Phenotypic Characterization of a Novel Long-QT Syndrome Mutation (R1623Q) in the Cardiac Sodium Channel

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Background—A heritable form of the long-QT syndrome (LQT3) has been linked to mutations in the cardiac sodium channel gene (SCN5A). Recently, a sporadic SCN5A mutation was identified in a Japanese girl afflicted with the long-QT syndrome. In contrast to the heritable mutations, this externally positioned domain IV, S4 mutation (R1623Q) neutralized a charged residue that is critically involved in activation-inactivation coupling.

Methods and Results—We have characterized the R1623Q mutation in the human cardiac sodium channel (hH1) using both whole-cell and single-channel recordings. In contrast to the autosomal dominant LQT3 mutations, R1623Q increased the probability of long openings and caused early reopenings, producing a threefold prolongation of sodium current decay. Lidocaine restored rapid decay of the R1623Q macroscopic current.

Conclusions—The R1623Q mutation produces inactivation gating defects that differ mechanistically from those caused by LQT3 mutations. These findings provide a biophysical explanation for this severe long-QT phenotype and extend our understanding of the mechanistic role of the S4 segment in cardiac sodium channel inactivation gating and class I antiarrhythmic drug action. (Circulation. 1998;97:640-644.)

Key Words: arrhythmia ■ sodium ■ long-QT syndrome

Patients with congenital long-QT syndrome are predisposed to syncope and sudden death from polymorphic ventricular tachycardia due to delayed ventricular repolarization. The syndrome has been linked to mutations in various genes encoding voltage-gated cardiac ion channels. In particular, three mutations in the cardiac sodium channel gene (SCN5A) have been linked to a heritable form of the long-QT syndrome (LQT3), and all induce a late component of sodium current sufficient to delay repolarization. All three mutations have intracellular positions and reside near critical gating loci that mediate the inactivation of the S4 segment (Fig 1A). Biophysical characterization has revealed a consistent phenotype: the rapid decay of sodium current is preserved or enhanced but is followed by a small plateau of inward current.

Recently, a de novo missense SCN5A mutation (R1623Q) was identified in a Japanese girl severely affected by a sporadic form of the long-QT syndrome. In contrast to the familial LQT3 mutations, R1623Q replaced a charged arginine residue with a neutral glutamine at an external position in the S4 segment of domain IV (Fig 1A). Mutagenesis studies of the analogous residue in skeletal muscle sodium channels (hSkM1 R1448) implicated IV-S4 in coupling between activation and inactivation, and two hSkM1 paramyotonia congenita mutations at this position (R1448H, R1448C) disrupted inactivation.

Here, we characterize the functional consequences of R1623Q in heterologously expressed human cardiac sodium channels (hH1). The mutation has novel effects on sodium channel inactivation gating that differ from the familial mutations.

Methods

Site-directed mutagenesis of the human cardiac sodium channel α-subunit was performed as described and was verified by dideoxy sequencing. Sodium channel α-subunits were coexpressed with an equimolar ratio of β-subunit cRNA in Xenopus oocytes as described or were transfected into CHO-K1 cells. For the latter, wild-type and R1623Q full-length cDNAs were subcloned from pSP64T into the HindIII-XbaI site of vector pGFPVRS for bicistronic expression of the channel protein and GFP reporter as described. Transfected CHO-K1 cells were cultured in Opti-MEM I reduced serum medium ( Gibco) in a 5% CO2 incubator at 37°C for 1 to 2 days, and cells exhibiting green fluorescence were chosen for electrophysiological analysis.
Whole-cell sodium currents ($I_{\text{Na}}$) were recorded from oocytes with a two-electrode voltage clamp as described in ND-96 solution containing (in mmol/L): NaCl 96, KCl 2, MgCl\(_2\) 1, and HEPES 5, at pH 7.6. For $I_{\text{Na}}$ recordings from CHO-K1 cells, external solutions were (in mmol/L): NaCl 140, KCl 5.4, glucose 10, MgCl\(_2\) 1, CaCl\(_2\) 0.1, and HEPES 10, at pH 7.4. Glass pipettes contained (in mmol/L): KCl 140, MgCl\(_2\) 1, MgATP 4, HEPES 10, NaCl 5, and EGTA 5, at pH 7.4; 60% to 80% of the series resistance ($\approx$6 M\(\Omega\)) was compensated. For cell-attached single-channel recordings, a bath solution was used to zero the membrane potential that contained (in mmol/L): KCl 140 and HEPES 10, at pH 7.4. Pipettes were filled with a recording solution containing (in mmol/L): NaCl 140 and HEPES 10, at pH 7.4. Single-channel currents were filtered at 2 kHz, sampled every 50 ms, and idealized by use of a half-height criterion with three-point detection. Pooled data are expressed as mean±SEM, and statistical comparisons were made by one-way ANOVA (Microcal Origin). Numerical integration methods were used to analyze gating models (Fig 2C) as described.\(^{16}\)

