Novel LQT-3 Mutation Affects Na⁺ Channel Activity
Through Interactions Between α- and β₁-Subunits


Abstract—The congenital long-QT syndrome (LQT), an inherited cardiac arrhythmia characterized in part by prolonged ventricular repolarization, has been linked to 5 loci, 4 of which have been shown to harbor genes that encode ion channels. Previously studied LQT-3 mutations of SCN5A (or hH1), the gene that encodes the human Na⁺ channel α-subunit, have been shown to encode voltage-gated Na⁺ channels that reopen during prolonged depolarization and hence directly contribute to the disease phenotype: delayed repolarization. Here, we report the functional consequences of a novel SCN5A mutation discovered in an extended LQT family. The mutation, a single A→G base substitution at nucleotide 5519 of the SCN5A cDNA, is expected to cause a nonconservative change from an aspartate to a glycine at position 1790 (D1790G) of the SCN5A gene product. We investigated ion channel activity in human embryonic kidney (HEK 293) cells transiently transfected with wild-type (hH1) or mutant (D1790G) cDNA alone or in combination with cDNA encoding the human Na⁺ channel β₁-subunit (hβ₁) using whole-cell patch-clamp procedures. Heteromeric channels formed by coexpression of α- and β₁-subunits are affected: steady-state inactivation is shifted by –16 mV, but there is no D1790G-induced sustained inward current. This effect is independent of the β₁-subunit isoform. We find no significant effect of D1790G on the biophysical properties of monomeric α- (hH1) channels. We conclude that the effects of the novel LQT-3 mutation on inactivation of heteromeric channels are due to D1790G-induced changes in α- and β₁-interactions. (Circ Res. 1998;83:141-146.)

Key Words: long-QT syndrome ■ genetics ■ Na⁺ channel

The congenital long-QT syndrome (LQT) is an inherited cardiac arrhythmia that is defined in part by prolonged ventricular repolarization, an association with recurrent syncope, and a propensity to polymorphous ventricular tachycardia (torsade de pointes) and sudden death. LQT has been linked to 5 loci, 4 of which have been shown to harbor genes that encode ion channels. These discoveries have created the unique opportunity to develop molecular therapeutic approaches to disease management based on specific functional changes in the channel proteins encoded by mutant genes. This has been possible in part because all previously studied LQT-3 mutations of SCN5A (or hH1), the gene that encodes the human Na⁺ channel α-subunit, have been shown to have a significant effect on inactivation of heteromeric channels. Previously studied LQT-3 mutations have been shown to cause functional changes in expressed channel activity that contribute directly to delayed ventricular repolarization. A base substitution at nucleotide 5519 of the SCN5A cDNA, is expected to cause a nonconservative change from an aspartate to a glycine at position 1790 (D1790G) of the SCN5A gene product. The D1790G mutation was found in all patients (n=24) of an extended LQT family but not in >200 chromosomes carried by healthy individuals.

Previously studied LQT-3 mutations of SCN5A have been shown to encode voltage-gated Na⁺ channels that fail to inactivate completely during prolonged depolarization and hence contribute directly to the disease phenotype: delayed repolarization. Because the position of glycine 1790 (D1790) is thought to be intracellular, near the C-terminus of the α-subunit, and not associated with major functional channel properties, extrapolation to changes in the properties of encoded Na⁺ channels is not obvious. Here we report the functional consequences of the D1790G LQT-3 mutation as revealed by measuring ion channel activity in transiently transfected human embryonic kidney (HEK 293) cells. We find that the D1790G mutation has little effect on the biophysical properties of monomeric α- (hH1) channels, but it significantly affects the properties of heteromeric channels formed by coexpression of α- and β₁-subunits. This result is important not only because it shows the functional consequences of a novel gene mutation linked to an inherited cardiac arrhythmia but also because it provides important insight into the physiological importance and key interaction residues for the human cardiac Na⁺ channel β₁-subunit. Furthermore, the data suggest that therapeutic strategies designed to treat carriers of previously described LQT-3...
mutations of SCN5A are not likely to be effective in treating carriers of the D1790G mutation of SCN5A.

