rectification. A genetically encoded Eag domain fragment (amino acids 1-135) was shown to restore slow deactivation to N-truncated channels. Our present study sought to further investigate Eag domain contributions to hERG gating kinetics. We coexpressed the genetically encoded Eag domain fragment (N1-135) with hERG channels bearing a deletion of the N-terminus in Xenopus oocytes and measured current with two-electrode voltage-clamp recordings. Here we report that coexpression with the N1-135 peptide led to a reduction in relative outward current and slowed recovery from inactivation resulting in channels with properties similar to those measured in wild-type hERG. Through regulation of deactivation and inactivation gating, the Eag domain determines the physiologically critical resurgent component of hERG current via a non-covalent interaction with the channel.

626-Pos

Mutations Within the S4-S5 Linker Alter Voltage Sensor Constraints During Activation and Deactivation of Herg K^+ Channels

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hERG channel gating is associated with relatively slow voltage sensor movement that limits the rate of channel opening and closing. The mechanistic basis underlying the constraints upon sensor movement in these channels is unclear. Here, we have used voltage clamp fluorimetry (VCF) to study the effects of mutations within the S4-S5 linker on voltage sensor movement and its coupling to the pore. Mutations at G546 had two separable effects on activation and deactivation gating. Substitution of G546 with residues possessing different physico-chemical properties all (with the exception of G546C) shifted activation gating by ~30mV in the hyperpolarizing direction. With the activation shift taken into account, the time constant of ionic current activation was also accelerated. In addition, a number of G546 mutants affected deactivation gating, although the effects of different mutations varied. In the most dramatic case, the G546V mutation induced biphasic deactivation with a pronounced slow component that was voltage-independent. Deletion of the N-terminus accelerated the fast component, but the slow component remained pronounced, suggesting that the slow component was not mediated by altered interaction with the N-terminus. VCF measurements of voltage sensor movement in G546V channels revealed fast and slow components of fluorescence change associated with deactivation, suggesting that the slow component of ionic current deactivation is due to slow voltage sensor return that is uncoupled from charge movement. Taken together, these data suggest: 1) reduced flexibility of the S4-S5 linker helix reduces constraints on voltage sensor movement during activation gating; 2) normal hERG channel closing involves at least two reconfigurations of the voltage sensor that are rate-limiting for pore closure.

627-Pos

Rescue of Gating in hERG1 Potassium Channels Containing LQT2 Mutations in the N-Terminal PAS Domain

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The human *ether-a-go-go*-related gene 1 (hERG1) encodes a voltage-dependent potassium (K^+) channel which underlies the cardiac delayed-rectifier K^+ current (IK_r). The closing rate of the channel is a major determinant of the amplitude of outward current, and is regulated by an N-terminal Per-Arnt-Sim (PAS) domain. Loss of function mutations in hERG1 result in a loss of IK_r and lead to congenital Long QT Syndrome 2 (LQT2). Only a small percentage of the PAS domain LQT2 mutations have been characterized in mammalian cells, and these exhibited a variety of defects. Therefore, it remains unclear as to how LQT2 mutations located in the PAS domain disrupt hERG1 function. To address this, we have selected 12 PAS domain LQT2 mutations and, using biochemistry and electrophysiology, examined their functional properties when expressed at physiological temperatures. Our data demonstrate that channels with LQT2 mutations located in the PAS domain exhibit a spectrum of deficiencies when cultured at 37°C. Western blot analysis indicated that some mutations are trafficking-deficient, evident by detection of only the immature form of the channel; others were indistinguishable from WT hERG1, with enriched expression of both the immature and mature forms; while the remaining exhibited intermediate levels of maturation. Whole-cell patch-clamp analysis revealed that the LQT2 PAS domain mutants produce functional channels at the cell surface with perturbed deactivation kinetics. Co-expression of a genetically-encoded N-terminal peptide with these gating-deficient mutants rescued the gating-deficiency and fully restored the WT phenotype. Taken together, these data are the first to characterize purely gating-deficient hERG1 PAS domain LQT2 mutations expressed in mammalian cells, and show that a genetically-encoded N-terminal peptide is able to fully restore the WT phenotype to the channels.

