hERG 1a LQT2 C-terminus truncation mutants display hERG 1b-dependent dominant negative mechanisms

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BACKGROUND The human ether-à-go-go–related gene (hERG 1a) potassium channel is critical for cardiac repolarization. hERG 1b, another variant subunit, co-assembles with hERG 1a, modulates channel biophysical properties and plays an important role in repolarization. Mutations of hERG 1a lead to type 2 long QT syndrome (LQT2), and increased risk for fatal arrhythmias. The functional consequences of these mutations in the presence of hERG 1b are not known.

OBJECTIVE To investigate whether hERG 1a mutants exert dominant negative gating and trafficking defects when co-expressed with hERG 1b.

METHODS Electrophysiology, co-immunoprecipitation, and fluorescence resonance energy transfer (FRET) experiments in HEK293 cells and guinea pig cardiomyocytes were used to assess the mutants on gating and trafficking. Mutations of 1a-G965X and 1a-R1014X, relevant to gating and trafficking were introduced in the C-terminus region.

RESULTS The hERG 1a mutants when expressed alone did not result in decreased current amplitude. Compared to wild-type hERG 1a currents, 1a-G965X currents were significantly larger, whereas those produced by the 1a-R1014X mutant were similar in magnitude. Only when co-expressed with wild-type hERG 1a and 1b did a mutant phenotype emerge, with a marked reduction in surface expression, current amplitude, and a corresponding positive shift in the $V_{1/2}$ of the activation curve. Co-immunoprecipitation and FRET assays confirmed association of mutant and wild-type subunits.

CONCLUSION Heterologously expressed hERG 1a C-terminus truncation mutants, exert a dominant negative gating and trafficking effect only when co-expressed with hERG 1b. These findings may have potentially profound implications for LQT2 therapy.

KEYWORDS hERG 1a; hERG 1b; KCNH2; I_{Kr}; LQT2; HEK293; Cardiomyocytes; Guinea pig; Potassium channel; Truncation mutants

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Introduction

Human ether-à-go-go related gene (hERG) or KCNH2 encodes the pore-forming subunits hERG 1a and hERG 1b of the rapid component of the delayed rectifier potassium current (I_{Kr}), critical for cardiac repolarization.[1] Congenital mutations in hERG that result in I_{Kr} inhibition, prolong action potential (AP) repolarization, and trigger cardiac arrhythmias called torsades de pointes associated with long QT syndrome 2 (LQT2).[2] To date, more than 600 LQT2-causing mutations have been identified and account for >25% of patients with genotype-positive LQT syndrome.[3] Major mechanisms underlying hERG channel dysfunction include abnormal channel folding/assembly, trafficking, and gating defects.[1,4]

hERG 1a homomeric currents display slow activation and a rapid inactivation.[5] Co-expression with hERG 1b increases hERG 1a density 2- to 3-fold, right-shifts the voltage dependence of activation, and increases the rate of channel activation and recovery from inactivation.[5–7] hERG 1a has a long N-terminus (376 residues), which contains the signal sensor Per-Arnt-Sim (PAS; 1–135 residues) domain critical for channel trafficking and deactivation.[1] The C-terminus (500 residues) has a putative cyclic nucleotide homology binding domain (cNhBD), and sequences for channel assembly and biophysical properties.[8]

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hERG 1b contains a shorter N-terminus, of which the first 36 amino acids are unique, and lacks the PAS domain with implications for its role in a faster deactivation of hERG 1a/1b currents.[4,7] Despite a critical role of hERG 1b for cardiac I_{Kr},[9] there is a paucity of studies that have investigated LQT2 in the context in which they exist in native cardiomyocytes. Thus, there are still questions as to whether the physiological consequences of hERG 1a mutations can be accurately depicted in the absence of hERG 1b.