Materials and Methods

HEK 293 cells (Cold Spring Harbor Laboratories) were grown under culture conditions and transfected by a lipofection procedure previously described by us.11 hH1 cDNA was subcloned into the vector pCNA3 (Invitrogen, Inc.). The D1790G mutation was engineered with the QuickChange Site-Directed Mutagenesis Kit (Stratagene, Inc) and confirmed by sequence analysis. Plasmid cDNA for coexpression of hβ1 with D1790G plus KPQ cDNA (right). The current traces in Figure 1A are shown at high gain to emphasize differences in small, sustained currents. Peak currents, too large to be resolved at this gain, were 1155 pA (D1849G) and 824 pA (KPQ). Thus, the sustained inward current through KPQ-hβ1 channels is on the same order of magnitude (2% to 4% of peak currents) as previously reported for monomeric KPQ channels.11,18 Despite similar expression levels of peak currents, maintained current could not be resolved for hβ1-D1790G channels in this or in any of 15 other similar experiments.

In contrast, when compared with hH1-hβ1 channel activity, D1790G-hβ1, but not KPQ-hβ1 channels alter steady-state inactivation (Figure 1B). Figure 1B, which illustrates effects for steady-state inactivation measured with 100-ms prepulses (similar results were obtained with 500 ms but are not shown), compares inactivation curves measured for hH1-hβ1

Because Na+ channels have been suggested to consist of α-(260-kDa) and β-(36-kDa) subunits,22-24 we first tested for unique functional effects of the D1790G mutation in cells expressing heteromeric channels consisting of test α- (hH1, wild-type, D1790G) and human heart β1-subunits (hβ1).25 Figure 1 shows functional consequences of the D1790G mutation that distinguish it from other previously described LQT-3 mutations, here illustrated by the SCN5A KPQ deletion mutant.7 Unlike previously described LQT-3 mutations of SCN5A,11,13,14,19 coexpression of hβ1 with D1790G encodes channels that do not conduct sustained inward current with prolonged depolarization (Figure 1A). Figure 1A compares currents recorded during maintained depolarization from cells cotransfected with hβ1 plus D1790G cDNA (left) and hβ1 plus KPQ cDNA (right). The current traces in Figure 1A are shown at high gain to emphasize differences in small, sustained currents. Peak currents, too large to be resolved at this gain, were 1155 pA (D1849G) and 824 pA (KPQ). Thus, the sustained inward current through KPQ-hβ1 channels is on the same order of magnitude (2% to 4% of peak currents) as previously reported for monomeric KPQ channels.11,18 Despite similar expression levels of peak currents, maintained current could not be resolved for hβ1-D1790G channels in this or in any of 15 other similar experiments.

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Results

Table 1. Summary of Effects of the D1790G Mutation and hβ1, Coexpression on Expressed Channel Activity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>hH1</th>
<th>hH1-hβ1</th>
<th>D1790G</th>
<th>D1790G-hβ1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak current density</td>
<td>-51.3±6.2 (n=14)</td>
<td>-63.8±6.7† (n=16)</td>
<td>-55.4±4.5* (n=16)</td>
<td>-52.8±5.0† (n=16)</td>
</tr>
<tr>
<td>Inactivation V1/2</td>
<td>-70.2±1.3 (n=17)</td>
<td>-58.7±1.2† (n=16)</td>
<td>-73.1±0.9* (n=9)</td>
<td>-75.0±1.6§ (n=20)</td>
</tr>
<tr>
<td>Inactivation slope factor</td>
<td>6.3±0.3 (n=16)</td>
<td>7.8±0.3† (n=16)</td>
<td>5.8±0.2* (n=10)</td>
<td>4.9±0.2§ (n=20)</td>
</tr>
</tbody>
</table>

*P<0.05 vs hH1.
†P<0.01 vs hH1.
‡P<0.05 vs hH1-hβ1.
§P<0.01 vs hH1-hβ1.
||P>0.05 vs D1790G.

Figure 1. Heteromeric channels: unique functional consequences of the D1790G mutation. Currents recorded in cells cotransfected with hβ1 and either D1790G (left) or KPQ (right) cDNA. A, High-gain records (recorded at -10 mV) show sustained inward current for KPQ (arrow) but not D1849G (260-kDa) and β-(36-kDa) subunits,11,13,14,19 coexpression of hβ1 with D1790G encodes channels that do not conduct sustained inward current with prolonged depolarization (Figure 1A). Figure 1A compares currents recorded during maintained depolarization from cells cotransfected with hβ1 plus D1790G cDNA (left) and hβ1 plus KPQ cDNA (right). The current traces in Figure 1A are shown at high gain to emphasize differences in small, sustained currents. Peak currents, too large to be resolved at this gain, were 1155 pA (D1849G) and 824 pA (KPQ). Thus, the sustained inward current through KPQ-hβ1 channels is on the same order of magnitude (2% to 4% of peak currents) as previously reported for monomeric KPQ channels,11,18 Despite similar expression levels of peak currents, maintained current could not be resolved for hβ1-D1790G channels in this or in any of 15 other similar experiments.
channels (open circles in both panels), D1790G-hβ1 channels (solid circles, left panel), and KPQ-hβ1 channels (solid circles, right panel). The D1790G mutation alters both the half-maximal voltage and slope conductance of this relationship (Table).

