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The N588K-HERG K^+ channel mutation in the 'short QT syndrome': Mechanism of gain-in-function determined at 37 °C

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Abstract

The idiopathic short QT syndrome (SQTS) is characterised by an abnormally short QT interval on the electrocardiogram and by an increased risk of arrhythmia and sudden death. One variant of the syndrome is linked to missense mutations that lead to a single amino-acid change (N588K; asparagine to lysine) in the S5-Pore linker region of the cardiac HERG K^+ channel. This study was performed in order to determine how the N588K mutation alters HERG channel current (I_{HERG}) kinetics at mammalian physiological temperature. The whole-cell current–voltage (I–V) relation for wild-type (WT) I_{HERG} measured from Chinese Hamster Ovary cells was maximal at ~0 mV and showed marked inward rectification positive to this. In contrast, N588K I_{HERG} showed marked rectification only at +60 mV and at more positive voltages. The voltage dependence of activation of N588K-HERG did not differ significantly from that of WT-HERG. However, N588K I_{HERG} had a significantly more positive inactivation $V_{0.5}$ (-8.14 ± 0.82 mV) than did WT I_{HERG} (-70.05 ± 0.82 mV; $P < 0.001$, unpaired t test; $n = 5$ for each). Its P_{Na}/P_K ratio was also greater. The instantaneous I–V relation for N588K I_{HERG} under action potential voltage clamp peaked at ~+40 mV, compared to ~−37 mV for WT-I_{HERG}. These findings underscore the importance of the S5-P linker in HERG channel function and indicate that N588K-HERG contributes increased repolarising current earlier in the ventricular action potential at physiological temperature due to a ~+60 mV shift in voltage dependence of I_{HERG} inactivation.

Keywords: Arrhythmia; HERG; I_{Kr}; Rapid delayed rectifier; Turret; CHO cells; Long QT syndrome; Potassium channel; Short QT syndrome; Sudden death

The idiopathic short QT syndrome (SQTS) was suggested to exist as an inherited syndrome in 2000 [1]. It is associated with a shortened QT interval on the electrocardiogram in the absence of structural heart disease and with an increased risk of atrial and ventricular tachycardias and of sudden death [1–3]. In 2004 and 2005, three genetic variants of the SQTS were identified that involve gain-in-function mutations to genes encoding different K^+ channel subunits. The SQT1 variant of the SQTS involves mutations to *KCNH2* (also known as *HERG*; '*Human ether-a-go-go-related gene*'), which encodes the α-subunit of channels responsible for the rapid delayed rectifier K^+ current (I_{Kr}) [4–6]. The SQT2 variant has been associated with a mutation to *KCNQ1* [7] the gene responsible for the α-subunit (KvLQT1) of the channel responsible for the slow delayed rectifier K^+ current, I_{Ks}. A third (SQT3) variant has recently been reported in which a defect occurs in the *KCNJ2* gene responsible for Kir2.1 (inwardly rectifying, I_{K1}) K^+ channels [8].

The genetic substrate for the SQT1 variant of SQTS was initially identified by genetic screening of three families with SQTS (exhibiting rate corrected QT intervals of ⩽300 ms) associated with sudden death [6]. In two

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families, two missense mutations were identified that led to an identical amino-acid substitution (asparagine to lysine) at position 588, which lies within the S5-P linker region of the HERG channel protein [6]. A third family has subsequently been identified with the N588K-HERG mutation and with markedly shortened atrial and ventricular refractory periods [9]. The steady-state current–voltage (I–V) relations for HERG/I_{Kr} normally show marked inward rectification at positive potentials due to an unusually fast 'C-type' inactivation process [10]. The S5-P linker region appears to be important in the inactivation gating of HERG channels [11–13], and recordings at ambient temperature from N588K-HERG showed this to lack the rectification of the I–V relation that is present in the wild-type channel [6]. It was proposed that as a result of abolishing rectification, the N588K mutation produces a gain in HERG/I_{Kr} current that leads to action potential and QT interval abbreviation [6]. A very recent study performed at ambient temperature has provided direct evidence that, when co-expressed with KCNE2 (MiRP1), N588K-HERG exhibits altered voltage-dependent inactivation kinetics [14]. However, while MiRP1 can co-assemble with HERG and has been proposed to act as an auxiliary subunit to recapitulate native I_{Kr} [15], it has also been shown to be able to interact with a wide range of channel α-subunits [16–18], and there is debate as to whether or not it functions as *the* auxiliary subunit in the I_{Kr} channel [19]. Moreover, I_{HERG} shows marked temperature sensitivity [20,21] and therefore data obtained at ambient temperature do not necessarily represent the situation at mammalian physiological temperature. Here, we report for the first time the characteristics of N588K I_{HERG} at 37 °C, in the absence of MiRP1, in order to define selectively the effects of the N588K mutation on voltage-dependent kinetic properties of I_{HERG}.

