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
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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
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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 P_o curves for 467Q, 467R and 467K. The P_o-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
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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 ($V_{0.5}=-53.1\pm1.13$ mV and -60.7 ± 1.15 mV for K897T and WT, respectively, $p<0.05$). 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-of-function 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_{Kr}
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Mutations in HERG not only reduce I_{Kr} 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^+ Occupancy of the Pore Critically Determines the Selectivity-Stability of K^+ Channels. A Study with Shab Channels
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Potassium channels are characterized by their ability to select K^+ excluding the smaller 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

parallel depending on the ions dwelling within the pore, but that (b) it is experimentally possible to dissociate the change in selectivity from that of stability, suggesting that the structural elements that determine either the selectivity or the stability of G_K are not identical. The functional and structural arguments that will be presented are not compatible with the notion of a rigid selectivity filter in which selectivity arises from protein structural elements alone.

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Biogenesis of Pore Architecture in Voltage-Gated K^+ Channels

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Although formation of the mature conducting pore of Kv channels necessitates tetramerization of individual subunits, it is possible that the first stages of pore formation occur early in biogenesis, prior to oligomerization of the transmembrane core. Our studies explore this hypothesis, specifically with respect to acquisition of secondary and tertiary structure. Using biogenic intermediates, a mass-tagging strategy, namely pegylation, and a molecular tape measure (Lu and Deutsch, 2005), we probed the pore domain of Kv1.3. The pore helix appears to form a compact secondary structure inside the ribosomal exit tunnel when located in the terminal 20Å of the tunnel whereas the turret is extended. Tertiary folding of the re-entrant pore loop was assessed by estimating pegylation rates of select cysteines engineered throughout the turret, the pore helix, and the loop preceding S6 in two monomeric constructs in the presence of ER membranes. The fastest rates were observed for turret and loop residues, whereas pore helix residues were 5-10 fold slower. To help interpret these observations, all-atom molecular dynamics simulations of a single monomer of the Kv1.2 channel pore domain were generated in a fully solvated lipid membrane. The two transmembrane helices S5 and S6 as well as the pore helix remain stable along the trajectory. These results are consistent with a tertiary re-entrant pore architecture being acquired at the monomer stage of Kv biogenesis, perhaps coincident with integration of transmembrane segments into the bilayer. [Supported by NIH grant GM 52302 and GM062342].

Reference: Lu and Deutsch, Biochemistry 44: 8230, 2005.

612-Pos

Does a Single Mutation in the P-Loop Open a Novel Current Pathway Beside the Central A-Pore in the Human Voltage-Gated Potassium Channel Kv1.3

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Voltage-gated potassium channels are membrane proteins containing a potassium-selective ion conducting central α-pore. Recent studies showed that mutations in the voltage sensor of the *Shaker* channel can disclose another ion permeation pathway through the voltage sensing domain (S1-S4) beside the α-pore, the so called ω-pore described by Tombola *et al.* 2005 (*Neuron***45**:379-388). We investigated a mutant voltage-gated *h*Kv1.3 channel where the substitution of a cysteine in the pore-loop at position 388 (*Shaker* position 438) generated a current through the α-pore, and an inward-current at hyperpolarizing potentials carried by different cations (Li^+~Na^+>NH_4^+>Cs^+>K^+~Rb^+). This observed inward current appeared similar to the ω-current and was not affected by the α-pore blocker CTX, in contrary to the currents through the α-pore of the *h*Kv1.3_V388C mutant channel. Verapamil, which is acting from the intracellular side, could block both, the α-current with an IC_{50} of 3.5 μM and the observed inward-current at hyperpolarizing potentials with an IC_{50} of 2.3 μM in the *h*Kv1.3_V388C mutant channel. Due to the block of inward-current by verapamil we suppose that the observed inward-current runs through the verapamil binding site in the cavity between S6-S6 of two adjacent subunits in the *h*Kv1.3_V388C mutant channel. We hypothesize that the *h*Kv1.3_V388C mutation generated a channel with a second ion conducting pathway distinct from the α-pore, but not identical to the ω-pathway, running through one part of the *h*Kv1.3 verapamil binding site. The entry of the novel pathway is presumably located at the backside of Y395 (*Shaker* position 445), proceeds plane parallel to the α-pore, ending between S5 and S6 at the intracellular side of one α-subunit. Supported by grants from the 4SC AG (Martinsried) and the DFG (Gr848/14-1).

