These results suggest that alprenolol prolongs the QT interval by direct inhibition of activated HERG channels.

Keywords: alprenolol, HERG channel

606-Pos

Mutations at the Intron 9 Donor Splice Site in hERG Lead to Cryptic Splicing in LQT2

Matthew R. Stump, Qiuming Gong, Zhengfeng Zhou.

OHSU, Portland, OR, USA.

Long QT syndrome type 2 (LQT2) is caused by mutations in the human ether-a-go-go-related gene (hERG). More than 30% of LQT2 mutations are nonsense, frameshift, or splice site mutations that may affect mRNA stability and splicing. To date, relatively few studies have focused on the pathogenesis of hERG splice site mutations. We characterized three LQT2 mutations in the 5' donor splice site of intron 9: 2398G>T, 2398+3A>T, and 2398+5G>T. G2398 is the last nucleotide of exon 9 and 2398G>T has been previously classified as a missense mutation (G800W). The functional consequences of these mutations were studied by RT-PCR analysis of RNA collected from HEK293 cells transfected with minigenes containing the wild-type or mutant genomic sequence spanning exon 8 to exon 11 of hERG. All three splice site mutants disrupt normal splicing and produce an aberrantly spliced transcript. Sequence analysis showed that this transcript results from the use of a cryptic 5' donor splice site in intron 9 located 54 nt downstream of the normal site. Translation of this transcript would result in an in-frame insertion of 18 amino acids in the cyclic nucleotide binding domain. A full length hERG cDNA construct including the 2398G>T mutation and the additional 54 nt from intron 9 was expressed in HEK293 cells. Patch clamp studies revealed that the splice mutant channels did not produce hERG current. Western blot analysis showed that the mutant expressed the immature form of the hERG protein indicating defective channel trafficking. These studies underscore the importance of RNA analysis in describing the pathogenesis of LQT2. The intron 9 donor splice site appears to be a localized hot-spot for LQT2 mutations.

607-Pos

Lysine Versus Arginine: RNA Editing In The Eag Potassium Channel Mary Y. Ryan¹, Rachel Maloney², Jeffrey Fineberg¹, Robert A. Reenan², Richard Horn¹.

¹Thomas Jefferson University, Philadelphia, PA, USA, ²Brown University, Providence, RI, USA.

Four RNA editing sites in eag, a Drosophila voltage-gated potassium channel, result in point mutations. One of these mutations, K467R, involves a highly conserved basic residue at the top of the S6 segment. We characterized wild-type and mutant channels using two-microelectrode voltage clamp and patch clamp in Xenopus oocytes. The homologous mutation is lethal in Shaker and hERG. Position 467 plays an important role in inactivation; the K467R mutation causes a 54% decrease in the fraction of inactivated current at +80 mV. The fraction of inactivated current is reduced at higher (10 mM) extracellular Mg⁺² concentrations; constructs with a lysine at 467 are more sensitive to changes in extracellular Mg⁺² than those with an arginine. Mutating position 467 to alanine, glutamine or cysteine resulted in intermediate inactivation phenotypes and a leftward shift of the peak current-voltage relationship, normalized at +80 mV. Using instantaneous IV measurements from cell-attached oocyte patches, we constructed normalized Po curves for 467Q, 467R and 467K. The Po-V curves for these mutations are superimposable, suggesting little effect on activation gating. However, 467Q and 467R produce inward rectification in instantaneous IV measurements, suggesting a change in ion permeation. Single channel current amplitudes at +40 mV, estimated from non-stationary noise analysis, are comparable for these mutants, which affect instantaneous rectification at more depolarized potentials. Preliminary experiments show no change in rectification between cell-attached and inside-out patches suggesting the permeation change is not due to block by cytoplasmic cations. Intracellular TBA (tetrabutylammonium) blocks 467R significantly better than 467K. Block by intracellular, but not extracellular, TEA (tetraethylammonium) interferes with inactivation. These results show that even a minor residue change can have a dramatic impact on channel biophysics.

608-Pos

Overlapping LQT1 and LQT2 Phenotype in a Patient with Long QT Syndrome Associated with Loss-of-Function Variations in KCNQ1 and KCNH2

Jonathan M. Cordeiro¹, Guillermo J. Perez¹, Ryan Pfeiffer¹, Elena Burashnikov¹, Martin Borggrefe², Christian Wolpert², Rainer Schimpf², Charles Antzelevitch¹.

¹Masonic Medical Research Laboratory, Utica, NY, USA, ²University of Mannheim, Mannheim, Germany.