Methods

Maintenance of cell lines expressing wild-type HERG and N588K-HERG channels. Experiments on wild-type (WT) HERG current (I_{HERG}) were performed on a Chinese Hamster Ovary (CHO) cell line stably expressing HERG in a pIRES-hyg vector. Cells were passaged using a non-enzymatic agent (Splitase, AutogenBioclear) and plated out onto small sterilised glass coverslips in 30 mm petri dishes containing Kaighn's modification of Ham's F12-K medium (Gibco), supplemented with 10% foetal bovine serum (Gibco) and 200 μg ml^{-1} gentamicin (Gibco). To make the mutated construct, the HERG transcript was cloned into pcDNA3 vector and was engineered with the mutation N588K using a Quick change II XL site-directed mutagenesis kit (Stratagene). The DNA was sequenced bidirectionally for the full length of the HERG insert to ensure that only the correct mutation had been made (MWG Biotech). CHO cells were transiently transfected with N588K-HERG, using previously described methods [22]. Briefly, CHO cells were plated onto small sterilised glass coverslips. After 24 h, the cells were co-transfected 2:1 with expression plasmids encoding N588K-HERG and green fluorescent protein (the latter in pCMX donated by Dr. Jeremy Tavare) using FuGene 6 Transfection Reagent (Roche Diagnostics) according to the manufacturer's instructions. After an incubation period of 5–6 h in serum containing medium, the medium in the petri dishes was replaced. After transfection, the cells were incubated at 37 °C for a minimum of 1 day prior to any electrophysiological study.

Electrophysiological recording. Glass coverslips onto which cells had been plated were placed in a bath (0.5 ml volume) mounted on an inverted microscope (Nikon Diaphot) and the cells were superfused with a standard Tyrode's solution which contained (in mM): 140 NaCl, 4 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 5 Hepes (titrated to pH 7.45 with NaOH). Patch-pipettes (Corning 7052 glass, AM Systems) were pulled (Narishige PP830) and fire-polished to 2.0–6.5 MΩ (Narishige, MF83). The pipette dialysis solution contained (in mM): 130 KCl, 1 $MgCl_2$, 5 EGTA, 5 MgATP, and 10 Hepes (titrated to pH 7.2 with KOH). The 'pipette-to-bath' liquid junction potential was measured for this filling solution and was −3.2 mV. Since this value was small, no corrections of membrane potential were made. Whole-cell patch-clamp recordings of membrane currents were made using an Axopatch 1D amplifier (Axon Instruments) and a CV-4 1/100 headstage. Between 80% and 90% of the electrode series resistance could be compensated. Voltage-clamp commands were generated using Clampex 8 (Axon Instruments). Data were recorded via a Digidata 1200B interface (Axon Instruments) and stored on the hard disk of a Viglen computer. Data digitisation rates were 10–25 kHz during all protocols and an appropriate bandwidth of 2–10 kHz was set on the amplifier [23].

Data analysis and presentation. Data were analysed using Clampfit 8 (Axon Instruments), Excel 2002, and Prism v.3 (Graphpad) software. Data are presented as means ± standard error of the mean (SEM). Statistical comparisons were made using a Student's t test. P values of less than 0.05 were taken as statistically significant.

The voltage dependence of I_{HERG} activation was determined by fitting the values of I_{HERG} tail currents (normalised to peak I_{HERG} tail value and plotted against voltage) with a modified Boltzmann equation of the form:

$$I = I_{Max} \Big/ \left(1 + \exp\left(\frac{(V_{0.5} - V_m)}{k}\right)\right), \quad (1)$$

where I is the I_{HERG} amplitude following test potential V_m, I_{Max} is the maximal I_{HERG} observed, $V_{0.5}$ is the potential at which I_{HERG} was half-maximally activated, and k is the slope factor describing I_{HERG} activation.

In order to estimate the amplitude of rapidly inactivating I_{HERG} tails at the onset of voltage commands, these were fitted with a mono-exponential equation [24] of the form:

$$y = A \times \exp\left(\frac{-x}{\tau}\right) + C, \quad (2)$$

where y represents current amplitude at time x, τ is the time constant for the decaying I_{HERG}, and A represents the initial current amplitude. C describes any residual current component following current decline.

Voltage dependence of I_{HERG} availability (inactivation) was determined by fitting normalised peak I_{HERG} tails by the equation:

$$I/I_{Max} = 1 - \left(1 \Big/ \left(1 + \exp\left(\frac{(V_{0.5} - V_m)}{k}\right)\right)\right), \quad (3)$$

where $V_{0.5}$ is the potential at which I_{HERG} was half-maximally available/inactivated, k is the slope factor describing I_{HERG} availability, and other terms have similar meanings to Eq. (1).

Results

Current–voltage relations and voltage dependence of activation

Wild-type (WT) and N588K I_{HERG} were elicited by 2-s duration depolarising voltage commands, as indicated