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