2305-Pos Board B291
Structural Basis of an Inherited hERG1 Long QT Mutant
Julio F. Cordero-Morales, Vishwanath Jogini, Anthony Lewis, Valeria Vasquez, David Medovoy, Martin Tristani-Firouzi, Eduardo Perozo.
Mutations in human ether-a-go-go related K+ channel gene hERG1 are a common cause of Long QT syndrome (LQTS), an electrical disorder of the heart that predisposes individuals to sudden death. Of these, changes to the pore domain are associated with a greater risk of life-threatening arrhythmias. One such mutation N629D, located adjacent to the selectivity filter, causes loss of function and altered ion selectivity. We have shown that the selectivity filter sequence of non-selective cation channel NaK shows striking similarity to that of hERG1, and that mutagenic transformation of the NaK filter into hERG1's generates a structurally canonical K+ filter. Here, using this hERG1-like NaK as a structural model, we reveal the molecular mechanisms that underlie channel dysfunction in the N629D mutant. The NaK/hERG N629D filter crystal structure obtained at 2.8 Å show that N629D induces a dramatic conformational rearrangements in the extracellular vestibule that extend to the filter, disrupting K+ binding sites to cause loss of ion selectivity. These changes involve a 180 deg rotation of the Phenylalanine 66 at the selectivity filter (equivalent to position Phe627 in hERG1) and an intrinsic sensitivity to the nature of the permeant ion. These findings provide insight into the hERG1 selectivity filter and offer a new biophysical tool for the study of the molecular mechanisms underlying LQTS pore mutants at atomic level.

2306-Pos Board B292
Gating Current of the KCNQ1 Voltage-Gated Potassium Channel
Katarina J. Ruscic, Francesco Miceli, Francisco Bezanilla, Steve A.N. Goldstein.
KCNQ1 pore-forming alpha-subunits are crucial to physiology, operating *in vivo* with KCNE1 and KCNE3 beta-subunits in the heart and stomach, respectively. Recently, the gating currents of KCNQ4 and KCNQ5 channels were characterized (Miceli, Channels, 2009); here, equivalent measurements are described for KCNQ1 channels formed in the absence of beta-subunits. Human KCNQ1 was studied in *Xenopus* oocytes with the cut-open oocyte voltage clamp technique. To record gating currents, cells were depleted of internal potassium ions and residual ionic current was blocked with tetraethyammonium and barium. At room temperature, gating currents were too small to resolve. At 28°C, ON gating current gave rise to peak charge movement at +40 mV of ~0.7 nC / μA of ionic current. The total charge movement at each test potential was conserved in the OFF-gating currents. Analysis of the charge-voltage (QV) relationship and conductance-voltage (GV) relationship showed a 10 mV hyperpolarizing shift of the half-maximal voltage of activation of the normalized QV curve with respect to the normalized GV curve. KCNQ1 ON-gating current decay constants were 4-fold slower than in KCNQ4 and required longer test pulses to fully resolve. Overlays of gating and ionic currents revealed that, as for KCNQ4, gating charges were still moving even after KCNQ1 channels started to open, indicating that charge movement was a rate-limiting factor in channel opening. These first characterizations of KCNQ1 gating currents and are an important step towards understanding mutations that lead to cardiac arrhythmias, such as long-QT syndrome, and the effects of beta-subunits on channel function. Supported by NIH GM030376, University of Chicago MSTP, and The Paul and Daisy Soros Fellowship for New Americans.

2307-Pos Board B293
Gating Currents from Neuronal K_V7 Channels Carrying BFNS-Causing Mutations in the S_4 Segment of the Voltage Sensing Domain
Francesco Miceli, Ernesto Vargas, Maria Roberta Cilio, Francisco Bezanilla, Maurizio Taglialatela.
K_v7 genes encode for channel voltage-gated potassium subunits with inhibitory function on electrical excitability. Mutations in $K_v7.1$ cause one form of long QT syndrome, whereas $K_v7.4$ mutations underlie a rare form of slowly progressive deafness; finally, mutations in $K_v7.2/K_v7.3$ genes have been identified in families affected by Benign Familial Neonatal Seizures (BFNS) and/or peripheral nerve hyperexcitability (PNH). Disease-causing mutations often affect residues in the voltage-sensing domain of K_v7 subunits and modify the gating properties of the macroscopic currents carried by these channels. To achieve a more detailed functional analysis of these gating changes, we previously recorded the gating currents from the neuronal $K_v7.4$ channel, using the cut-open vaseline gap technique. In the present work, we have characterized the ionic and gating currents from homomeric $K_v7.4$ channels carrying mutations homologous to BFNS-causing mutations in $K_v7.2$ (R213Q/W, D218G and R219W), as well as the non-BFNS mutant R219Q ($K_v7.4$ numbering).
Channels carrying the D218G, R219Q or the R219W mutations in the C-terminal part of S_4 show the following salient properties: +30 mV shift of the G/V curves and faster activation/deactivation kinetics. Gating currents from these channels showed: gating charge conservation between Q_{ON} and Q_{OFF}, slight (5-13 mV) right-shift of the Q/V curves, and a fast Q_{OFF} decay with no rising phase. Neutralization of R213 caused a G/V right-shift associated to slower activation/deactivation channel kinetics. Interestingly, upon pore current blockade, in $K_v7.4$ channels carrying the R213Q/W mutations causing PNH, a persistent outward current was recorded that increases on depolarization, indicating a gating pore leak in the active state of the sensor. These data may help elucidate the molecular pathogenesis of BFNS and associated diseases.Supported by Telethon GP07125 (MT), E-Rare JTC 2007 (MRC), and NIH GM30376 (FB).

