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mutagenesis and voltage clamping to explore this issue. We learn the following: (1) KCNE2 differs from KCNE1 in the extracellular juxtamembranous region (helix vs unstructured loop). Cys substitution in this region has distinct impact on the gating kinetics and/or pore conductance of the KCNQ1/KCNE2 channel complex, but has little or no effects on the KCNQ1/KCNE1 channel function. (2) KCNE2 & KCNE1 sequences diverge in the carboxyl end. Truncating this region of KCNE1 (93 −129) does not interfere with its ability to modulate KCNQ1, while truncating the corresponding region of KCNE2 (98-123) abolishes its function as a KCNQ1 modulator. Intracellular application of a peptide corresponding to KCNE2 aa 98-123 reduces currents through KCNQ1, increases currents through KCNQ1/KCNE2, but has no effects on currents through KCNQ1/KCNE1. (3) Cys substitution along the TM helices of KCNE1 and KCNE2 affects the gating kinetics and/or pore conductance of the KCNQ1/KCNE channel complexes. Structural alignment suggests a rotation of KCNE2 relative to KCNE1 in terms of helical faces interacting with the pore domain and voltage-sensing domain of the KCNQ1 channel. We propose that while both KCNE subunits utilize their TM helices to interact with KCNQ1, the more rigid helical structure of KCNE2 allows it to allosterically modulate the KCNQ1 gating and ion permeation properties by the extracellular juxtamembranous and cytoplasmic carboxyl domains.

2311-Pos Board B297

Pore Determinants of KCNQ3 K^+ Current Expression

Frank Choveau, Sonya Bierbower, Mark Shapiro.

Our laboratory has suggested the pore region of KCNQ channels to be implicated in governing current amplitudes, in which large currents are possible by an interaction between a threonine and an isoleucine at the 315 and 312 positions, respectively. Consistent with this, replacement of A315 in KCNQ3 with a threonine or serine increased current amplitudes (Zaika et al., 2008. *Biophys J*, 95). Here, we find several mutations in KCNQ3 at position 312 (I312V, I312E and I312R) abolished the homomeric current. Co-expression of KCNQ3 I312V and I312E with wild-type (WT) KCNQ2 resulted in smaller currents *vs*. WT KCNQ2+3 channels, but did not modify channel voltage dependence. Evidence that the I312V and I312E mutants were expressed in the heteromers includes a shifted TEA sensitivity, compared to KCNQ2 homomers. Molecular modeling suggests the lack of current in I312V and I312E KCNQ3 channels to be due to a destabilization of the pore structure. Another lab has suggested the C-terminus KCNQ1-3 channels is critical in determining KCNQ current amplitudes (Schwake et al., 2006. *J. Neurosci*., 26). Moreover, the crystal structure of the KCNQ4 coiled-coil ‘‘D-helix’’ highlighted three positions (V619, M629 and C643) critical for KCNQ4 channels oligomerization, which are divergent in KCNQ3 (Howard et al., 2007. *Neuron*, 53). We find H_2O_2-induced oligomerization of KCNQ4 subunits, reported by native PAGE, to localize to a cysteine located at the end of the D-helix at position 643, at which only KCNQ3 possesses a histidine at the analogous position. As a probe for the role of H646 in KCNQ3 expression, we tested the H646C mutant. However, homomeric or heteromeric channels containing this mutation produced smaller currents, ruling out this divergent residue as underlying low amplitude of KCNQ3 currents. Our results confirm that the pore region is predominant in governing KCNQ channel expression.

2312-Pos Board B298

A Spectrum of Functional Phenotypes Associated with LQT1 Mutations Identified in Patients with Early-Onset Atrial Fibrillation

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Mutations in *KCNQ1*, the gene encoding the voltage-gated K^+ channel α-subunit that underlies the slowly activating delayed rectifier K^+ current (I_{Ks}) in the heart, are linked to Type I Long QT Syndrome (LQT1) and Familial Atrial Fibrillation (FAF). Compared to the background prevalence of 0.1%, early-onset atrial fibrillation (patients < 50 years) was observed in ~2% of LQT1 patients (Johnson et al., Heart Rhythm, 2008). We expressed several LQT1 mutations (P7S, R231H, and T322A) identified in patients with early-onset AF in HEK293 to better understand their functional phenotype. Cells were transfected with cDNA for the auxiliary K^+ channel subunit KCNE1 (E1) and WT-, P7S-, R231H-, or T322A-KCNQ (Q1). Whole-cell Q1E1 currents (I_{Q1E1}) were measured using the patch-clamp technique and holding potential of −80mV. We measured I-V relations from these cells by prepulsing from a holding potential of −80 mV to 70 mV in 10-mV increments for 5 seconds, followed by a test-pulse to −50mV for 5 seconds. The peak I_{Q1E1} measured during the test-pulse was plotted as function of the pre-pulse and the data were described using the Boltzmann equation to calculate the maximally activated I_{Q1E1} (I_{MAX}), the midpoint potential ($V_{1/2}$), and slope factor (*k*). Each mutation generated different functional phenotypes. Compared to cells expressing WT (n=7), cells expressing P7S (n=10) did not alter I_{MAX} (WT=99 ± 9 pA/pF, P7S=93 ± 17 pA/pF), $V_{1/2}$ (WT=26 ± 3mV, P7S=24 ± 3mV), or *k* (WT=16 ± 1mV/e-fold ΔI, P7S=16 ± 1mV/e-fold ΔI). Cells expressing R231H (n=9) generated constitutively active I_{Q1E1} (R231H=64.07 ± 27.48 pA/pF) at −80 mV that was similar to previously described FAF-linked mutations, and I_{MAX} was not altered (80.09 ± 15.58 pA/pF). In contrast, cells expressing T322A (n=7) generated no I_{Q1E1}. These data demonstrate there is no characteristic I_{Q1E1} phenotype for LQT1 mutations identified in patients with early-onset AF.

