Drug Trapping in hERG Channels does not Require Closure of theActivation Gate

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Human ether-a-go-go related gene (hERG) channel inhibitors can be trapped in the channels at rest. The structural peculiarities of hERG blockers that enable trapping or alternatively resting state dissociation are currently unknown. Propafenone (small molecule, MW 341 g/mol) is efficiently trapped in the closed hERG channel pore (1). To investigate whether the size of the blocking molecule plays a role in trapping we synthesized bulky propafenone derivatives containing benzoyl and trimethylphenyl side chains, attached by piperazine linkers, with molecular weights of 500 (Fba212) and 650 g/mol (Fba213) respectively. hERG channels were expressed in Xenopus laevis oocyte and potassium current inhibition was studied using the two - microelectrode voltage clamp technique. It was found: first, both compounds are potent hERG blockers with IC50 3.7 μM (Fba212) and 52μM (Fba213). Secondly, channel block by Fba212 and 213 was prevented by mutations Y652A and F656A as previously shown for propafenone. Third, both propafenone derivatives were trapped at rest. To obtain insights into the molecular mechanism of channel block docking experiments with Fba212 and Fba213 in closed and open conformation were performed. Both compounds interact with the propafenone binding site (Y652A and F656A). Fba213 was found to exceed the size of the closed channel cavity of our hERG homology model. We conclude that drug trapping in hERG channels does not necessarily require full closure of the activation gate.


Drug Trapping in hERG Channels by Increasing the Dynamic Range of Potassium Currents

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The human Ether-a-go-go Related Gene (hERG) encodes Kv11.1 and underlies the rapidly activating delayed rectifier K+ current in the heart, and loss-of-function hERG mutations cause the type 2 long QT syndrome (LQT2). The majority of LQT2-linked missense mutations decrease the trafficking of Kv11.1. An important finding is that the two compounds bind to Kv11.1 and block current (IKv11.1) can correct the trafficking for most of these mutations (pharmacological correction). We tested the hypothesis that pharmacological correction increases the trafficking of mutant LQT2 channels from the Endoplasmic Reticulum (ER). Voltage-clamping and Western blotting experiments of HEK293 cells expressing the trafficking-deficient LQT2 mutation G601S showed that pharmacological correction still occurred in cells treated with the protein synthesis inhibitor cycloheximide. Confoasals analysis of HEK293 cells stably expressing wild type Kv11.1 or G601S showed that G601S is selectively stored in an intermediate ER compartment with BAP31 treatment. The intermediate BAP31 compartment does not overlap with the perinuclear ER compartment, or the ER Golgi Intermediate Compartment.

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Inactivation Properties of hERG Currents in E496A and E842A Mutations in Large Conductance Ca2+ Channel[KCNQ1]

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The large conductance Ca2+ channel [KCNQ1] represents the major potassium conductance in rod photoreceptors. KCNQ1 mutations cause autosomal dominant nocturnal blindness. A number of KCNQ1 mutations have been characterized, including those causing autosomal dominant nocturnal blindness. In this study, we have characterized the inactivation properties of hERG currents in E496A and E842A mutants. The results suggest that these mutants have a decreased dynamic range of potassium currents in comparison to wild type KCNQ1. These results provide insights into the molecular mechanisms of KCNQ1 inactivation and may have implications for the treatment of inherited retinal degenerations.

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Rehabilitation Studies for withdrawn Drugs from the Market: Derivation of Non-hERG Channel Blocker Cisapride Analogues using Multi-Faceted Approaches

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The human ether-a-go-go related gene 1 (hERG1) potassium ion channel is a key element for the cardiac delayed rectified potassium current (IKr) and it plays an important role in the normal repolarization of the action potential. Diverse types of organic compounds used both in common cardiac and noncardiac medications can reduce the IKr and may lead to ventricular arrhythmia. Therefore, several approved drugs have been withdrawn from the market because of their effects on QT interval prolongation. Since most of these drugs have high potency for their principal targets, “rehabilitation” studies for decreasing their hERG1 blocking affinities while keeping them active at the binding site of their targets may lead re-use of these drugs. For this aim, a gastrointestinal agent, cisapride, is studied. Cisapride is withdrawn from the market because of its high hERG1 blocking affinity. In this study, analyses of fragment interactions of cisapride at the human A2A adenosine receptor and hERG1 central cavities is investigated using silico simulations (molecular docking, molecular dynamics simulations, de novo drug design) and novel and safer (with low hERG1 blocking affinities) of cisapride derivatives are proposed for their synthesis and their biological tests.