We examined the impact of 2 nonsense LQT2 hERG 1 mutations (G965X and R1014X) with regard to the gating and trafficking properties of I_{Kr} mediated by hERG 1a/1b subunits in HEK293 cells and cultured adult guinea pig ventricular myocytes. Mutants lacked the RXR signal (residues 1005–1007, G965X), which is important for trafficking,[10] and/or the tetramerization coiled-coiled (TCC; residues 1036–1074, G965X, R1014X), a domain critical for channel assembly.[11] These mutations have been previously studied[12–14] and are associated with both mild and severe phenotypes in patients,[13,15,16] yet there are deficits in how diverse LQT2 mutations depress I_{Kr} in heart. An important objective of this study is to assess the role of hERG 1b, and determine whether common mechanisms underlie the loss of I_{Kr} mediated by distinct LQT2 mutations. In HEK 293 cells and cultured adult guinea pig cardiomyocytes, we found that mutants used biophysical and trafficking mechanisms to suppress hERG 1a/1b currents, and displayed dominant negative propensities to different extents.

Methods

Generation of plasmid constructs

hERG 1 constructs were generously provided by Dr Gail Robertson (University of Wisconsin). hERG 1 mutants (G965X/R1014X) were generated as described in the Supplemental Methods (available online).

Generation of adenoviruses

Adenoviral vectors were generated using the pAdEasy system (Agilent, Santa Clara, CA) as previously described, with modifications.[17] Detailed descriptions of viral amplification, expansion, and purification are provided in the Supplemental Methods.

Cell culture and transfection

HEK293 cells (ATCC Manassas, VA) were maintained in DMEM at 37°C. HEK293 transfection, cardiomyocyte enzymatic dissociation. and viral infection protocols are detailed in the Supplemental Methods.

Electrophysiology

Whole-cell recordings in HEK293 cells and cultured adult guinea pig ventricular myocytes were performed as described in the Supplemental Methods.

Confocal imaging

Live fluorescence images of HEK293 cells were obtained using a Zeiss LSM 510 META scanning confocal microscope, as described in the Supplemental Methods.

Immunoprecipitation and immunoblotting

HEK293 cells were co-transfected with mCherry-tagged wild-type (wt) or mutant hERG 1 and yellow fluorescent protein (YFP)-tagged wt and mutant subunits. Cardiomyocytes were virally infected with wt and mutant hERG 1a/1b subunits. Cells were harvested and prepared for Western blot analysis, as detailed in the Supplemental Methods and previously described.[18]

Fluorescence resonance energy transfer measurements

Three-cube fluorescence resonance energy transfer (FRET) technique, adapted from Chen et al,[19,20] was used to assess co-assembly of fluorescently (ECFP or EYFP) tagged wt hERG 1a or hERG 1b, and mutant hERG 1a subunits, as detailed in the Supplemental Methods and previously reported.[17]

Data analyses

Electrophysiological data were analyzed off-line using built-in functions in Fitmaster (HEKA Holliston, MA) and Origin software. Details of analysis, including voltage dependence of activation and time constants for activation and deactivation, are provided in the Supplemental Methods.

Statistical analyses

Data are reported as means ± SEM. Statistical differences were determined using 1-way ANOVA with Bonferroni post hoc analysis or 2-tailed unpaired t test for comparisons between groups and considered significant at $P < .05$.

Results

Biophysical properties of homomeric hERG 1a C-terminus truncation mutants

We evaluated the impact on gating and trafficking of 2 LQT2 mutants, G965X and R1014X, corresponding to serial deletions of the last 195 and 146 amino acids of the hERG 1 C-terminus, respectively. We used the whole-cell patch clamp technique to assess functional properties of each mutant, expressed as homomers in HEK293 cells. Compared to wt hERG 1a (6 μg), mutants revealed a variety of behaviors that ranged from no change to significant changes in hERG 1a current density (Figure 1, and Table S1, available online). HEK293 cells were transfected with either wt or mutant hERG 1a alone or a combination of both subunits (Figure 1A). We found that cells expressing wt hERG 1a homomeric channels displayed voltage-dependent, outward whole-cell currents that were absent in nontransfected and hERG 1b alone expressing cells (Figure 1B–G). Compared to wt hERG 1a, homomeric G965X (6 μg)