kinetics. The mechanism by which the eag domain regulates gating remains unclear. Recent evidence suggests the intracellular loop between the S4 and S5 transmembrane domains (S4-S5 linker) may be important in regulating both activation and deactivation, and that modulation of gating by the eag domain may act via the S4-S5 linker. Here we sought to investigate the role of the S4-S5 linker using site-directed mutagenesis and a combination of electrophysiology and Förster Resonance Energy Transfer (FRET). We found that channels with alanine mutations in the S4-S5 linker exhibited altered gating. All the S4-S5 mutant channels caused an acceleration of deactivation kinetics, except for S543A, which had significantly slowed deactivation. Co-expressing an eag domain fragment (N-eag) with S4-S5 mutant channels which additionally lacked a native eag domain (∆eag) failed to restore slow deactivation kinetics to the mutant channels. FRET analysis revealed that eag domains tagged with a CFP were in close proximity to each of the S4-S5 mutant channels tagged with a Citrine. Replacement of the entire S4-S5 linker with alanines (hERG [S4-S5]Ala) produced channels with altered gating, including fast deactivation and a far left-shifted steady-state activation curve. Co-expression of hERG ∆eag[S4-S5]Ala channels with N-eag did not alter channel gating; however, FRET analysis revealed that N-eag was in close proximity to the mutant channels. Together, these findings suggest that an intact S4-S5 linker is necessary to transduce eag domain-dependent regulation of gating, but it is not required for the eag domain to bind to the channel.

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A Proline Scan Approach to Investigate the Activation Gate of hERG Channels
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In Shaker channels, the activation gate is formed at the bundle crossing by the S4-S5 linker and is characterized by unusually slow activation and deactivation gating kinetics compared to Shaker-like voltage-gated K+ (Kv) channels. In Shaker-like Kv channels movement of the voltage sensor upon depolarization is mechanically transduced by the a-helical S4-S5 linker to S6 activation gate opening. Given the unique gating properties of hERG channels, the details of voltage sensor coupling with the S6 activation gate opening are of significant interest. We have recently shown that substitution of a glycine residue (G546) within the S4-S5 linker (from position 539 to 552) in the G546L background to determine the intracellular pore gate and how it is coupled to S4 movement is less clear. Recent evidence suggests the intracellular loop between the S5 and S6 segments of adjacent hERG1 subunits. Thus, modulate voltage-dependent gating. F290 is conserved in hERG (F463), and are apparently limited by slow movement of the S4 voltage sensor. In fast-activating Shaker channels, a putative gating charge transfer centre formed in the activation and deactivation gating kinetics, since K6R dramatically slowed and right-shifted activation. Taken together, these data suggest a role for F463 in mediating the closed-open equilibrium, similar to that described for F290 in Shaker. Also consistent with results from Shaker, the hERG KIR mutation left-shifted the G-V relationship and stabilized the open state. In contrast to a predicted stabilization of the open state, R5K caused a moderate right-shift of the G-V and closed-state stabilization. Intriguingly, the neighbouring K6 residue was more important than R5 in hERG voltage sensing, since K6R dramatically slowed and right-shifted activation. hERG channels, the gate was trapped open, but upon strong hyperpolarization channels slowly activated into a distinct voltage-dependent open state, reminiscent of the well-studied hERG mutation, D540K. The presence of voltage-dependent gating in this mutant suggests that the opened closed phenotype is due to uncoupling of gate closure from voltage sensor gating, rather than an immobilization of sensor movement. Moreover, the activation of V659P channels upon hyperpolarization suggests a ‘down’ configuration of the voltage sensor that is distinct from that occupied at −80 mV and that leads to hyperpolarization-activated pore opening, as in HCN channels.

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Examining the Regulation of Voltage-Dependent S4 Movement in hERG Potassium Channels
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Unlike many Kv channels, hERG channel activation and deactivation are slow and are apparently limited by slow movement of the S4 voltage sensor. In fast-activating Shaker channels, a putative gating charge transfer centre formed in part by F290 has been proposed to interact with S4 charges (e.g. R1, K5) and, thus, modulate voltage-dependent gating. F290 is conserved in hERG (F463), but the relevant residues in the hERG S4 are reversed (K1, R5) and there is an extra positive charge adjacent to R5 (K6). We have examined whether hERG channels possess a transfer centre similar to that in Shaker and if these S4 charge differences underlie slow gating in hERG channels. Of five hERG F463 hydrophobic substitutions tested, only F463W and F463Y shifted the G-V relationship to more depolarized potentials and dramatically (>20x) slowed channel activation. With the S4 residue reversals (i.e. K1, R5) taken into account, this closed S4 stabilization suggests a motif that is similar to that described for F290 in Shaker. Also consistent with results from Shaker, the hERG KIR mutation left-shifted the G-V relationship and stabilized the open state. In contrast to a predicted stabilization of the open state, R5K caused a moderate right-shift of the G-V and closed-state stabilization. Intriguingly, the neighbouring K6 residue was more important than R5 in hERG voltage sensing, since K6R dramatically slowed and right-shifted activation. Taken together, these data suggest a role for F463 in mediating the closed-open equilibrium, similar to that proposed for F290 in Shaker channels.
Furthermore, the hERG channel open state appears to be stabilized by the unique configuration of R5 and K6 side chains. Both charges play important roles, but that of K6 appears more critical at stabilizing the activated state of the channel.
PD increases hERG1 current by multiple effects, including a shift of inactivation to a more positive voltage, slowing of deactivation and an increase in single channel block probability. The efficacy of PD was increased in proportion to the WT:L646E subunit ratio. The Hill coefficient was 1.40 - 1.55 for all the heteromeric concatamers, suggesting a weak positive cooperativity for PD binding. At a fully effective concentration of PD (10 μM), each WT binding site contributes ~20% towards the maximum response of 80% enhancement of current.