628-Pos

Block of Herg by Trapped Drugs Shows a Different Dependency on Extracellular Potassium Compared to Block of Herg by Drugs That are Not Trapped

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Block of the cardiac potassium channel HERG by a number of drugs has been shown to decrease with an increase in the extracellular potassium concentration. This dependency on extracellular potassium can be explained by at least two mechanisms: 1) destabilization of the drug by the permeant ion 2) differential binding to the inactivated state. We have previously shown that block of HERG by quinidine, a drug that is not trapped after channel deactivation, correlates better with the permeant ion than with inactivation, indicating that quinidine block is destabilized by the permeant ion.[1] We show here that block of HERG by terfenadine and bepridil, drugs shown to be trapped in the channel after channel deactivation[2], is not altered with an increase in the extracellular potassium concentration. Furthermore block by both terfenadine and bepridil of the HERG mutant D540K, which opens with both depolarization and hyperpolarization, is decreased with increased extracellular potassium, similar to the effect of extracellular potassium on block of WT HERG by quinidine. In addition, the decrease in block of D540K by bepridil is less with an increase in extracellular cesium compared to an increase in extracellular potassium ($P_{Cs}/P_K = 0.33$). Finally, preliminary data indicate that block by bepridil of a number of HERG inactivation deficient mutants does not depend on extracellular potassium. Together these results suggest that the permeant ion is not able to destabilize a trapped drug but is able to destabilize a drug that is not trapped and suggest a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.

[1]Barrows et al. (2009) Channels: **3(4)** :239-248.
[2] Stork et al. (2007) *BJP***151**:1368-1376.

629-Pos

Conformational Flexibility of the hERG K+ Channel Pore Domain

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Malfunction of hERG K+ channels, due to inherited mutations or inhibition by drugs can cause long QT syndrome, which may lead to life-threatening arrhythmias. A 3-dimensional hERG structure is a prerequisite to understand the molecular basis of hERG malfunction. To achieve a consensus model we have carried out an extensive analysis of hERG models, based on different alignments of helix S5. The consensus model was validated using a combination of geometry/packing/normality validation, as well as molecular dynamics simulations and molecular docking. The model is confirmed by a recent mutation scanning experiment.1 Subsequently, the refined model was used to study the conformational flexibility of the hERG pore domain. Extensive molecular dynamics simulations revealed that the aromatic side-chains, lining the inner cavity can adopt a wide variety of conformations. Detailed knowledge of the hERG channel plasticity will be crucial to help interpreting differences in channel block of different drugs, since many drugs selectively block certain channel states.

1 Ju, P., Pages, G., Riek, R. P., Chen, P.C., Torres, A. M., Bansal, P. S., Kuyucak, S., Kuchel P. W, Vandenberg, J.I. (2009) J. Biol. Chem. 284, 1000-1008.

630-Pos

Substitution Scan of the S4-S5 Linker Region in KCNQ1 Channel: Structural Scaffold for Critical Protein Interactions

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KCNQ1 α-subunits are composed out of six transmembrane segments (S1-S6) that tetramerize into a functional channel. *In vivo*, KCNQ1 α-subunits associate with the β-subunit KCNE1 to generate the slowly activating cardiac I_{Ks} and consequently mutations in KCNQ1 are linked to the congenital LQT1 syndrome. Similar to other Kv channels, the S1-S4 segments form the voltage sensing domain that senses the membrane potential and that controls the