**Results**

Fig 1B shows $I_{\text{Na}}$ recorded from CHO-K1 cells expressing wild-type or R1623Q sodium channels. Although a small noninactivating current is present at the end of the 100-ms depolarization (<3% of peak current, similar to the LQT3 mutants),\(^{4,5,9}\) a more striking difference between the mutant and wild-type currents is the slowed rate of macroscopic current decay. This contrasts with the familial long-QT mutations, which do not slow the sodium current decay.\(^{4,5,9}\) This effect was also apparent in $I_{\text{Na}}$ from *Xenopus* oocytes (Fig 2A, top), in which time from peak $I_{\text{Na}}$ to 50% decay was prolonged.

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**Figure 1.** A, Topological map of domains III and IV of the hH1 sodium channel. B, Normalized whole-cell currents in CHO-K1 cells depolarized to −20 mV from a holding potential of −100 mV. Inward current magnitudes were −6.4 nA (wildtype) and −2.9 nA (R1623Q). C, Unitary currents from CHO-K1 cell-attached patches repeatedly depolarized to −20 mV for 100 ms at 1 Hz (holding potential, −100 mV). Sweeps were selected to emphasize prolonged openings and reopenings. Patches contained $\approx$2 active channels. Biexponential fits [\(y = A_1 \exp(-t/t_1) + A_2 \exp(-t/t_2)\)] to normalized open-time distributions revealed a more prominent slow component in R1623Q recordings (R1623Q: \(A_1 = 0.7, A_2 = 0.3, t_1 = 0.1\) ms, \(t_2 = 1.1\) ms; wildtype: \(A_1 = 0.8, A_2 = 0.2, t_1 = 0.2\) ms, \(t_2 = 1.1\) ms). D, Ensemble-average currents at −20 mV composed from cell-attached patches shown in C. Traces were assembled from 100 (wildtype) and 212 (R1623Q) consecutive sweeps.
was prolonged threefold (Fig 2B; wildtype, 0.94 ± 0.08 ms, n=14; R1623Q, 3.07 ± 0.11 ms, n=28; P<.001).

Cell-attached patch recordings in CHO-K1 cells (Fig 1C) revealed that, unlike wild-type channels (left), the R1623Q mutant (right) exhibited multiple, prolonged openings early in the depolarization period. The R1623Q ensemble-average current (Fig 1D) decayed slowly, consistent with whole-cell recordings (Fig 1B). The number of openings per depolarization (corrected for channel number) was 0.63 ± 0.05 for R1523Q and 0.51 ± 0.08 for hH1. Single-exponential fits to the open-time distributions revealed just a modest increase in R1623Q (τ at −20 mV was 0.5 ms versus wildtype, 0.4 ms); however, the open-time distribution in both wildtype and R1623Q was dominated by brief openings (<0.3 ms). Biexponential fitting revealed a second, longer component (τ2=1.1 ms) that was more prominent in the R1623Q recordings (see legend, Fig 1C). Thus, the slowed rate of R1623Q current decay resulted from both an increased number of openings during depolarization and prolonged individual opening events.

Class IB antiarrhythmic compounds are effective in treating polymorphic ventricular arrhythmias in animal models of the long-QT syndrome."5,17 For LQT3-related arrhythmias, the effectiveness of class IB agents is consistent with their high potency in suppressing the plateau of noninactivating sodium current."5,18,19 However, a major

Figure 2. A, Whole-cell currents in Xenopus oocytes depolarized from −100 to −20 mV. For wildtype and R1623Q, paired observations from a single oocyte are shown before (top) and during (bottom) exposure to 200 μmol/L lidocaine. B, Summarized τ50 data before (open bars) and after (solid bars) exposure to 200 μmol/L lidocaine (see text). C, Markov model of sodium channel gating. Model A (wildtype): k1=2000 s⁻¹, k₂=2.9 s⁻¹, kₐ=497 s⁻¹. Model B (LQT3 mutants): k₁=2000 s⁻¹, k₂=55 s⁻¹, kₐ=497 s⁻¹. Increasing k₂ renders the inactivated state nonabsorbing, allowing persistent openings and a plateau of noninactivating current. Model D (R1623Q): k₁=665 s⁻¹, k₂=55 s⁻¹, kₐ=497 s⁻¹. Because k₁ is reduced to a magnitude similar to kₐ, channel openings are prolonged, and channels may close and reopen before inactivation. Models C and E (identical to wildtype), lidocaine increases kₐ (reversing effects of R1623Q) and decreases k₂ (reversing effects of LQT3 mutations).
additional feature of the R1623Q mutation is a slowed rate of current decay. Fig 2A (bottom) shows wild-type and R1623Q \( I_{\text{Na}} \) after lidocaine exposure. With lidocaine (200 \( \mu \text{mol/L} \)), the rate of R1623Q current decay approached that of the wild-type channel. Fig 2B shows the effect of lidocaine on \( \tau_{\text{Na}} \). In paired observations, lidocaine significantly hastened the rate of R1623Q \( I_{\text{Na}} \) decay (\( P<.001 \) versus predrug) but had no effect on wild-type \( I_{\text{Na}} \) decay.

