of G601S-hERG. After nocodazole treatment the G601S-hERG immunostaining pattern changed from perinuclear to one that consisted of focal aggregates located throughout the cytosol. Nocodazole treatment also increased the glycolytic processing of G601S-hERG similar to that of wild-type hERG. In contrast, nocodazole treatment did not alter the immunostaining or glycolytic processing of R752W-hERG. These data suggest that the tdLQT2 phenotype for G601S-hERG but not R752W-hERG is regulated by microtubule function. We conclude that microtubule dependent and independent mechanisms may regulate the tdLQT2 phenotype.

621-Pos

Mink Dictates the Alpha Subunit Composition of Surface-Expressed N-Type Potassium Channels

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Homomeric and heteromeric complexes formed by members of the Kv3 subfamily of voltage-gated potassium (Kv) channel alpha subunits generate currents essential for the high-frequency firing of mammalian neurons. Kv3.1 and Kv3.2 alpha subunits generate delayed rectifier currents, whereas Kv3.4 generates fast-inactivating currents. This 'N-type' fast-inactivation occurs via an N-terminal 'ball' domain, which blocks the channel pore directly after opening, preventing K^+ ion flux. Heteromeric channels containing Kv3.4 with either Kv3.1 or Kv3.2 exhibit N-type inactivation with a rate dependent upon the number of Kv3.4 alpha subunits in the tetramer. As Kv channel inactivation and inactivation recovery rates are important determinants of excitable cell action potential morphology and refractory period duration, the stoichiometry of these heteromeric complexes is expected to be tightly regulated. Here, using channel subunits cloned from rat and transiently expressed in CHO cells, we show that Kv3.4 current is significantly suppressed (>90%) by the ancillary beta subunit MinK (KCNE1) and that the suppression can be rescued by co-expression of Kv3.1. Through use of dominant-negative pore mutants and N-terminal A and B box (NAB) intra-subfamily binding domain mutants, we demonstrate that MinK ensures that Kv3.4 alpha subunits can only reach the surface as part of a heteromeric complex with Kv3.1. Thus, by acting as a molecular matchmaker, MinK governs Kv channel inactivation rate and, potentially, cellular excitability and refractory periods.

622-Pos

Pharmacological-Induced Increase in the Functional Expression Of hERG Current

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The human Ether-a-go-go Related Gene (hERG) K^+ channel is linked to type 2 Long QT Syndrome (LQT2), and most LQT2 mutations decrease hERG current (I_{hERG}). Since LQT2 follows an autosomal dominant inheritance pattern, increasing the functional expression of WT-hERG may have therapeutic potential. The goal of this study was to identify ways to increase I_{hERG} without altering gating. We tested the hypothesis that nocodazole (noc), an antimicrotubule agent, and cytochalasin D (cytoD), an antimicrofilament agent, would increase I_{hERG} because they can alter the kinetics of protein trafficking to and from the membrane. We cultured HEK293 cells stably expressing WT-hERG in noc (20∈1/4M) or cytoD (5∈1/4M) for 18-22 hours and measured I_{hERG} using the whole-cell patch clamp technique. Using a holding potential of −80 mV, cells were pre-pulsed to 50 mV in 10 mV increments for 5 seconds, followed by a test-pulse to −50 mV for 5 seconds for control, noc, or cytoD treated cells. The peak I_{hERG} measured during test-pulse was plotted as a function of the pre-pulse. The data were described using a Boltzmann equation to calculate the maximal current density (I_{MAX}), midpoint potential for activation ($V_{1/2}$), and the slope factor (k) for I_{hERG} activation. Noc treatment did not alter any of these parameters. CytoD treatment increased I_{MAX} (control=93 ± 8 pA/pF, n=5; cytoD=156 ± 15 pA/pF, n=7) but did not alter not $V_{1/2}$ or k. CytoD also did not alter the voltage-dependent rates of I_{hERG} deactivation (n=6, per group). These data suggest that the functional expression of WT-hERG is increased by inhibition of microfilaments but not microtubules. We conclude that targeting microfilaments and/or microfilament-dependent proteins may represent a novel strategy for increasing the functional expression of WT-hERG without altering I_{hERG} function.

