Arrhythmogenic Mechanism of an LQT-3 Mutation of the Human Heart Na⁺ Channel α-Subunit
A Computational Analysis

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Background—D1790G, a mutation of SCN5A, the gene that encodes the human Na⁺ channel α-subunit, is linked to 1 form of the congenital long-QT syndrome (LQT-3). In contrast to other LQT-3–linked SCN5A mutations, D1790G does not promote sustained Na⁺ channel activity but instead alters the kinetics and voltage-dependence of the inactivated state.

Methods and Results—We modeled the cardiac ventricular action potential (AP) using parameters and techniques described by Luo and Rudy as our control. On this background, we modified only the properties of the voltage-gated Na⁺ channel according to our patch-clamp analysis of D1790G channels. Our results indicate that D1790G-induced changes in Na⁺ channel activity prolong APs in a steeply heart rate–dependent manner not directly due to changes in Na⁺ entry through mutant channels but instead to alterations in the balance of net plateau currents by modulation of calcium-sensitive exchange and ion channel currents.

Conclusions—We conclude that the D1790G mutation of the Na⁺ channel α-subunit can prolong the cardiac ventricular AP despite the absence of mutation-induced sustained Na⁺ channel current. This prolongation is calcium-dependent, is enhanced at slow heart rates, and at sufficiently slow heart rate triggers arrhythmogenic early afterdepolarizations.

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Key Words: sodium • calcium • arrhythmia • torsad de pointes • action potentials

The congenital long-QT syndrome (LQTS) is an inherited cardiac disorder that is defined in part by prolonged ventricular repolarization, an association with recurrent syncope, a propensity to polymorphous ventricular tachycardia (torsade de pointes), and sudden death.1,2 Multiple genes that encode ion channel subunits have now been shown to be linked to LQTS,3 and in most cases, the functional properties of the mutant gene products are consistent with the disease phenotype, ie, increase of inward or decrease of outward plateau current.4,5

LQT-3 is linked to the gene encoding the α-subunit of the cardiac voltage-gated sodium channel (SCN5A on chromosome 3).6 Functional analysis of initially reported SCN5A mutations revealed mutant Na⁺ channels that fail to inactivate completely on prolonged depolarization7,8 and produce a small but functionally important enhancement of inward plateau current sufficient to delay repolarization and increase vulnerability of the heart to arrhythmias.9 All subsequently reported LQT-3 mutations with the exception of D1790G (DG) promote sustained Na⁺ current and are expected to prolong repolarization through this common mechanism.9–14

The purpose of the present study was to use a computational approach to determine whether or not the biophysical properties of DG mutant Na⁺ channels might affect the cellular action potential (AP) through mechanisms other than a direct contribution to maintained Na⁺ channel plateau current. We modeled the cardiac ventricular AP using parameters and techniques described by Luo and Rudy15 as our control and modified only the properties of the voltage-gated Na⁺ channel according to our patch-clamp analysis of DG channels. Our results indicate that the DG mutation can prolong AP duration (APD) despite the absence of mutation-induced inward Na⁺ channel current during the plateau phase. Instead, the mutation indirectly affects other electrogenic pathways, which have a common interdependence on altered cellular calcium homeostasis. The computations predict DG mutation–induced AP prolongation, which is heart rate–dependent and driven by subsequent changes in the intracellular calcium transient. These results are important not only for the novel fundamental insight into the mechanistic basis of inherited arrhythmias but also because they suggest novel targets (ie, calcium-handling proteins) as therapeutic agents.

Methods

Computer-Generated AP Reconstruction

The AP was calculated by solving the differential equation

\[ I_{\text{ion}} = -C_m \frac{dV_m}{dt} \]

where \( I_{\text{ion}} \) is the total transmembrane ionic current.
(µA/cm²). Cm is the specific capacitance of the membrane (1 µF/cm²), and Vm is the transmembrane potential (mV). All ionic currents were formulated according to the Luo-Rudy model of a ventricular AP, except that the sodium current was formulated to reproduce our patch-clamp experiments in HEK 293 cells. The differential equation was solved numerically with a discretization time step of 10 µs.

