

conformation. Since KcsA represents a structural model for the pore domain of  $K^+$  channels, it is obvious that a detailed understanding of the molecular basis of inactivation is not limited to this prokaryotic channel, but offers new directions into how inactivation gating might proceed in other  $K^+$  channels. Here, using patch clamp experiments, EPR spectroscopy, functional assays, molecular dynamics, and X-ray crystallography we show that interactions involving residues Trp67, Tyr78, and Asp80 in KcsA, conserved in most potassium channels, also constitute critical contacts between the selectivity filter and its adjacent pore helix which determines the rate and extent of C-type inactivation in *Shaker*, Kv1.2, and hERG. Substitution of a tryptophan or tyrosine at the pore helix to phenylalanine in these channels decreases the rate and extent of inactivation, pointing this position as key modulator of gating. Furthermore, by substituting equivalent amino acids critical for hERG inactivation in KcsA we were able to create a non-conducting KcsA mutant with normal pH activated lower gate. These results suggest commonalities in inactivation gating mechanism of eukaryotic channels, and provide evidence that the hydrogen bond network and Van der Waals interactions between the pore helix, selectivity filter, and external vestibule serve as the basis for C-type inactivation in the  $K^+$  channel family.

#### 975-Pos Board B854

##### Mapping the hERG Channel Activation Gate Using SCAM

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hERG channels, important for cardiac repolarization, are susceptible to block by a wide range of therapeutic drugs intended for other targets. The S6 activation gate contributes to the efficacy of drug block, yet its location is unknown. We used cysteine mutagenesis and expression in *Xenopus* oocytes to identify S6 domain residues important in gating, and mapped these residues in energy-minimized homology models based on the crystal structures of MlotK1 and Kv1.2 for the closed and open states, respectively (Wynia-Smith et al., J. Gen. Physiol., 2008). The predominant mutant phenotype was slowed channel closing and/or constitutive conductance at negative potentials. Focusing initially on cysteine mutations with wild-type behavior and thus little structural perturbation, we are using MTS reagents to identify residues selectively accessible in the open state. Mutants S654C, F656C, S660C and L666C span a region homologous to the activation gate in Shaker channels as well as a separate region predicted by our molecular models to form a closing gate. These mutants reacted with MTSET and displayed current inhibition that reversed upon addition of DTT to the bath. The control channel showed no reactivity to MTSET. S654C exhibited a progressive development of current block during pulses to positive voltages, suggestive of state-dependent block. Experiments under way to determine rates of state-dependent modification will further define the residues forming the occluding gate in hERG channels.

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##### LQT2 Linked Mutations E444D And P451L In The S1-S2 Linker Lead To Biophysical Abnormalities Of Herg Channels

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Human ether-a-go-go related gene (hERG) encodes the pore-forming  $\alpha$ -subunits that underlie the rapidly activating delayed rectifier  $K^+$  current (IKr). Mutations in hERG reduce IKr to cause type 2 long QT syndrome (LQT2). The  $\alpha$ -subunits contain six transmembrane domains (S1-S6), with the S1-S4 domains acting as a voltage sensor and the S5-S6 domains forming the pore and selectivity filter. The N- and C-terminal segments are suggested to regulate channel gating and assembly. However, the functional role of linker regions remains largely unknown. We hypothesize that LQT2 mutations E444D and P451L in the S1-S2 linker modulate biophysical properties of hERG channels. We heterologously expressed wild type (WT), E444D or P451L in HEK293 cells. IhERG was measured by the whole-cell patch clamp and protein processing by Western Blot analysis. Western Blots showed normal protein trafficking with the presence of 135 and 155 kDa bands for WT, E444D and P451L. Tail current densities at -50 mV were  $147.4 \pm 30.0$ ,  $148.2 \pm 24.8$  and  $108.8 \pm 31.8$  pA/pF, respectively ( $n=4$ ,  $p>0.05$ ). Fit with the Boltzmann equation, the voltage dependence of activation for WT and E444D showed V1/2 values of  $-11.1 \pm 2.2$  and  $-8.6 \pm 2.4$  (k =  $6.9 \pm 0.2$  and  $7.6 \pm 0.4$ ), whereas P451L was shifted positively to  $1.1 \pm 2.2$  mV (k =  $7.6 \pm 0.7$ ). The inactivation rates for E444D between -40 and 60 mV were accelerated up to two-fold compare to WT, whereas P451L inactivation rates were not different from WT at most voltages. For both mutations, the rates of recovery from inactivation and deactivation were similar. Thus, E444D and P451L alter the biophysical properties of hERG channels differently. These findings suggest that linker regions have a functional role in channel gating, and support the hypothesis that LQT2 mutations in the S1-S2 linker regions predominately result channel biophysical abnormalities.

