may modify cardiac action potential duration.


603-Pos  Board B383
Block of HERG by Extracellular Calcium and other Divalent Ions
Andrew Nguyen, Alice Wong, Angad Oberoi, Thiuyv Le, Alan Miller.
Basic Sciences, Touro University - California, Vallejo, CA, USA.
Reduction of the current carried by the cardiac potassium channel HERG can lead to Long QT syndrome, an arrhythmia characterized by a rapid heart rate and reduced cardiac output, which can, in certain situations, be fatal. The effect of extracellular electrolytes on the biophysical properties of the HERG channel have been studied in some detail. In particular, increases in extracellular calcium, magnesium and hydrogen have been shown to slow channel