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 MlotiK1 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.

976-Pos Board B855

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 α-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 α-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 ± 30.0, 148.2 ± 24.8 and 108.8 ± 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 ± 2.2 and -8.6 ± 2.4 ($k= 6.9 \pm 0.2$ and 7.6 ± 0.4), whereas P451L was shifted positively to 1.1 ± 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.

977-Pos Board B856

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 *ltk-* 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 τ ($\tau_{turnover}$) of 13 hr, while Q1-associated E1 (co-immunoprecipitated with Q1) has a $\tau_{turnover}$ 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

vesicles from COS-7 cells expressing E2 (with an extracellular epitope tag) into Q1-expressing oocytes leads to a decrease in current amplitude and appearance of cell surface epitope. Thus, vesicle-delivered E1 or E2 peptides can associate with lone Q1 channels and modulate their function. **Proposal:** In cardiac mycoytes coexpressing Q1, E1 & E2, both KCNE subunits can associate with Q1 and modulate cardiac I_{Ks} channel function in a dynamic fashion.

980-Pos Board B859

Using Disulfide Trapping to Probe KCNQ1/KCNE1 Interactions During the I_{Ks} Channel Gating

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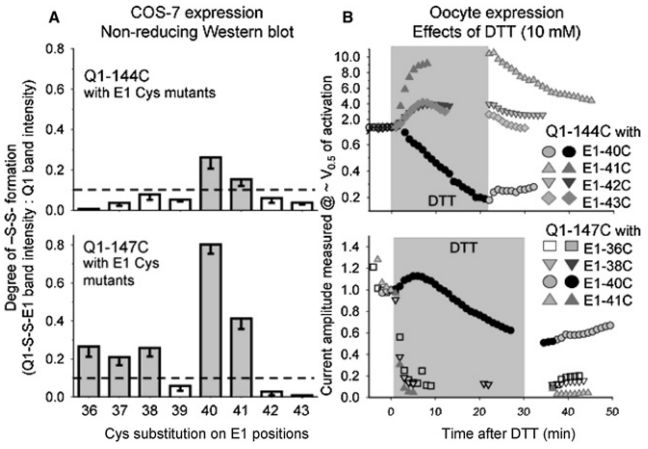
Background: (KCN)E1 association with (KCN)Q1 markedly slows Q1 activation. The mechanism is not clear. **Methods:** We introduce Cys(teine) into Cys-less Q1/E1 background. Double Cys-substituted Q1/E1 pairs are expressed in COS-7 cells to detect spontaneous disulfide formation (by non-reducing Western blotting), and in oocytes to probe state-dependence/rate of disulfide formation/reduction (by voltage clamping). **Results:** There are 'hot spots' of Q1/E1 interactions: Q1 position 147 with E1 positions 36 to 41, and E1 40 & 41 with Q1 144 & 147 (**Fig. A**). Oocyte experiments confirm that all constructs retain wild-type like function. We test effects of 10 mM DTT on current amplitudes measured at $V_{0.5}$ of activation (**Fig. B**). A decrease in current reflects a depolarizing shift in $V_{0.5}$/preferential disulfide bond formation in activated states. An opposite effect reflects a hyperpolarizing shift in $V_{0.5}$/preferential disulfide formation in the resting state. Rate of DTT effect indicates rate of disulfide reduction: slower rates indicate stronger disulfide between positions in closer proximity. **Conclusion:** Our data suggest that the extracellular S1-S2 linker of Q1 and the extracellular end of E1 transmembrane domain may rotate/swing, and glide through each other during I_{Ks} gating transition.



981-Pos Board B860

Physical Interactions Between The Cytoplasmic Domains Of KCNQ1 And KCNE1 Channel Subunits

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KCNE1 associates with KCNQ1 to form the slowly activating K+ current (IKs) that is critical for myocardial repolarization. Like other voltage-gated K+ channels, it is tetrameric with N- and C-termini oriented intracellular. The C-terminus (CT) is large and predicted to contain various functional properties influencing channel folding, assembly, trafficking and gating. Interactions between the transmembrane segments of KCNE1 and KCNQ1 govern rates of activation and channel conductance. Less is known regarding possible interactions of the C-termini. Many Long QT (LQT) mutations occur in the C-termini of both KCNE1 and KCNQ1. We investigated possible physical interactions between the C-termini of KCNQ1 and KCNE1. Recombinant proteins (KCNE1-CT and KCNQ1-CT), expressed in HEK293 cells are capable of co-precipitation and co-localization within the cell. The direct physical interaction between KCNQ1-CT and KCNE1-CT expressed and purified from E.Coli indicated a direct interaction. Analysis of purified subdomains of KCNQ1-CT further localized the binding region for KCNE1-CT to a region just after the last transmembrane segment, close to the inner membrane surface (349-398). This KCNQ1 segment (Q1C1) was sufficient for account for KCNQ1-CT biding to KCNE1-CT since subdomains C-terminal to this region did not physically interact with KCNE1. The kinetics studies of the interaction between C-terminal cytoplasmic domains of KCNQ1 and KCNE1 proteins, as exhibited by surface plasmon resonance analysis, indicate that Q1C1 region contributes to the bimolecular interaction with dissociation constant of ~ 4 μM. LQT mutants of KCNE1-CT, D76N and W87F retained binding to Q1C1 with the similar affinity, indicating that disease-causing mutations do not disrupt the association. Our results indicate that the C-termini of KCNQ1 and KCNE1 comprise and interaction domain contains that may play a role in IKs channel behavior.