We next compared the time course of inactivation of currents measured during depolarizing pulses for cells transfected with hH1 cDNA plus hβ1 cDNA and D1790G cDNA plus hβ1 cDNA. The results of these experiments are summarized in Figure 2A. Here, we analyzed the time course of currents in response to voltage pulses positive to threshold for Na channel activation only. Shown are results for hH1 + hβ1 (open symbols, n=6) and D1790G + hβ1 (solid symbols, n=6) channels. Traces: normalized currents recorded at -10 mV. Calibration: 5 ms. B, Peak current- (normalized to total cell capacitance) voltage relationship of hH1 + hβ1 (left, mean±SEM, n=16) and D1790G + hβ1 (right, n=16) channels. Smooth curves are described below. C, Normalized mean conductance vs voltage relationships for D1890G + hβ1 (■) and hH1 + hβ1 (▲). Data were obtained from mean data of Figure 2A with Vrev50 mV. Smooth curve is best-fit Bolzmann relationship (V1/2=-22 mV, slope factor=6.6 mV) to data. Smooth curves in A were obtained by scaling this relationship by appropriate maximal conductances and driving forces.

Figure 2. The D1790G mutation does not affect kinetics of onset of inactivation or voltage dependence of activation. A, Fast (left) and slow (right) time constants (mean±SEM) of onset of inactivation vs test-pulse voltage obtained by best fits of data to functions with 2 exponential components. Voltage pulses were chosen positive to threshold for Na channel activation only. Shown are results for hH1 + hβ1 (open symbols, n=6) and D1790G + hβ1 (solid symbols, n=6) channels. Traces: normalized currents recorded at -10 mV. Calibration: 5 ms. B, Peak current- (normalized to total cell capacitance) voltage relationship of hH1 + hβ1 (left, mean±SEM, n=16) and D1790G + hβ1 (right, n=16) channels. Smooth curves are described below. C, Normalized mean conductance vs voltage relationships for D1890G + hβ1 (■) and hH1 + hβ1 (▲). Data were obtained from mean data of Figure 2A with Vrev50 mV. Smooth curve is best-fit Bolzmann relationship (V1/2=-22 mV, slope factor=6.6 mV) to data. Smooth curves in A were obtained by scaling this relationship by appropriate maximal conductances and driving forces.
Because the location of the D1790G mutation is thought to be intracellular, near the C-terminus, and Makita et al.\textsuperscript{26} have shown that the carboxy-terminal half of the \( \alpha \)-subunit may be important for coassembly of cardiac \( \text{Na}^+ \) channel \( \alpha \)- and \( \beta \)-subunits, we investigated the possibility that functional changes induced by the D1790G mutation in heteromeric channels might depend on subunit interactions. We thus compared the effects of the D1790G mutation on heteromeric (\( \alpha \) and \( \beta \)) and monomeric (\( \alpha \)) channels. As was the case for heteromorphic channels, the D1790G mutation does not affect the kinetics of the onset of inactivation, the voltage dependence of activation, or reversal potential (data not shown). However, surprisingly, neither does the D1790G mutation affect the voltage dependence of steady-state inactivation (Figure 3) of monomeric channels. Thus, the D1790G-induced shift in inactivation (Figure 1B) of heteromeric channels depends on the presence of the \( \beta \)-subunit.

Does the D1790G mutation affect \( \alpha \)- and \( \beta \)-interactions? To test this, we focused on steady-state inactivation and compared the effects of coexpression of h\( \beta \), with both hH1 and D1790G \( \alpha \)-subunits. Coexpression of h\( \beta \), and hH1 causes a positive shift in \( V_{1/2} \) and increases the slope factor of the inactivation curve compared with expression of only hH1 subunits, and these effects are statistically significant (Table, Figure 4A). In contrast, although not statistically significant, coexpression of the \( \beta \)-subunit with D1790G \( \alpha \)-subunit, if anything, causes small opposite effects on steady-state inactivation: a slight positive shift in \( V_{1/2} \), and a decrease in the slope (Figure 4B and Table).