Experimental parameters used in the model were determined from experimental data for voltage-dependence of steady-state activation and inactivation and the kinetics of the onset and recovery from inactivation. Parameters for Boltzmann relationships and time constants were extracted as previously described. Rate constants were adjusted with temperature, assuming Q10 factors (the factor is usually between 2 and 3 for rate constants between different states in ionic channels).

To simulate wild-type (WT) channels, we used \( m_\alpha = 1/\{1 + \exp[-(V_m+32.5)/9]\} \) and \( h_\alpha = 1/[1 + \exp(V_m+57.87)/7]\). To simulate DG channels, \( m_\alpha = 1/[1 + \exp(V_m+29.10.5)] \) and \( h_\alpha = 1/[1 + \exp(V_m+74.3)/6.5] \) were used. In the DG channels, the time constants of onset of inactivation (\( r_1 \)) and the slow inactivation gate (\( n \)) were half of those used in the WT channels. The maximum sodium conductance was 27 mS/cm². In all simulations, the maximum conductance of the slow component of the delayed rectifier potassium current, \( I_K_s \), was reduced by 40% to uncover AP differences between cells with WT and DG channels. These APs thus simulate endocardial or M-cell activity. APs were initiated by intracellular 0.5-ms current pulses (100 µA/cm²). Initial conditions were established by stimulating cells with WT and DG channels once per second for a 3-minute period. Then, cells were stimulated 20 times at a constant cycle length. Cycle lengths between 500 and 4000 ms were used.

Expression of Recombinant Na⁺ Channels

Na⁺ channels were expressed in HEK 293 cells as previously described. Transfections were carried out with equal amounts of Na⁺ channel α-subunit cDNA (WT or DG, respectively), with β1-, and/or β2-subunit cDNA subcloned individually into the pcDNA3 (Invitrogen) vector (total cDNA 2.5µg). Control experiments (data not shown) indicated no significant differences in channel activity for these subunit combinations.

Electrophysiology

Membrane currents were measured with whole-cell patch-clamp procedures with Axopatch 200B amplifiers (Axon Instruments) as previously described. Unless noted otherwise, recordings were made at room temperature (22°C) with previously published solutions. Data acquisition and analysis were carried out with pClamp7 (Axon Instruments), Excel (Microsoft), and Origin (Microcal Software). Steady-state inactivation was determined after application of 500-ms conditioning pulses applied once every 2 seconds. Recovery from inactivation was measured in paired pulse experiments, with a test pulse applied at variable times after a 100-ms conditioning pulse to −10 mV. Holding potentials were −80 mV.

Results

Biophysical Properties of DG Mutant Channels: Computational Parameter Set

Figure 1 shows families of experimental records of WT and DG mutant channels expressed in HEK 293 cells as well as analysis of the experimentally determined voltage-dependence of the time constants of the onset of inactivation. As illustrated in Figure 1A, we compared experimental recordings of WT and DG channels at 22°C (top) and 29°C (bottom). Clearly, increasing temperature speeds the kinetics of both WT and DG mutant channels, as expected, but it neither changes the relationship between WT and DG mutant channel time constants nor promotes DG-induced sustained inward current. Figure 1B summarizes time constants obtained by fitting experimental records with functions containing 1 exponential component. Although in a limited number of experiments, we previously reported no effect of the DG mutation on the kinetics of the onset of inactivation, in a more complete analysis we have found that, in fact, this mutation speeds the onset of inactivation (Figure 1) and slightly alters the voltage-dependence of activation (Figure 3). Using these data, we extrapolated the temperature coefficient (Q10) of 2.1 for inactivation kinetics for both WT and DG mutant channels to compute the effects of the mutation on APs at physiological temperatures.

Figure 2 shows the simulated changes in Na⁺ channel currents and the time constants of the onset of inactivation generated by the computer-based model. As is the case for the experimental data, the simulated currents reflect the speeding of the onset of inactivation as a function of membrane potential but do not exhibit enhanced sustained current.