Cellular Mechanism for the Pharmacological Correction of hERG Mutations Linked to the Long QT Syndrome

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The human Ether-a-go-go Related Gene (hERG) encodes Kv11.1 and underlies the rapidly activating delayed rectifier K+ current in the heart, and loss-of-function hERG mutations cause the type 2 long QT syndrome (LQT2). The majority of LQT2-linked missense mutations decrease the trafficking of Kv11.1. An important finding is that the two compounds bind to Kv11.1 and block current (IKv11.1) can correct the trafficking for most of these mutations (pharmacological correction). We tested the hypothesis that pharmacological correction increases the trafficking of mutant LQT2 channels from the Endoplasmic Reticulum (ER). Voltage-clamping and Western blotting experiments of HEK293 cells expressing the trafficking-deficient LQT2 mutation G601S showed that pharmacological correction still occurred in cells treated with the protein synthesis inhibitor cycloheximide. Confocal analysis shows that HEK293 cells stably expressing wild type Kv11.1 or G601S shows that G601S is selectively stored in an intermediate ER compartment with BAP31 treatment. The intermediate BAP31 compartment does not overlap with the perinuclear ER compartment, or the ER Golgi Intermediate Compartment. Additional experiments showed that treating cell in E4031 for as little as 30 min was sufficient to cause the pharmacological correction of IKv11.1 for many hours. Together these data demonstrate that a steady-state subpopulation of LQT2 channels is stored separately in the BAP31 transitional ER compartment and their functional expression is readily corrected by E4031 treatment.
C39X and C44X channel proteins are trafficking defective. We show that C39X and C44X channels undergo translation reinitiation at M60 which deletes the hERG N-terminus including the first 34 residues of the Pore, Arnt, and Sim (PAS) domain. In contrast to the reinitiation of Q81X channels at M124, in which the PAS domain is nearly completely deleted, reinitiation at M60 disrupts the folding of this highly structured domain, precluding the efficient folding and trafficking of the mutant channels. The RNase protection assay, western blot analysis and electrophysiology were used to characterize the LQT2 mutants at the RNA, protein, and functional level. Our findings indicate that translation reinitiation may give rise to trafficking as well as functional defects of mutant hERG channels associated with LQT2 nonsense mutations.

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Non-Coding Data in KCNH2 mRNA Control Translation and Trafficking Efficiencies
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The KCNH2 gene encodes a K+ channel important to normal cardiac electrophysiology. Its significance is underscored by its link to both hereditary (locus LQT2) and acquired Long-QT cardiac arrhythmias. Over 400 deleterious mutations have been reported throughout the KCNH2 length. Why the channel is so susceptible to missense mutations is unclear. More than 50% of LQT2 mutations result in defective assembly and trafficking. There is evidence that even wild type channels are processed inefficiently. It has also been reported that the channel protein may reach the surface via atypical pathways. Most attention has logically focused on the protein for these processes.

Much less is known about mRNA-dependent factors in channel processing. We analyzed the coding mRNA sequence of KCNH2. It has 66% GC content and 60 potential hair-pin loop segments. We re-synthesized the cDNA to achieve 50% GC-content and reduce the number of potential hair-pin loops while maintaining identical amino acid coding. Cellular expression of the codon-modified cDNA (CM-KCNH2) produced ionic currents comparable to native cDNA. Channel protein was expressed in a dramatically different pattern. Whereas the native protein (NT-KCNH2) is usually more abundant (immature form) in ER/Golgi compartments than on the surface, CM-KCNH2 showed a preponderance of the mature form, indicating channels at the surface. Immunofluorescence analysis confirmed this localization. From these results we hypothesize that KCNH2 translation efficiency, determined by RNA sequence-specific elements independent of coding, affects downstream protein assembly and/or trafficking an unusual occurrence. As a corollary to this, we postulate a synergy between the inherently inefficient biosynthesis of KCNH2 and LQT2 mutations that contribute to the pathogenesis of hereditary LQT2. Further investigation of mechanisms underlying mRNA-dependent processing of KCNH2 channels may lead us to reconsider approaches to hereditary and acquired arrhythmia syndromes.

