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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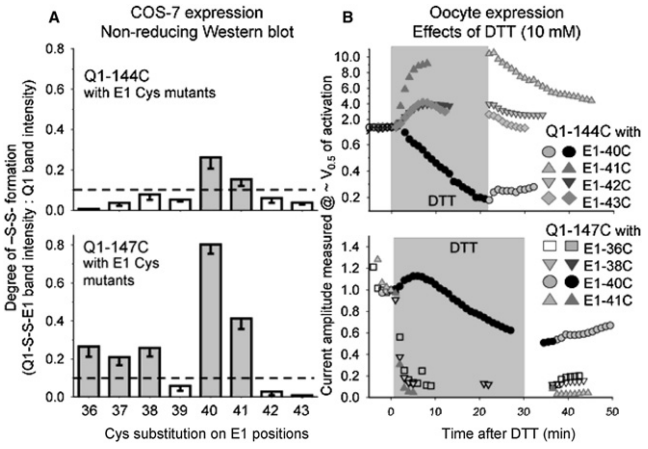
Background: (KCN)E1 association with (KCN)Q1 markedly slows Q1 activation. The mechanism is not clear. **Methods:** We introduce Cys(teine) into Cys-less Q1/E1 background. Double Cys-substituted Q1/E1 pairs are expressed in COS-7 cells to detect spontaneous disulfide formation (by non-reducing Western 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.

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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.