**Discussion**

Three SCN5A mutations (\( \Delta \text{KPQ}1505 \) to 1507, N1325S, and R1644H) that underlie the autosomal dominant disorder (LQT3) exhibit striking phenotypic similarities.4,5,6 The rapid decay of sodium current is preserved but is followed by a persistent plateau of inward current. These mutations reside near proposed III-IV linker docking sites (N1325S, R1644H) or on the linker itself (\( \Delta \text{KPQ} \); Fig 1A). In contrast, the mechanism by which R1623Q and the analogous D454 mutations (hSKM1 R1448C, R1448H) slow \( I_{\text{Na}} \) decay may involve uncoupling activation from inactivation.11,12 The coincidence of mutations involving the same residue in disparate disease states (long-QT syndrome, paramyotonia congenita) is curious and suggests that uncoupling of linked gating processes may not be an uncommon pathological event.

Fig 2C presents a simplified kinetic scheme that rationalizes the functional consequences of these diverse structural defects. Single-channel studies of the LQT3 mutations reveal that the ability of the open channel to inactivate is retained but the inactivated state is rendered nonabsorbing (increase in \( k_\text{-i} \)), allowing openings to occur throughout depolarization. This defect induces a persistent plateau of noninactivating current (Fig 2C, model B) but does not slow the rate of ensemble-average current decay.4,5 A structural interpretation consistent with nonabsorbing inactivation is that the unbinding rate of the III-IV linker from its docking site is increased. Conversely, our results, as well as those derived from single-channel studies of the analogous domain-IV, S4 paramyotonia mutations,13 suggest that a different mechanism must underlie the prolonged decay of ensemble-average R1623Q current. Consistent with a deficiency in positioning the S4 segment for docking the inactivation gate,11 R1623Q may reduce the forward rate of inactivation from the open state (\( k_i \); Fig 2C, model D). Although it prolongs openings, a reduction in \( k_i \) also renders the two rate constants exiting the open state (\( k_i, k_\text{-i} \)) similar in magnitude, allowing mutant channels to reopen in the early depolarization period before eventually inactivating (eg, Fig 1C). Hence, reducing \( k_i \) slows the rate of ensemble-average current decay. In addition, reducing the magnitude of \( k_i \), relative to \( k_\text{-i} \), increases the potential for measurable steady-state (noninactivating) current, consistent with the small persistent current plateau in our R1623Q recordings (Fig 1B and 1D).

Analogous to the effect of lidocaine on R1623Q decay (Fig 2A and 2B), we have previously shown that lidocaine enhances the rate of macroscopic current decay when inactivation is destabilized by III-IV linker mutations.16 Lidocaine not only accelerated the rate of \( \mu 1\text{-F1304Q} \) current decay by eliminating reopenings but also delayed recovery from inactivation, suggesting that the drug may function like an allosteric effector that both increases the forward rate and decreases the return rate from the inactivated state.18 Under this paradigm, lidocaine would increase the forward rate of inactivation (\( k_i \)) for the R1623Q mutant, speeding the decay of ensemble-average current (Fig 2C, model E), and would also decrease the return rate (\( k_\text{-i} \)) from a previously nonabsorbing inactivated state (eg, the LQT3 mutants; Fig 2C, model C). Given the effects of lidocaine on the R1632Q and the LQT3 mutants,18 the local anesthetics seem to be capable of “repairing” diverse kinetic deficiencies in inactivation gating. Consistent with earlier reports,19 lidocaine also suppressed the persistent component of steady-state current through wild-type channels (Fig 2A), an effect congruent with a drug-induced reduction in \( k_\text{-i} \).

Although future studies will be necessary to explore the effects of clinically relevant lidocaine concentrations, these intriguing effects of the class IB agents on gating may underlie their salutary effects in patients with long-QT–associated ventricular arrhythmias. Interestingly, the index patient has been effectively treated for several years with mexiletine, an orally bioavailable congener of lidocaine.20

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**References**


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