2308-Pos Board B294
Histidine 562 on S5 is a pH Sensor for HERG Gating
Glenna C.L. Bett, MiMi Liu, **Randall L. Rasmusson**.
In the HERG channel there are at least two binding sites for extracellular protons. One site alters gating, and the other alters conductance. One of these sites shifts activation and inactivation voltage dependence. The only two histidines on the extracellular loops of the channel have been shown to be uninvolved in the pH sensitivity of gating. We hypothesized that a histidine residue located at nearly the midpoint of S5 (H562) could be the pH sensor for gating kinetics. We substituted a glutamine at this point and produced a HERG current with kinetics shifted nearly 20 mV negative and rendered gating pH_o-insensitive. This analysis was extended to a HERG construct with all three histidines removed. Although removal of the histidine from S5 in HERG resulted in reduced pH sensitivity of deactivation time constants, this disruption could occur via several mechanisms. Therefore, we tested whether inserting a histidine into the middle of the S5 region of the Kv1.4 channel would confer pH sensitivity. Because of an intrinsic pH sensitivity, we made the S5 mutation in a double construct in which the external pH sensing histidine at position 508 in Kv1.4 was mutated to a glutamine. Introduction of a histidine at position G489 on the S5 voltage sensor of the H508Q mutant channel induced pH dependent gating. Changing pH_o from 7.8 to 6.2 caused an approximate −15 mV shift in the threshold for activation of Kv1.4[G489H][H508Q]. The half activation time was pH_o dependent for a shift from pH 7.4 to 6.2. The G489H mutation restored the coupling between extracellular pH and N-terminal binding that was disrupted by the H508Q mutation. These studies suggest that an important pH sensitivity of gating is conferred by an S5 cleft space histidine in HERG.

2309-Pos Board B295
Modeling HERG Gating Transitions
QinLian Zhou, **Glenna C.L. Bett**.
HERG (human ether-à-go-go related gene, Kv11.1, KCNH2) is a voltage-gated potassium channel with unique gating characteristics. HERG has fast voltage-dependent inactivation, relatively slow deactivation, and fast recovery from inactivation. This combination of gating kinetics makes study of HERG difficult without using mathematical models. Several HERG models have been developed, with fundamentally different organization and properties.
We programmed five distinct HERG models and tested their behavior under voltage-clamp and guinea-pig ventricular myocyte action potential clamp. Four models used Markov formalisms, and one used Hodgkin-Huxley formalism. HERG behavior cannot be replicated using a Hodgkin-Huxley formalism. The Markov Models had 2 or 3 closed states, 1 open state, and 1 inactivated state. A voltage-independent activation step is required in order to replicate the experimentally observed voltage-independent rate limiting step of activation. A fundamental difference between models is the presence or absence of a transition directly from the closed state to the inactivated state. Two of the Markov models had transitions directly from the closed state to the inactivated state. Our analysis demonstrates that the only models which effectively reproduce HERG experimental data require that the closed-inactivated transitions are absent or are effectively zero compared to the closed to open transitions, rendering the closed-inactivation transition superfluous. Furthermore, the models make significantly different predictions about the behavior of the HERG during action potentials and premature stimuli.
Our simulations indicate there is no direct transition between the pre-activated closed state and the inactivated state, although inactivation from a "flicker" open state is possible. The use of a model with a direct transition between closed and inactivated states with rates that are effectively zero is potentially misleading in understanding HERG gating.

2310-Pos Board B296
KCNE2 uses More Domains than KCNE1 to Modulate KCNQ1 Channel Function
Yuhong Wang, Min Jiang, Mei Zhang, Pan Li, Xuanyu Meng, Dimitar P. Zankov, Meng Cui, Changlin Tian, **Gea-Ny Tseng**.
KCNE1 & KCNE2 are both single membrane-passing peptides with amino- & carboxyl ends in extra- & intra-cellular compartments. They also share high sequence homology in the transmembrane (TM) and juxtamembranous regions. Yet the two have distinctly different effects when associated with the KCNQ1 channel. Understanding the structural basis for differential KCNQ1 modulation by the 2 KCNE subunits is prerequisite to drug design targeting such interactions. We use the techniques of NMR, cysteine (Cys)-scanning