623-Pos

hERG Heteromeric 1A/1B and Homomeric 1A Channels Exhibit Differential Pharmacological Sensitivities

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The majority of hERG screens aiming to minimize the risk of drug-induced long QT syndrome have been conducted using heterologous systems expressing the hERG 1a subunit, yet both hERG 1a and 1b subunits contribute to the channels producing the repolarizing current I_{Kr}. Previous studies show that differences in gating in heteromeric 1a/1b vs. homomeric 1a channels markedly increase repolarizing current during the ventricular action potential and protect against QT prolongation in computational models. We conducted a pharmacological analysis of 50 compounds targeting hERG channels and selected for their chemical diversity to evaluate differences in sensitivity that may influence safety margins or contribute to a stratified risk analysis. Experiments were carried out using the IonWorks™ plate-based electrophysiology device. Non-cumulative, 8-point concentration effect curves were generated, with each point representing data from 20 to 30 cells. Potency was determined as IC_{50} values (∈1/4M) obtained from data normalized to vehicle and 100% blocking levels and fitted to the Hill equation. To minimize possible sources of variability, compound potency was assessed using test plates arranged in alternating columns of 1a and 1a/1b cells. Although most compounds had similar potencies at both variants, some surprising differences were observed. For example, fluoxetine (Prozac) was 6-fold more potent at blocking hERG 1a/1b compared to 1a channels. The results were robust when compounds were tested against the hERG 1a and 1a/1b cell lines in parallel, but statistical analysis encompassing longitudinal variation indicates such differences may not be sufficient to warrant routine use of hERG 1a/1b in preclinical high throughput screens. However, our findings have uncovered several important candidates for further risk evaluation as we learn more about native subunit composition in different populations or changes in subunit composition during development.

624-Pos

A New Mechanism for Long QT Syndrome: Polypeptides Encoded by hERG1a Non-Sense Mutations Regulate hERG1a/1b Channels

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hERG potassium channels are critical for cardiac action potential repolarization. Disruption of channel function by inherited mutations in the gene encoding hERG have been shown to cause type 2 long QT syndrome (LQT2) by perturbing trafficking, assembly, selectivity and activation gating. To date, most mutations have been studied in heterologous systems expressing the hERG 1a subunit, yet both hERG 1a and 1b subunits contribute to the channels producing the repolarizing current IKr. hERG 1a and 1b subunits are structurally identical except for the N terminal region, which is unique and much shorter in the 1b subunit. Differences in gating result in markedly increased repolarizing current in heteromeric 1a/1b vs. homomeric 1a channels during the ventricular action potential and protect against QT prolongation in computational models. We examined nonsense mutations giving rise to truncations at different points in the amino terminus of hERG 1a. Surprisingly, these fragments had little or no effect on maturation of hERG 1a and 1b subunits expressed in HEK-293 cells. Instead, they altered gating and increased rectification as if the channels were homomers of hERG 1a subunits. Thus, by ''complementing'' the hERG 1b subunit (and its short N terminus), the mutant 1a fragments reduce the repolarization capability of the channel and mediate a novel mechanism of type 2 long QT syndrome.

625-Pos

The Eag Domain Regulates Outward Current Density and Recovery from Inactivation of the hERG K^+ Channel Through a Non-Covalent Interaction

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The Human Ether-á-go-go Related Gene (hERG) encodes a voltage-activated K+ channel. hERG contributes to repolarization of the ventricular action potential as the primary component of the cardiac delayed rectifier K^+ current (I_{Kr}) and has also been shown to modulate neuronal firing frequency. hERG gating is characterized by rapid inactivation upon depolarization and rapid recovery from inactivation and slow closing (deactivation) upon repolarization. These factors combine to create a resurgent hERG current, where the amplitude of the current is paradoxically larger with repolarization than with depolarization. These gating transitions also determine the timing and amplitude of the resurgent current. Previous data has suggested that the hERG N-terminus regulates gating kinetics, however the molecular mechanisms are not fully understood. Deletion of the N-terminus (amino acids 2-354) has been shown to speed channel deactivation and recovery from inactivation compared to that of wild-type hERG. Relative outward current amplitude is also increased during the depolarization phase of N-truncated channels, leading to reduced current

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