Background: Long QT Syndrome (LQTS) is an inherited disorder characterized by prolonged QT intervals and potentially life-threatening arrhythmias. Mutations in several ion channel genes are responsible for LQTS. Here we describe a patient with LQTS who has a mutation in KCNQ1 as well as a polymorphism in KCNH2. Methods and Results: The proband (MMRL0362), a 32 yo female, exhibited multiple ventricular extrasystoles and episodes of syncope. Her ECG (QTc=518ms) showed an LQT2 morphology in leads V4-V6 and LQT1 morphology in leads V1-V2. Genomic DNA was isolated from lymphocytes. All exons and intron borders of 7 LQTS susceptibility genes were amplified and sequenced. Variations were detected predicting a novel missense mutation (V110I) in KCNQ1 as well as a common polymorphism in KCNH2 (K897T). We expressed WT or V110I KCNQ1 channels in CHO-K1 cells co-transfected with KCNE1 and performed patch clamp experiments. In addition, WT or K897T KCNH2 were studied by patch clamp. Current-voltage (I-V) relations for V110I showed a significant reduction in both developing and tail current densities compared to WT at potentials >+20 mV (p<0.05), suggesting a reduction in I_{Ks} currents. K897T-HERG channels displayed a significantly reduced tail current density compared to WT-HERG at potentials >+10 mV. Interestingly, channel availability assessed using a triple-pulse protocol was slightly greater for K897T compared to WT $(V_{0.5} = -53.1 \pm 1.13 \text{ mV} \text{ and } -60.7 \pm 1.15 \text{ mV} \text{ for K897T and WT, respec-}$ tively, p<0.05). Comparison of the fully activated I-V revealed no difference in the rectification properties between WT and K897T channels. Conclusions: We report a patient with a loss-of-function mutation in KCNQ1 and a loss-offunction polymorphism in KCNH2. Our results suggest that a reduction of both I_{Kr} and I_{Ks} underlies the combined LQT1 and LQT2 phenotype in this patient.

609-Pos

Divergent Effects of AF- or LQTS-Associated HERG Mutations on Endogenous $I_{\rm Kr}$

Jianguo Han, Franck Potet, Wen Shuai, Dan M. Roden, Dawood Darbar, Sabina Kupershmidt.

Vanderbilt University, Nashville, TN, USA.

Mutations in HERG not only reduce IKr to cause QT syndrome (LQTS) but have also been associated with atrial fibrillation (AF). The mechanisms in AF are unknown. To identify genetic defects conferring AF susceptibility, we screened HERG in 375 patients with typical and lone AF, and identified three probands with rare, non-synonymous HERG variants absent in control populations (284). The first was a C-terminal HERG variant (R1047L), previously reported in LQTS, in 2 probands. One proband was part of a kindred that included 2 other family members with AF or palpitations, and all 3 were mutation carriers; no family was available in the 2nd proband. A second variant (R954C) located only six residues from a previously identified LQTS variant (S960N) was also identified in a lone AF proband. In mutation carriers, QT intervals during sinus rhythm were normal. These variants are particularly interesting because AF and LQTS mutations are likely to be located in close structural proximity. We compared the functional effects of these mutations and WT in two heterologous cell systems: HEK cells stably expressing endogenous HERG (HERG-HEK) or 'empty' HEK cells. R1047L caused a 1.4 fold increase in current amplitude in HERG-HEK. In empty HEK cells, there was no difference between R1047L and WT. R954C generated currents that were similar to WT in both HERG-HEK and empty HEK cells, although the nearby S960N variant reduced current 1.6 fold in HERG-HEK and 2 fold in empty HEK cells. These results suggest that relative expression levels of normal and mutant alleles determine net effect on ionic current and action potential controls. Variability in these mechanisms, across or within chambers, may contribute to phenotypes that manifest in only one chamber.

610-Pos

 K^\pm Occupancy of the Pore Critically Determines the Selectivity-Stability of K^\pm Channels. A Study with Shab Channels

Imilla I. Arias-Olguín¹, Manuel Soriano-García², Froylan Gomez-Lagunas¹. School of Medicine, UNAM, Mexico City, Mexico, ²Institute of Chemistry, UNAM, Mexico City, Mexico.

Potassium channels are characterized by their ability to select K^+ excluding the smaller $\mathrm{Na^+}$ ions. Based on crystallographic images of the pore this selectivity is commonly explained in terms of protein structural elements alone. On the other hand, it is well known that some pore properties such as the stability of the K^+ conductance itself critically depend on the K^+ occupancy of the pore. Here it will be shown functional data demonstrating that (a) both the stability and the selectivity of the pore of Shab K^+ channels change in