2313-Pos Board B299

Long QT-Linked HERG Mutations at R531 of the S4 Alter the Gating Properties of Wt-HERG

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Mutations in the *human Ether-a-go-go Related Gene* (*hERG*) are linked to Type 2 Long QT Syndrome (LQT2). LQT2-linked mutations at R531 disrupt a conserved basic residue on the fourth transmembrane segment (S4) of the voltage-sensor. To understand the impact that these mutations have on hERG function, we expressed WT-, R531Q-, or R531W-hERG in HEK293 cells. Since LQT2 follows a dominant-inheritance pattern, we also transfected cells with equal amounts of WT- and R531Q-hERG or WT- and R531W-hERG. The whole-cell patch-clamp technique was used to record hERG current (I_{hERG}). I-V relations were measured by pre-pulsing cells in 10-mV increments to 100mV for 5s, followed by a test-pulse to −50mV for 5s. The peak tail I_{hERG} measured during the test-pulse was plotted as a function of the pre-pulse potential. The data were fit with the Boltzmann equation to calculate the maximal I_{hERG} activation, midpoint potential for maximal activation ($V_{1/2}$), and slope factor (*k*). Maximal I_{hERG} was not significantly different among the groups; however, there was an increase in the *k* and a positive shift in the $V_{1/2}$ of currents recorded from cells expressing mutant channels compared to those expressing WT-hERG. The voltage-dependence for rates of I_{hERG} deactivation, development of inactivation, and recovery from inactivation were measured by calculating the time constants (τ) associated with I_{hERG} decay for a broad range of test potentials. Cells expressing R531Q- and R531W-hERG exhibited a faster mean τ for I_{hERG} deactivation and recovery from inactivation, but a slower τ for development of inactivation. Cells expressing WT-hERG and R531Q- or R531W-hERG tended to have an intermediate effect on the I-V relations and τ. These data suggest that R531Q- and R531W-hERG produce functional channels with altered gating, which persist in the presence of WT-hERG.

2314-Pos Board B300

The Trafficking of Mutant HERG K+ Channels Linked to Long QT Syndrome are Regulated by a Subdomain in the Endoplasmic Reticulum

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Type 2 Long QT syndrome (LQT2) is caused by *human Ether-a-go-go Related Gene* (*hERG*) mutations, and studies suggest that most LQT2 missense mutations are retained in pre-Golgi compartments. We tested the hypothesis that trafficking-deficient LQT2 (tdLQT2) channels are regulated by quality control mechanisms in specialized subdomains of the Endoplasmic Reticulum (ER) and/or the ER Golgi Intermediate Compartment (ERGIC). An inherent problem with studying the localization of proteins to ER subdomains and the ERGIC is that they are in close proximity and it is difficult to distinguish them from one another. We found that acutely treating cells with nocodazole (a microtubule depolymerizing agent) and brefeldin (bfa, an inhibitor of Coat Protein I vesicular transport) allowed us to delineate specialized ER subdomains and the ERGIC using confocal microscopy. We assessed whether the tdLQT2 mutation G601S-hERG colocalized with the different ER subdomains and/or the ERGIC in stably expressing HEK293 cells. Imaging data show that G601S-hERG accumulated in the peripheral ER subdomains and did not colocalize with markers for perinucler ER subdomains, transitional ER subdomains, or the ERGIC. Treating cells in E-4031, a drug that increases G601S-hERG trafficking in the secretory pathway, prevented G601S-hERG from accumulating in the peripheral ER subdomains and promoted the movement of G601S-hERG into the ERGIC. The data suggest that cellular quality control mechanisms within specialized ER subdomains are primarily responsible for the ER retention of G601S-hERG, and that E-4031 facilitates the movement of G601S-hERG out of the specialized ER subdomains and into the ERGIC.

2315-Pos Board B301

Gating Modulation of KCNQ2/3 via N-C termini Interaction

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M-channels are slowly activated, non-inactivating, voltage-dependent potassium channels. Heteromeric assembly of subunits, encoded by two members of the KCNQ gene family KCNQ2 and KCNQ3, recapitulate the functional properties of the M-current. KCNQ2 and KCNQ3 are co-expressed on the cell body and dendrites of hippocampal and cortical neurons. Importantly,