opening or closure of the channel gate through an electromechanical coupling. In other channels a direct interaction between the S4-S5 linker and bottom part of S6 has been shown to constitute this electromechanical coupling. We previously identified residues in the C-terminal part of S6 that are critical for KCNQ1 gating. To investigate if these residues interact with the S4-S5 linker, we performed an alanine/tryptophan substitution scan of the S4-S5 linker sequence. Based on their impact on channel gating, we categorized these substitutions as either ''high'' or ''low impact''. The pattern of ''high impact'' positions was consistent with an α-helical configuration and clustered on one side of the S4-S5 linker. Since substitutions at these positions markedly impaired channel gating, they are good candidates to contact residues in the bottom part of S6. Indeed, replacing valine 254 in the S4-S5 linker by a leucine resulted in channels that were partially constitutively open but channel closure could be rescued by combining V254L with the S6 mutation L353A that by itself displayed a similar phenotype as V254L. The observation that all known LQT1 mutations in the S4-S5 linker map on the ''high impact'' side further strengthens the proposal that this face of the S4-S5 linker contacts the C-terminal S6 segment and constitutes part of the electromechanical coupling in KCNQ1 channels.

631-Pos

Estimating Conformational Changes of KCNQ1 Channels During Gating Using Molecular Dynamics Simulations

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Although the structure of an ion-channel protein may be known through crystallography or homology modeling, measuring its conformational changes during gating is extremely difficult if not impossible. Alternatively, molecular simulations is a powerful tool for estimating conformational changes. Movement of the positively charged S4 segment is a known conformational change of voltage-gated ion-channels, observed by measuring the accessibility of different amino-acid residues.

A previously determined structure of KCNQ1 was used. Movement of the S4-S3 complex with 3 translational and 2 rotational degrees of freedom was assumed to be the major conformational change during gating. More than 1 million conformations were considered. This expanded configuration space (compared to 1 translational and 1 rotational degree of freedom and about 2000 conformations used previously) enabled more accurate analysis of stable conformations. Conformations with steric overlap were eliminated. The electrostatic energies of the remaining conformations for various membrane potentials were computed and used to determine the probability of the ion-channel residing in each conformation (state residency). Conformations with small state residencies were eliminated.

Two regions in the configuration space with high state residency were identified: one associated with open conformations (outward position of S4-S3) and the other with closed conformations (inward position of S4-S3). These regions were separated by a narrow region with low state residency (an energy barrier). The open state consists of a large number of conformations while the energy barrier and its adjacent closed state (intermediate closed) consist of only few conformations. Channel conformations may branch from the intermediate closed conformation into two trajectories toward different subsets of closed conformations (deep closed). The simulated steady state open probabilities at various membrane potentials were consistent with experimental channel activation curves.

632-Pos

Neighboring Alpha-Subunit (KCNQ1) Mutations with a Gain-of-Function IKs Phenotype Show Differential Dependence on Presence of Beta-Subunit (KCNE1)

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The IKs cardiac potassium channel forms through co-assembly of KCNQ1, a 6 transmembrane-(TM) spanning voltage-gated potassium channel α subunit and KCNE1, a single TM spanning accessory protein. Two mutations in the S1 transmembrane helix of KCNQ1, S140G and V141M, have been shown to cause a hyperpolarizing shift in the voltage dependence of channel activation and to disrupt deactivation, resulting in accumulation of open channels and a gain-of-function phenotype during repetitive activity that is causally related to congenital human atrial fibrillation. Initial reports suggested that the phenotype of these mutants depends on the presence of the accessory protein KCNE1, which has been shown to be close in proximity to KCNQ1 S1, raising the possibility that KCNE1 directly interacts with KCNQ1 position 140 and/or 141. Here, we show that a Cys substituted at KCNQ1 position 141 spontaneously crosslinks with cysteines introduced in two positions in KCNE1, but a Cys substituted at position 140 does not crosslink to any Cys-substituted KCNE1 residues tested. Co-expression of KCNE1 with either S140G or V141M KCNQ1 slows deactivation and causes similar negative shifts in channel activation. However, in whole-cell patch clamp experiments using isotonic potassium to explore channel deactivation across a wide range of hyperpolarized potentials, we find that the V141M channel activity is indistinguishable from WT while the S140G mutation shifts the V1/2 of activation -30mV and drastically slows deactivation (tau ~1500ms vs. ~150ms) when compared with wild-type KCNQ1. Taken together, our results support: 1) an orientation in which KCNQ1 residue V141, but not S140, points toward and is in close proximity to KCNE1 and 2) a direct effect of S140G on channel gating but an allosteric effect of V141M on channel gating that requires the presence of KCNE1.