Figure 3 illustrates experimental and computational data showing the effects of the DG mutation on the voltage-
dependence of steady-state inactivation and activation and on the time course of recovery from the inactivated state. The mutation causes a marked negative shift of the voltage-dependence of inactivation with relatively minor changes in the voltage-dependence of activation, as we previously reported.11 The mutation also causes a small but significant speeding of the recovery process. The results obtained in experiments (A) were incorporated into the simulated channels (B).

Simulation of Cellular Electrical Activity: AP Prolongation That Is Heart Rate–Dependent

Figure 4 shows the consequences of these mutation-induced changes in channel properties on cellular electrical activity that is predicted by the computer-generated model. At a basic cycle length (BCL) of 1000 ms, there is little effect of the DG mutation despite a significant APD prolongation. At a BCL of 3000 ms, however, it causes dramatic APD prolongation, which results in the generation of early afterdepolarizations (EADs). The results of similar calculations repeated over a series of BCLs are summarized in Figure 5, in which APD is plotted versus BCL for both WT and DG channels. The expression of mutant DG channels markedly alters this relationship. Figure 5 shows that even at moderately long BCLs (1000 to 2000 ms), the DG mutation prolongs APs compared with cells expressing WT channels. This effect becomes very pronounced (note change in vertical scale) as heart rate slows further, and at a BCL of 3000 ms, as illustrated above, the mutation-induced action prolongation is sufficient to induce EADs (arrow). Similarly, the DG mutation induces EADs after pauses in AP activity (Figure 6).

Ionic Basis of DG Cellular Phenotype:
A Role for [Ca\textsuperscript{2+}]

Figures 7 and 8 show the effects of the DG mutation on several key ionic pathways underlying the computed APs. These computations reflect steady-state conditions during a BCL of 3000 ms, but the patterns revealed are the same as those that occur in DG-induced pause-dependent AP prolongation (data not shown). Figure 7 focuses on computed Na\textsuperscript{+} channel currents and illustrates current during the AP plateau (A) as well as, on an expanded time scale, during the upstroke (B). As predicted by the voltage-clamp data, the DG mutation does not promote sustained inward Na\textsuperscript{+} current that would account for AP prolongation (A, lower row). In fact, the
computations show that the overall effect of this mutation is to reduce the contribution of Na$^+$ channel activity to the initial upstroke and overshoot (arrow) of the AP.

Figure 8 illustrates DG mutation–induced changes in other pathways that occur during the duration of the AP. The computations reveal an increase in Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels, an increase in the magnitude of the transient change in [Ca$^{2+}$]$_i$, changes in Na$^+$/Ca$^{2+}$ exchange current, and an initial reduction followed by prolonged activation of the slowly activating K$^+$ channel current, $I_{Ks}$. The mechanisms underlying these changes are discussed below.

**DG-Induced Changes in [Ca$^{2+}$]$_i$ Determine Frequency-Dependent ADP Prolongation**

Perhaps the most prominent feature of the DG cellular phenotype predicted from these computations is the marked dependence of APD on heart rate (Figure 5). Figure 9 shows marked DG mutation–induced changes in the calcium transient that are steeply frequency-dependent. At low stimulation frequencies (long BCLs), the calcium transient in cells expressing DG Na$^+$ channels is increased in both amplitude and duration. At faster heart rates (BCLs on the order of 1000 ms), there is little difference between calcium transients of Figure 5.

Figure 6. Effect of DG mutation on pauses in stimulation. Last 2 APs in a train of 20 at BCL of 500 ms followed by 1500-ms pause and postpause AP. Pause in activity induces an EAD (arrow) in cells expressing DG but not WT channels.

Figure 7. DG-induced AP prolongation is steeply heart rate-dependent. Plotted is relationship between APD and BCL. At BCL of 3000 ms, mutation-induced action prolongation is sufficient to induce EADs (arrow) at BCL >2500 ms.