ICA increases hERG1 current by a profound attenuation of inactivation. An increase in the WT:F557L subunit ratio enhanced the agonist activity of ICA (10 μM and 30 μM) in a synergistic fashion. For 2WT:2F557L concatenated channels, synergy required adjacent (as opposed to diagonal) WT subunits. In summary, four intact binding sites per channel are required to fully activate hERG1 by PD or ICA.

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Drug Trapping in hERG Channels does not Require Closure of the Activation 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 oocytes 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 3.7μM (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.


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Inactivation Properties, not Binding Site Differences, Govern Reciprocal Functional Responses to ICA105574 in EAG and ERG Potassium Channels Vivek Garg¹, Anna Stary-Weininger², Michael C. Sanguinetti³.

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ICA105574 (3-Nitro-N-(4-phenoxycphenyl)-benzamide) activates hERG1 (Kv11.1) channels by reducing inactivation, but inhibits hEAG1 (Kv10.1) channels by inducing inactivation. Using chimeric EAG-ERG constructs, we found that the channel-specific response to ICA105574 is mediated by the pore region (S5-pore helix-S6). Simulated docking of ICA105574 on a molecular model of hEAG1 coupled with alanine mutagenesis indicates that similar to hERG1, ICA105574 binds to a hydrophobic region confined by the S5/S6/ pore helix of one subunit and S6 of adjacent subunit in hEAG1 channels. Based on the putative ICA binding site and protein sequence alignment, a triple mutant of hEAG1 (M431F/M458L/L463M hEAG1) was designed to make the ICA binding region in hEAG1 homologous to hERG1. ICA inhibited the triple mutant similar to WT-hEAG1, indicating that the amino acid differences within the putative ICA binding region do not determine the reciprocal functional response to ICA. A single mutation in S5 (F557L), pore helix (L622C) or S6 (Y652A) of hERG1 eliminates the activity of ICA on hERG1 (Garg, et al 2011, Mol. Pharm.). The equivalent mutations in hEAG1 affect inactivation differentially and result in very different responses to ICA. First, Y464A induced inactivation of hEAG1 channels and this gating was accentuated by ICA. Second, ICA is a partial agonist of F359L and L434C mutant channels. We recently proposed a tripartite model of slow inactivation for hEAG1 that involves interaction of the same three residues. We conclude that ICA binds to the same site in hEAG1 and hERG1 channels, and that the functional consequences are determined by the underlying (fast or slow) mode of selectivity-filter mediated inactivation.

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Rehabilitation Studies for withdrawn Drugs from the Market: Derivation of Non-hERG1 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 indications 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 high 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 gastroprokinetic 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 in silico applications (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.

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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 drugs that bind to Kv11.1 and block current (IK1.1) can correct the trafficking for most of these mutations (pharmacological correction). We tested the hypothesis that pharmacological correction increases the trafficking of mutant LTQ2 channels from the Endoplasmic Reticulum (ER). Voltage-clamping and Western blotting experiments of HEK293 cells expressing the trafficking-deficient LTQ2 mutation G601S showed that pharmacological correction still occurred in cells treated with the protein synthesis inhibitor cycloheximide. Confocal analyses of HEK293 cells stably expressing wild type Kv11.1 or G601S showed that G601S is selectively stored in an intermediate ER compartment with BAP31. The intermediate BAP31 compartment does not overlap with the perinuclear ER compartment, transitional ER compartment, or the ER Golgi Intermediate Compartment. Trafficking-deficient cells in E-4031, a drug that corrects G601S trafficking, decreased G601S co-localization with intermediate BAP31 compartment and increased G601S immunostaining at the cell surface membrane. Additional experiments showed that treating cell in E-4031 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 LTQ2 channels is stored separately in the BAP31 transitional ER compartment and their functional expression is readily corrected by E-4031 treatment.

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LQT2 Nonsense Mutations Generate Trafficking Deficient N-Terminally Truncated Channels by the Reinitiation of Translation

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Long QT syndrome type 2 (LQT2) is caused by mutations in the human ether-a-go-go related gene (hERG). The reinitiation of translation has recently been reported as a novel pathogenic mechanism of LQT2 by which early PTC mutations generate N-terminally truncated channels with altered gating properties. We have previously shown that hERG transcripts containing the Q81X mutation were translated at the downstream M124 codon following premature termination and generated channels that exhibited increased deactivation kinetics and decreased resurgent outward current during action potential repolarization. We have now identified two additional LQT2 nonsense mutations C39X and C44X that escape nonsense mediated mRNA decay by the reinitiation of translation. Unlike the Q81X channels, which are expressed at the cell surface,