633-Pos

Physical and Functional Interactions Between the KCNQ1 and KCNE1 C-Terminal Domains

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KCNE1 is a regulatory subunit that associates with pore-forming subunits of KCNQ1 to form a channel complex that carries the slowly activating delayed rectifier current, IKs. Interactions between the transmembrane regions of KCNE1 and KCNQ1 S6 control activation kinetics of IKs, while interactions between the intracellular C-termini of the two subunits may dictate deactivation kinetics. Numerous Long QT Syndrome mutations occur in the C-termini of KCNQ1 and KCNE1, indicating the functional importance of these regions. We have located the proximal third of the KCNQ1 C-terminus (KCNQ1-CT) as a site of direct interaction with the KCNE1 C-terminus (KCNE1-CT) via co-immunoprecipitation studies, in vitro pull-down assays and Surface Plasmon Resonance analyses of purified recombinant proteins. Electrophysiological studies employing co-expressed soluble KCNE1-CT with full length KCNQ1 now provide functional evidence that support the physical association findings. When KCNE1-CT is co-expressed with KCNQ1 in CHO cells and analyzed by whole-cell patch clamp, deactivation kinetics of the KCNQ1 current are accelerated. Similarly, deactivation of IKs is accelerated by co-expressing KCNE1-CT with KCNQ1 and full-length KCNE1. KCNE1-CT also shifts the voltage dependence of activation of KCNQ1 current but not IKs current, and has no significant effect on activation kinetics of either current. Thus, excess soluble KCNE1-CT is capable of interacting with KCNQ1 and can perturb the KCNQ1/KCNE1-C-terminal interactions determining deactivation rates of IKs. Work in the laboratory now focuses on identifying the interacting residues of the KCNQ1 and KCNE1 C-termini. Preliminary experiments using Hydrogen-Deuterium Exchange coupled to Mass Spectrometry show that deuterium incorporation into purified KCNE1-CT is slowed by the addition of KCNQ1-CT, implying that binding between the two peptides protect certain residues of KCNE1-CT from being deuterated. Further experiments will work towards delineating the precise structural nature of this interaction.

634-Pos

Partial Restoration of the Cardiac KCNQ1 Mutant A341V by the KCNE1 Auxiliary Subunit

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Dysfunction of the cardiac potassium channel, IKs, can lead to the prolongation of the QT interval in the electrocardiogram, a potentially life-threatening condition known as the long QT syndrome (LQTS). The IKs consists of a pore-forming subunit, encoded by KCNQ1, and an auxiliary subunit, encoded by KCNE1. One of the most common mutations associated with the inherited form of LQTS is the substitution of an alanine residue to a valine at position 341 in KCNQ1. In the present study, we investigated the impact of the KCNE1 subunit on the A341V mutant. The whole-cell patch clamp technique was used to record current from transiently transfected HL-1 cells, a cardiac cell line derived from mouse atrial cells. In contrast to the non-functional A341V, the cotransfection of A341V with KCNE1 (A341V+KCNE1) resulted in a functional channel. Though the resultant current was qualitatively similar to that of native IKs, A341V+KCNE1 resulted in a significantly smaller mean current density, longer time to half-activation and a rightward shifted voltage dependence of activation with a steeper slope than the wild-type KCNQ1+KCNE1. To determine whether the observed functional restoration of A341V by KCNE1 involved trafficking of the mutant construct, HL-1 cells were transfected with a GFP-tagged A341V with and without KCNE1 and visualized by confocal microscopy. No differences