**Discussion**

These results thus support the view that the D1790G mutation, discovered by linkage analysis and subsequent sequence analysis of LQT-3 in an extended LQT family,\textsuperscript{17} markedly affects the voltage dependence of inactivation by altering the interaction between the \( \text{Na}^+ \) channel \( \alpha \)- and \( \beta \)-subunits. The fact that the mutation has little or no effect on other channel properties suggests that it causes specific changes in the interaction between the 2 \( \text{Na}^+ \) channel subunits and not major conformational changes in the channel protein. Future experiments probing \( \alpha \)- and \( \beta \)-interaction domains should focus on this region of the cardiac \( \text{Na}^+ \) channel \( \alpha \)-subunit.

Experiments designed to identify the functional roles of the cardiac \( \beta \)-subunit in other expression systems have produced inconsistent results. Some groups report no effects.\textsuperscript{25,27} Others report that coexpression of \( \alpha \)- and \( \beta \)-subunits enhances expression of \( \text{Na}^+ \) channel currents with slight changes in gating kinetics and gating voltage dependencies\textsuperscript{28,29} and modifies expressed channel sensitivity to lidocaine block.\textsuperscript{30} Our experiments show consistent and significant effects of coexpression of hH1 and h\( \beta \), on steady-state inactivation (Table) that are not seen when h\( \beta \) is coexpressed with D1790G in HEK 293 cells. We found that these effects were the same if we substituted the rat (\( \tau \beta \)-)\textsuperscript{31} for the human (h\( \beta \)) isoform or if we measured inactivation in full (135 mmol/L) [\( \text{Na}^+ \)]\textsubscript{o} (data not shown). It should be pointed out that the composition of native cardiac \( \text{Na}^+ \) channels has not been firmly established, because message\textsuperscript{28} for \( \beta \)-has been reported in heart but immunoprecipitation of \( \beta \)-protein has not been demonstrated.\textsuperscript{32}

Our results indicate that the D1790G mutation does not affect the voltage dependence of activation of heteromeric channels. Gating of cardiac \( \text{Na}^+ \) channels differs from gating of neuronal \( \text{Na}^+ \) channels in that evidence has been presented for voltage-dependent transitions from open to inactivated states for cardiac but not neuronal channels.\textsuperscript{33-36} In addition, for both cardiac and neuronal channels, multiple closed states precede transitions into the open state, and transitions from

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Insensitivity of steady-state inactivation of monomeric (\( \alpha \)-subunit) \( \text{Na}^+ \) channels to D1790G mutation. Bottom, Steady-state inactivation (mean±SEM; ○, hH1, \( n=17 \); ●, D1790G, \( n=9 \)). Curve: Boltzmann function (\( V_{1/2} = -72 \text{ mV} \); \( V_b = +5.8 \text{ mV} \)). Top, Currents at –10 mV after conditioning pulses (100 ms, –130 to –10 mV, 10-mV increments). Calibration: 2 ms, 100 pA.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Effects of D1790G mutation on h\( \beta \)-induced changes in steady-state inactivation. Steady-state inactivation determined as in Figures 1 and 3. Shown in plots are normalized inactivation curves (mean±SEM). A, hH1 (○), \( n=17 \); hH1+h\( \beta \) (●), \( n=16 \). B, D1790G (○), \( n=10 \); D1790G+h\( \beta \) (●), \( n=20 \). Parameters for smooth curves are summarized in the Table.
each of these closed states can occur directly into the inactivated state. D1790G-induced alteration in the rate constants linking these early closed- to inactivated-state transitions or voltage-dependent transitions between open and inactivated states would be consistent with our experimental data. Furthermore, because our data strongly suggest that the D1790G mutation alters \( \alpha \) - and \( \beta \)-subunit interactions, our results imply that the \( \beta \)-subunit specifically affects transitions between these states of the channel.