613-Pos

Conductance and Concentration Relationship in a Reduced Model of the K Channel

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K^+ ions move rapidly through potassium channels more or less ignoring Na^+. The mechanism of selectivity is thought to depend on the solvation of K^+ and its electrostatic interactions with carbonyl dipoles of the channel wall, made of the side chains Thr Val Gly Tyr Gly TVGYG in many types of potassium channels. We calculate the conductance of the tetrameric KcsA prokaryotic K^+ channel measured in solutions of different K^+ concentration. The 3D model used here consists of two regions of different dielectric constant, one representing the protein and one representing a bath of implicit water. The geometry of the model is loosely based on the 'open' MthK crystal structure of Jiang's laboratory in which the intracellular half of the channel has a wide (~1.2 nm) pore radius. Ions are represented as hard spheres with Pauling radii. The surface charge on the protein is calculated using the induced charge computation method of Gillespie and collaborators. A Grand Canonical Monte Carlo approach developed by Boda maintains system neutrality while keeping bath concentrations fixed at values comparable to experiments. The Metropolis algorithm maintains a Boltzmann distribution to keep the system in thermodynamic equilibrium. The spatial density distribution of the ions allows an estimate of a characteristic slope conductance, for small driving force. Four pairs of GLU71/ASP80 ionizable residues lie directly behind the K^+ selectivity filter and have a substantial effect on potential energy profile along the selectivity filter. The model will be used to investigate the relationship of the protonation state of the residues, the composition of the bathing solutions, and the slope conductance.

614-Pos

High-Resolution Structural Modeling of Ion Channel Pore-Forming Domains Using Rosetta

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Ion channels play a key role in many biological processes. Electrical signals conducted by ion channels are essential for rapid transmission of information in brain, cardiac muscle contraction, sensation of the environment, and secretion of hormones. Despite recent progress in determining the high-resolution structures of several members of ion channel family, the high-resolution structures of majority of ion channels remain unknown. We developed a novel approach to generate high-resolution structural models of pore-forming domains of ion channels using Rosetta symmetry-loop *de novo*/homology computational method. In this approach, the selectivity filter region residues were modeled using Rosetta *de novo* loop modeling method and the pore-forming transmembrane helices were modeled using Rosetta homology method. We tested our method on several known high-resolution ion channel structures, including KcsA (pdb: 1K4C and 1K4D), NaK (pdb: 3E86), and Kv1.2 (pdb: 2R9R) channels. Results show that Rosetta symmetry-loop modeling method is able to predict the selectivity filter region of ion channels with high-resolution (1.0-1.5 Å root mean square deviation of backbone atoms from the native structure) and that Rosetta full-atom scoring function is able to discriminate well the best models in the majority of tested cases. Our results show that the Rosetta symmetry-loop *de novo*/homology modeling method is a powerful new approach for high-resolution structure prediction of pore-forming domains of ion channels. Supported by University of Washington Royalty Research Fund grant (to V.Y.-Y), HHMI grant (to D.B.), and NIH Grant R01 NS15751 (to W.A.C.).

615-Pos

Can Bubble Gates Be Seen in Experiments?

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Bubble gating is the hypothesis that the flux through an ion channel can be controlled by the formation or breaking of a bubble in a narrow hydrophobic gate [1]. If a bubble forms, liquid water is transformed into the gas phase and ions face a large dielectric boundary, which stops the flux through the gate.

The physics model of the bubble gate [1] allows one to systematically look for experimentally verifiable signs of bubbles. Here I discuss two possibilities.

1. Bubble formation is a small scale pseudo phase transition form a liquid into a gas. A liquid has a large internal energy, but a small entropy, while the gas has a large entropy, but a small internal energy. When a bubble forms, the free energy changes only slightly, because the large change in internal energy is almost balanced by a large change in entropy.

2. Hydrophobic gases, such as nobel gases, have a small solubility in water. If a bubble forms, it is filled by the hydrophobic gas [2].

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