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##### Transfer Of rolf S3-S4 Linker To hERG Eliminates Activation Gating But Spares Inactivation

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A recent study in Shaker, a voltage-dependent potassium channel, suggests a coupling between activation and inactivation. This coupling is controversial in hERG, a fast-inactivating voltage-dependent potassium channel. To address this question, we transferred to hERG the S3-S4 linker of the voltage-independent channel, rolf, in order to selectively disrupt the activation process. This chimera shows an intact voltage-dependent inactivation process consistent with a weak coupling, if any, between both processes. Kinetic models suggest that the chimera presents only an open and an inactivated states, with identical transition rates as in hERG. The lower sensitivity of the chimera to BeKm-1, a hERG preferential closed-state inhibitor, confirms that the chimera exists only in open or inactivated conformations. This chimera allows determining the mechanism of action of hERG blockers, as exemplified by the test on ketoconazole.

#### 978-Pos Board B857

##### KvLQT1's S3 involvement in LQTS

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Hereditary Long QT syndrome (LQTS) is characterized by a prolonged QT interval in the electrocardiogram and an increased likelihood of serious ventricular arrhythmias. It has been reported to arise from missense mutations in the S3 segment of KCNQ1's S3. This is not surprising as tetramers of KCNQ1 subunits co-assemble with KCNE1 accessory subunits to form the slowly activating and deactivating  $I_{Ks}$  in the heart, which modulates repolarization of cardiac action potentials, particularly during sympathetic activation. However, the means by which these mutations, specifically in the S3 segment, cause LQTS remains poorly understood. The cause is often assumed to be decreased protein folding and trafficking to the cell membrane, but here we have investigated the properties of reported S3 mutations in a heterologous system to understand the potential biophysical basis for inherited LQTS. Functional channel properties were studied in transfected mouse *Itk-* cells utilizing whole cell patch clamp. Specifically, the voltage dependence of activation and deactivation was determined by tail current analysis at 35°C. Additionally, cardiac ionic current generated by an applied ventricular action potential clamp was measure at 1 and 3 Hz. Wild type KCNQ1 with KCNE1 subunits for  $I_{Ks}$  were compared with the KCNQ1 mutants. All mutants were expressed and trafficked to the cell surface. The majority of these mutations caused a dramatic depolarizing voltage shift in voltage dependence of activation, coupled with an acceleration of channel deactivation which would suppress  $I_{Ks}$ . This is expected to impair the physiological ability of this  $I_{Ks}$  channel current to summate in response to a rapid heart rhythm and lead to a reduction of the overall repolarization reserve in affected hearts

#### 979-Pos Board B858

##### Dynamic Partnership between KCNQ1 and KCNE Subunits

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**Background:** (KCN)Q1 and (KCN)E1 associate to form  $I_{Ks}$  channels important for cardiac electrical stability. (KCN)E2 is also expressed in the heart and can associate with Q1 or Q1/E1 to suppress the current amplitude. What regulate the partnerships of Q1/Ex ( $x = 1$  or 2) and their stability are not clear. **Methods:** We use COS-7 & oocyte expression systems to test (a) Q1/Ex stability by pulse-chase experiments, (b) ability of E1 or E2 to traffic to cell surface without/with Q1, and (c) ability of free E1 or E2 peptides to associate with lone Q1 channels in cell membrane. **Results:** Pulse chase-experiments in COS-7 cells show that Q1 has a mean turnover ( $\tau_{\text{turnover}}$ ) of 13 hr, while Q1-associated E1 (co-immunoprecipitated with Q1) has a  $\tau_{\text{turnover}}$  of 6 - 7 hr, suggesting E1 turnover in Q1/E1 complexes. Biotinylation experiments show that E1 expressed alone in COS-7 traffics to the cell surface inefficiently. Q1 coexpression leads to a 5-fold increase in cell surface E1. In contrast, E2 expressed alone in COS-7 traffics more efficiently to cell surface, and Q1 coexpression does not further increase cell surface E2. Injecting vesicles made from E1-expressing COS-7 cells into control oocytes (expressing native Q1) or oocytes pre-injected with Q1 cRNA produces an  $I_{Ks}$ -like current after a few hours, while injecting 'empty' vesicles from untransfected COS-7 cells has not effects. Likewise, injecting