1372-Pos Board B264
Interactions of Extracellular Potassium, Calcium, Magnesium and Hydrogen with the outer Pore of the Cardiac Potassium Channel hERG
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Reduction of the current carried by the cardiac potassium channel HERG can lead to Long QT syndrome, an arrhythmia characterized by a rapid heart rate and reduced cardiac output, which can, in certain situations, be fatal. The effect of extracellular electrolytes on the biophysical properties of the HERG channel have been studied in some detail. In particular, increases in extracellular calcium, magnesium and hydrogen have been shown to slow channel activation, increase channel deactivation, and shift the G-V curve to more positive voltages. A number of reports have also shown that hydrogen can reduce HERG current by a mechanism that does not involve an effect on channel deactivation and which likely involves pore block. We show here that reducing extracellular potassium results in a significant increase in HERG current reduction by extracellular calcium, hydrogen, and magnesium. Current reduction by calcium appears to be significantly greater than current reduction by magnesium. Furthermore, the reduction in HERG current by extracellular hydrogen depended on the extracellular calcium concentration. The Drosophila voltage-gated potassium channel Shaker, showed a much smaller decrease in current by extracellular calcium and this effect was not dependent on extracellular potassium. In addition there was no difference in current reduction by calcium between WT HERG and the inactivation deficient mutant S631A, either in low or high extracellular calcium. These results suggest that calcium, hydrogen, and magnesium can block the HERG potassium channel and that calcium, hydrogen, magnesium, and potassium may interact at the outer pore of the HERG channel.

1373-Pos Board B265
Structural Basis for Differential KCNQ1 Interactions with KCNE1 and KCNE2 in the Extracellular Juxtamembrane Region
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KCN1 and KCNE2 are both expressed in human heart and can associate with KCNQ1. KCN1 and KCNE2 share transmembrane topology and sequence homology, yet they differ in KCNQ1 modulation (KCN1 slows KCNQ1 Closed-to-Open transition, while KCNE2 slows KCNQ1 Open-to-Closed transition) and overall cell biology (KCNQ1/KCNE2 is 5 and 10 fold less sensitive than KCNQ1/KCNE1 to niflumic acid, I_{Ks} activator, and azimilide, I_{Ks} suppressor). Previous work has shown that the extracellular juxtamembrane (EJM) region of KCN1 interacts with the extracellular surface of KCNQ1 to modulate gating kinetics and to form I_{Ks} activator binding sites. This prompts us to compare the EJM of the two KCN1 subunits in terms of KCNQ1 interaction. We apply cysteine (Cys) scanning mutagenesis to EJMs of KCN1 and KCNE2 and analyze the patterns of functional perturbation when coexpressed with KCNQ1. We use methanethiosulfonate (MTS) reagents to probe the relationship between EJMs of KCNE1 and KCNQ1. Finally we probe diulixide formation between Cys engineered into the EJMs of KCNE1 subunits and those engineered into extracellular surface of KCNQ1. The EJM of KCN1 makes frequent contacts with KCNQ1, so that MTS modification of exposed Cys side chains can affect channel function. KCNQ1 is largely insensitive to MTS modification of exposed Cys side chains engineered to EJM of KCNQ1. However, MTS can slowly access 7 consecutive hydrophobic positions in the beginning of KCNQ1 transmembrane domain, as if there is a crevice between KCNQ1 and KCNE2. Disulfide trapping experiments suggest that KCNQ1 is leaning more toward KCNQ1 S2 than KCNE1. We propose that KCNQ1 interferes with C2S2 interactions during Cys posttranslational modification and thus slows deactivation. This weakens sensitivity to niflumic acid and azimilide by affecting binding site directly or by an allosteric mechanism.