Unlike previously reported SCN5A mutations, we find that the D1790G mutation does not alter channel properties in a manner that would be expected to promote sustained inward Na\(^+\) channel current during the ventricular action potential. Hence, a causal link between the D1790G mutation and prolonged ventricular repolarization remains unclear. The facts that our data indicate that this mutation alters the interactions between Na\(^+\) channel \( \alpha \) - and \( \beta \)-subunits and that the subunit composition of native Na\(^+\) channels has not been firmly established add to the paradox of our results. Furthermore, it is possible that heterologous expression of the D1790G mutation with and without h\( \beta \)\(_2\) in HEK 293 cells is not sufficient to express the phenotype of the mutation that is present in myocardial cells.

This new LQT-3 mutation (D1790G) was identified in a single large (n = 131) family that originates in the isle of Jerba near Tunis. In this family, there have been 3 cases of sudden cardiac death (1 documented) and currently only a single symptomatic case (recurrent syncope) out of 26 mutation carriers. In addition, episodes of sinus arrest have been documented by Holter recording in 3 mutation carriers. Clinical and ECG data have been collected for 92 members of this family for >10 years, and blood samples for genetic analyses were available for 75 members. Strict ECG criteria for QT-interval prolongation, derived from a normal ECG database, were used for phenotypic classification. To accommodate uncertainty in phenotypic classification at intermediate QTc values, all family members were characterized according to 3 phenotypic subsets: affected, unaffected, and equivocal. To account for age and sex differences, separate phenotypic definitions were used for 3 predefined demographic subsets: children (<16 years old), adult (>16 years old) men, and adult women. To account for QTc variability over time, each family member was classified according to his or her mean QTc value that was typically calculated from 4 to 6 QTc values measured in several ECGs recorded over several years. According to this phenotypic classification scheme, 40 family members were classified as unaffected, 23 as affected, and 12 as equivocal. Further genetic analysis identified the new D1790G mutation in all affected family members and in 3 members with an equivocal phenotypic status. The mutation was excluded in all unaffected members and in 300 unrelated control subjects. Hence, the mutation is clearly linked to the disease.

The D1790G inactivation shift in Figure 1B predicts that in ventricular cells in which resting potentials are expected to be near –90 mV (holding potentials in our experiments), the D1790G mutation will have little effect on channel availability. This prediction is supported by clinical data showing little change in impulse conduction (QRS duration) in carriers of the D1790G mutation (J.B. et al, unpublished observations). Consequently, our results suggest that the D1790G mutation prolongs ventricular repolarization through an indirect effect on cardiac electrical activity. One possibility is that voltage-gated Na\(^+\) channels may participate in electrical activity in or near the sinoatrial node (SAN) and hence contribute to pacing, as has been suggested in several animal studies. Chronic depolarization of the nodal area (SAN) compared with the ventricular cells would make cells of the SAN more susceptible to marked changes in D1790G-induced reduction in Na\(^+\) channel availability and hence pacing. Another possibility is that Na\(^+\) channels in sympathetic neurons innervating the sinus node may also express channels carrying the D1790G mutation, which in neuronal cells exhibiting repetitive activity would be expected to inhibit excitability and consequently sympathetic stimulation of the node. In either case, the subsequent reduction in heart rate would indirectly be expected to prolong ventricular repolarization. Interestingly, carriers of the D1790G mutation do in fact have low heart rates, and in some, sinus arrest has been documented (J.B. et al, unpublished observations). Confirmation of this possibility must await future experiments in which the effects of the D1790G mutation can be tested more directly in cells of the SAN region, perhaps in genetically altered murine models.

Finally, our results have important implications for strategies to treat LQT with a gene-specific approach. It is now clear that local anesthetic (lidocaine or mexiletine) treatment of LQT-3 in carriers of previously reported SCN5A mutations may prove to be a unique and specific therapeutic strategy to treat these gene defects. The results of our investigation of the D1790G SCN5A mutation predict that such treatment will be ineffective in controlling this LQT-3 arrhythmia, because this mutation does not promote sustained inward current but instead causes a negative shift in steady-state inactivation. In fact, such treatment might even exacerbate rhythm disturbances in carriers of this gene defect by further shifting the Na\(^+\) channel inactivation curve. Preliminary clinical data indicate that lidocaine does not correct QT prolongation in carriers of the D1790G mutation (J.B., unpublished observations). Thus, our results clearly show the importance of carrying out cellular functional studies before generalizing a molecular therapeutic approach to management of specific gene defects. Here we present examples of mutations in the same gene (SCN5A) linked to the same disorder (LQT-3) but for which distinct therapeutic strategies need to be developed.

References


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