**DG mutation affects calcium-sensitive pathways.** Simulation of effects of DG mutation on APs and key underlying pathways that occur at BCL of 3000 ms. Shown are following computations for cells expressing WT (dotted lines) and DG (solid lines) Na$^+$ channels (top to bottom): membrane potential; L-type calcium channel currents; intracellular free calcium; Na$^+$ / Ca$^{2+}$ exchange current; and delayed rectifier current, $I_{Ks}$. Arrow in second row indicates peak Ca$^{2+}$ influx with WT channel.
WT and DG-expressing cells (A). The computations thus suggest a complex calcium-dependent pathway regulating APD that becomes altered, as described above, by DG-induced changes in calcium entry. Because the effects of these changes on the calcium transient are predicted to be steeply frequency-dependent, the calculations suggest a strong interdependence on the filling and subsequent emptying of intracellular calcium stores in this process.

**Discussion**

**Sustained Na\(^+\) Channel Current Is Not Necessary to Prolong the Ventricular AP**

The major finding reported in this study is the fact that biophysical properties of DG mutant Na\(^+\) channel \(\alpha\)-subunits prolong computer-simulated ventricular APs, even though this mutation does not promote sustained Na\(^+\) channel current during the AP plateau phase. This study thus extends our understanding of the molecular basis of the disease phenotype, the prolonged QT intervals, and confirms the importance of computational studies in bridging information relating functional changes in individual ion channel subunits to predictions of cellular and even system phenotypes.

The plateau phase of the cardiac ventricular AP is maintained by a delicate balance between inward and outward movement of ions, and even very small changes in ionic currents during the plateau are expected to have marked effects on APD.\(^1\)\(^-\)\(^2\)^2\(^3\) The functional properties of channels encoded by DG mutant \(\alpha\)-subunits have defied interpretation within a framework that requires direct mutation-dependent increase in Na\(^+\) channel current during the AP plateau, because this mutation does not promote sustained Na\(^+\) channel activity in the face of cellular depolarization.\(^1\)^1 Instead, the most prominent characteristics of DG channels are a negative shift in channel availability as a function of membrane potential and a speeding of the kinetics of the onset of inactivation. However, our computations reveal the importance of these biophysical changes in channel properties to events that determine the duration of the AP and show that events that occur during the initial 5 ms of the AP can have profound effects on electrical activity that occurs during the following several hundred milliseconds.

**DG-Induced Decrease in Na\(^+\) Channel Activity Leads to an Increase in the \([\text{Ca}^{2+}]\) Transient**

Figures 7, 8, and 9 provide insight into the mechanism(s) by which the DG mutation delays AP repolarization. Because of the changes in Na\(^+\) channel kinetics, fewer channels are available for activation at the cellular resting potential, and once opened, these channels inactivate faster than WT channels. This results in a reduced Na\(^+\) channel current, slower upstroke, and more importantly, a less positive overshoot of the AP (Figure 7, arrow). The change in dV/dt and overshoot occurs during the very early stages of ventricular depolarization, as seen in Figure 8, and in turn causes an increase of Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels during this crucial period of electrical activity. The primary mechanism underlying this effect is not a change in gating parameters for Ca\(^{2+}\) channel activation and inactivation, but the effect of the DG mutation on the AP overshoot and subsequent change in the driving force for Ca\(^{2+}\) entry via L-type Ca\(^{2+}\) channels (not illustrated). With this alteration in Ca\(^{2+}\) entry, the model predicts that the subsequent Ca\(^{2+}\) transient will be altered in both time and magnitude, and as a result, all \([\text{Ca}^{2+}]\)-dependent processes will subsequently be altered (Figure 8). Two important ionic pathways, the Na\(^+\)/Ca\(^{2+}\) exchanger\(^2\(^4\) and the slowly activating delayed K\(^-\) channel current, \(I_{Ks}\),\(^2\(^5\) are affected (Figure 8). The result is a net increase of inward plateau current and corresponding increase in APD. The fundamental difference between the generation of the cellular phenotype in the case of the DG mutation compared with previously described LQT-3 mutations is that the increase of inward current does not come from a direct contribution of altered Na\(^+\) channel activity but rather from other pathways.