982-Pos Board B861

Mechanism Of I_{Kr} Loss In Mutant T421M-hERG Expressing Rat Ventricular Myocytes

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The *human ether a-go-go-related gene* (*hERG*) encodes rapidly activating delayed rectifier K^+ current (I_{Kr}) in heart. Missense mutations in *hERG* lead to loss in I_{Kr} in patients with type 2 long QT (LQT2) syndrome. In native adult rat ventricular myocytes (ARVMs), which intrinsically lack I_{Kr}, we studied hERG current (I_{hERG}) by expressing WT or T421M mutation-hERG protein using an adenoviral infection system. The T421M mutation was identified in the S1 transmembrane region of *hERG* in a 32-yr-old woman with LQT2. Isolated ARVMs were infected with WT or T421M-expressing adenovirus and I_{hERG} was recorded from infected myocytes 4 days after infection. Expression of WT or T421M-hERG in ARVMs produced I_{hERG} with peak tail current (prepulse to 50 mV, tail current recorded at -50 mV) of 9.9 ± 1.7 and 1.6 ± 0.1 pA/pF (mean ± SEM) respectively. Western blot analysis shows that T421M-hERG protein traffics similar to WT-hERG indicated by the presence of 135 and 155 kDa protein. The voltage dependence of activation for the T421M-hERG showed a marked positive shift of 38 mV ($V_{1/2}$, 24.5 ± 2.3 mV) compared to WT (-13.5 ± 3.0 mV, $p<0.05$). The deactivation time constants were derived by fitting tail current decay as a double exponential at hyperpolarizing potentials and were 5-10 fold faster for T421M than WT-hERG. We conclude that in native ventricular myocytes, T421M channels traffic normally and undergo minimal voltage-dependent activation during cardiac action potential repolarization, which is a novel mechanism for loss of I_{Kr}. Our results emphasize the importance of S1 region in modulating gating properties of hERG channels. Our adenoviral-mediated, over- expression ARVM model may be exploited to study the changes in cardiac action potential waveforms in cardiomyocyte's native background.

983-Pos Board B862

Trafficking-deficient LQT2 Mutations Disrupt Different Steps of hERG Channel Transport

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The *human Ether-a-go-go Related Gene* (*hERG*) encodes the voltage-gated K^+ channel α-subunit that forms the pore of the rapidly activating delayed rectifier K^+ current. *hERG* mutations are associated with type 2 Long QT syndrome (LQT2). Most missense LQT2 mutations are trafficking-deficient, and reduce complex-glycosylation in the Golgi apparatus (Golgi processing) and plasmalemmal expression. Golgi processing and plasmalemmal expression of LQT2 channels can be increased by culturing cells in drugs that block hERG current (I_{hERG}) (pharmacological correction) or by culturing cells at 27°C (temperature correction). LQT2 channels have different 'patterns' of correction, for example, G601S-hERG undergoes pharmacological and temperature correction, whereas R752W-hERG only undergoes temperature correction. These data suggest that these mutations may disrupt different steps in hERG trafficking. To test this, we used confocal microscopy to examine the localization of WT-hERG, G601S-hERG, or R752W-hERG, stably expressed in HEK293 cells. We stained cells using intracellular protein markers for the ER (calnexin), the Golgi (58K), or the endosomes (mannose-6-phosphate receptor or M6PR). We found that cells expressing WT-hERG showed plasmalemmal and intracellular staining that co-localized with calnexin, 58K, and M6PR. Cells expressing G601S- or R752W-hERG showed primarily intracellular hERG staining, but their staining patterns were different. G601S-hERG showed diffused intracellular staining that colocalized with calnexin, but not 58K or M6PR. Cells expressing R752W-hERG showed strong co-localization with the calnexin and M6PR, but not 58K. We conclude G601S-hERG appears to be retained primarily with the ER, whereas R752W-hERG is retained in the ER and endosomes. These data are surprising because they suggest that R752W-hERG may exit the ER and traffic to the endosomes without undergoing Golgi processing. These are the first data to show that trafficking-deficient LQT2-linked mutations, with different patterns of correction, colocalize to different intracellular compartments.