1374-Pos Board B266
Dynamic Control of I_{Ks} Current Amplitude by the ‘Late-Assembly’ Strategy of Channel Subunit Association
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Background: Ion channels composed of α (pore-forming) and β (auxiliary) subunits use an ‘early-assembly’ strategy (Kir6.x/SUR assembly in ER) to control cell surface expression, or a ‘late-assembly’ strategy (α/β2 of rat brain Nav channels, both subunits independently traffic to cell surface) to allow dynamic control of current amplitude/gating kinetics. Slow delayed rectifier (I_{Ks}) channel is composed of KCNQ1 (Q1, α) and KCNE1 (E1, β) subunits, and functions as ‘repolarization-reserve’ in human heart. It is not clear which of the 2 assemblies strategies Q1 and E1 use in forming I_{Ks}. Methods: We express Q1 and E1 tagged with fluorescent-protein (Q1-GFP and E1-dsR) or extracellular epitope (Q1-4COS and E1-40C) and use confocal imaging to track Q1 & E1 movements in cells. Results: In COS-7 cells 3 hr after transfection, Q1 and E1 travel in separate transport intermediates without mixing/fusion. E1 reaches the cell surface before Q1. By 24 hr, Q1 and E1 are colocalized on cell surface. Brefeldin-A (blocking protein export from ER) prevents surface expression of Q1-147C/E1-40C and reduces diulixide formation between the two (as a measure of functional Q1/E1 assembly). In NRVM during in vitro development, Q1-GFP and E1-dsR travel in distinctly different transport intermediates. As NRVM develops into mature-like phenotype, Q1-GFP and E1-dsR are colocalized on the cell surface, with a separate cytosolic Q1-GFP pool colocalized with α-actinin and calnexin (z-line and ER/SR markers, respectively). Conclusions: Q1 and E1 use the ‘late-assembly’ strategy to afford dynamic control of I_{Ks} current amplitude. This explains why native Q1 and E1 in adult ventricular myocytes are often not well colocalized. We propose that cardiac myocytes regulate I_{Ks} amplitude dynamically by adjusting the degree of Q1/E1 colocalization on cell surface.

1375-Pos Board B267
A Mutation in the Voltage-Sensor of Kv7.1 Prevents PKA Activation of IKs to Elicit Concealed Type I Long QT Syndrome during Stress
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Long QT syndrome is associated with prolongation of the corrected QT interval (QTc) and increased risk for ventricular arrhythmias. LQT1 is caused by slow delayed rectifier (IKs) activator binding sites. This prompts us to compare the EJM of the two KCN1 subunits in terms of KCNQ1 interaction. We apply cysteine (Cys) scanning mutagenesis to EJMs of KCN1 and KCNE2 and analyze the patterns of functional perturbation when coexpressed with KCNQ1. We use methanethiosulfonate (MTS) reagents to probe the relationship between EJMs of KCNE1 and KCNQ1. Finally we probe diulixide formation between Cys engineered into the EJMs of KCNE1 subunits and those engineered into extracellular surface of KCNQ1. The EJM of KCN1 makes frequent contacts with KCNQ1, so that MTS modification of exposed Cys side chains can affect channel function. KCNQ1 is largely insensitive to MTS modification of exposed Cys side chains engineered to EJM of KCNQ1. However, MTS can slowly access 7 consecutive hydrophobic positions in the beginning of KCNQ1 transmembrane domain, as if there is a crevice between KCNQ1 and KCNE2. Disulfide trapping experiments suggest that KCNQ1 is leaning more toward KCNQ1 S2 than KCNE1. We propose that KCNQ1 interferes with C2S2 interactions during Cys posttranslational modification and thus slows deactivation. This weakens sensitivity to niflumic acid and azimilide by affecting binding site directly or by an allosteric mechanism.

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