The computations also reveal a critical role of \(I_{Ks}\) in the DG-induced cellular phenotype (Figure 8) as a result of the reduction in this current that occurs as a consequence of mutation-induced changes in \(V_{m}\). Thus, although the disease-linked mutation is in the \(SCN5A\) gene, the cellular phenotype is due in part to changes in activity of channels encoded by LQT-1– and LQT-5–linked genes.\(^2\(^6\)^2\(^7\)
relationship between cellular and clinical phenotypes

The work presented here is the result of incorporation of biophysical properties of human WT and DG mutant Na\(^+\) channels expressed in a mammalian cell line into a computational model that integrates experimental data obtained from a variety of cell types and species. Extrapolation of these results to a precise understanding of human pathophysiology is not possible and goes beyond the scope of this study. Qualitative, but not quantitative, conclusions may be drawn from this work. Nevertheless, comparison to appropriate clinical parameters that have been measured for carriers of the DG mutation is important, and in fact, a stringent test of the validity of the methodology.

The model suggests that under steady-state pacing conditions, the Na\(^+\) current is smaller in myocytes expressing DG mutant channels next to WT channels. This will most likely lead to a reduced rate of rise of the AP upstroke (dV/dt) as predicted for the cellular model, and this, in turn, would be expected to be reflected in a widening of the QRS complex on the ECG of mutation carriers. Indeed, DG carriers tend to have wider QRS complexes than control patients. The computational work clearly indicates that for this mutation, bradycardia will potentiate APD prolongation. It is interesting to note that heart rates of DG carriers have been found to be slower than those of noncarriers. In fact, in several members of the DG family that have been studied, sinus slowing and even arrest may have been as significant as APD prolongation. This raises the interesting and important question as to a causal relationship between pacing and the DG mutation, a question that certainly is beyond the scope of the present study.

Role of \(I_{\text{Na}}\) in Modulating the Effect of the DG Mutation on APD

In the computations that we have carried out and reported, we have not included a contribution of the transient outward current \((I_{\text{to}})\). We did, however, test for its effects in calculations, which we have not illustrated. We find, as might be expected, that if expressed at sufficiently high levels, this current will tend to offset the effects of the DG mutation and modify its influence on APD. Thus, cells expressing \(I_{\text{Na}}\) at the highest densities (epicardial cells) would be expected to have substantially shorter APDs than cells in which \(I_{\text{to}}\) is expressed at the lowest densities (endocardial cells), because the additional contribution of DG mutant channels would then follow the same anatomic pattern. This pattern of channel expression would thus be expected to favor enhanced T-wave dispersion, which, interestingly, is what is observed in carriers of the DG mutation. Heterogeneity in the expression of ion channel genes, no doubt, remains an important area of investigation that will be needed to provide a causal link between expression of specific gene mutations and generation of the systemic disease phenotype.

Novel Therapeutic Strategies for LQT-3

Our analysis has revealed that beat-dependent changes in intracellular calcium that occur as a consequence of the DG LQT-3 mutation should be considered major factors in generating the disease phenotype (delayed ventricular repolarization) in carriers of this gene defect. This work therefore strongly suggests that a therapeutic strategy that includes inhibition of L-type calcium channel activity may be beneficial for carriers of the DG gene defect.

In summary, our computations show that the biophysical properties of DG mutant channels are sufficient to account for a cellular phenotype consistent with LQT-3: prolongation of the ventricular AP. This occurs despite the absence of mutation-induced sustained Na\(^+\) current. This finding not only is a necessary step in understanding the molecular basis of QT prolongation in carriers of the DG mutation but also raises the possibility that previously overlooked functional properties of other LQT-3 SCN5A mutations may also contribute to the disease phenotype and may require further investigation. Furthermore, our work suggests that novel therapeutic strategies may include modulation of calcium as well as sodium channel activity.

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