A novel SCN5A mutation associated with long QT-3: altered inactivation kinetics and channel dysfunction

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Rivolta, Ilaria, Colleen E. Clancy, Michihiro Tateyama, Huajun Liu, Silvia G. Priori, and Robert S. Kass. A novel SCN5A mutation associated with long QT-3: altered inactivation kinetics and channel dysfunction. Physiol Genomics 10: 191–197, 2002. First published July 16, 2002; 10.1152/physiolgenomics.00039.2002.—Mutations in the gene (SCN5A) encoding the α-subunit of the cardiac Na⁺ channel cause congenital long QT syndrome (LQT-3). Here we describe a novel LQT-3 mutation I1768V (I1768V) located in the sixth transmembrane spanning segment of domain IV. This mutation is unusual in that it is located within a transmembrane spanning domain and does not promote the typically observed sustained inward current corresponding to a gain of function (bursting). Rather, I1768V increases the rate of recovery from inactivation and increases the channel function (bursting). Rather, I1768V increases the sustained inward current corresponding to a gain of function (bursting). The entire coding region of all known LQTS-related genes (chromosome 3p21–23), may contain genetic defects that alter channel properties and underlie disease syndromes including long QT, Brugada, and conduction abnormalities (4, 6, 13, 24, 26, 30). Cardiac sodium current (I_{Na}) is primarily responsible for the generation and propagation of the cardiac action potential, crucial for contractile synchronization (20). The elucidation of a role of Na⁺ channel defects in seemingly idiopathic syndromes is relatively recent (15). Electrophysiological characterization of such defects promotes understanding of the relationship between genotype and phenotype and provides insight into the discrete structures that are involved in channel gating.

In this paper we report the functional consequences of a new mutation identified in a patient with congenital long QT syndrome (LQT-3). The mutation is at position 1768, located within the sixth transmembrane spanning region in domain IV of the cardiac sodium channel α-subunit. The mutation results in the substitution of a valine in place of a highly conserved isoleucine (1768V). We have previously reported a mutation in a different region (E1295K) (1) that has properties similar to those reported here. Namely, both mutations act to increase channel availability and increase the rate of channel recovery from inactivation, which results in a “window” of voltages over which nonactivation-related activity is observed and is distinct from bursting activity (3, 12, 23, 27). Similar changes were also observed in an SCN5A mutation recently reported (29) associated with sudden infant death syndrome (SIDS). Here we show that the I1768V mutation-induced changes in the kinetics of recovery from inactivation can account for all of the biophysical changes in the channel and consequently are likely to be key to mutation-induced rhythm dysfunction.

METHODS

Description of a new LQT-3 mutation in a patient with QT prolongation. The proband was a 15-yr-old male who experienced a syncopal episode at rest. He was then admitted to the hospital where a self-terminating polymorphic ventricular tachycardia with Torsade de Pointes morphology was observed (the K⁺ plasma level at admission was 4.3 meq/l). After sinus rhythm was restored spontaneously, QT interval prolongation was observed (QT = 455 ms, RR = 800 ms, QTc = 510 ms, lead II). All the possible causes of acquired QT interval prolongation (QT prolonging drugs, electrolyte imbalance, etc.) were excluded and therefore a congenital form of LQT syndrome (LQTS) was diagnosed. Genetic analysis of the proband performed by single-strand conformational polymorphisms (SSCP) (17) demonstrated an abnormal conformation identified in the exon 28 of the SCN5A gene. Subsequent DNA sequence analysis showed a single nucleotide transition (A→G at position 5302) leading to a nonconservative amino acid change with a valine replacing an isoleucine at codon 1768. The mutation occurs in the transmembrane segment S6 of domain IV of the channel. The entire coding region of all known LQT3-related genes (KCNE1, KCNE2,
\textbf{RESULTS}

\textbf{Biophysical characterization of the I1768V mutation.} HEK 293 cells transiently cotransfected with h\textsubscript{\(\beta\)}1 plus either WT or I1768V cDNA expressed sodium currents, which had no obvious biophysical differences (Fig. 1A). Comparison of the properties of WT and I1768V channels revealed no significant difference between the peak current, voltage dependence of channel activation, or current density as reflected in the current voltage relationships shown in Fig. 1B. Unlike the majority of previously characterized LQT-3-linked mutations, I1768V did not result in a decrease in sustained current due to channel bursting compared with WT as shown in Fig. 2 [\(|0.24\, \text{pA/pF}| = 0.06 \pm 0.02\%\), \(n = 8\), for WT; \(0.24\, \text{pA/pF}| = 0.06 \pm 0.02\%\) of peak, \(n = 5\), for I1768V (not significant)]. We confirmed this result by measuring the frequency of bursting in single channels and found that the WT channels burst with the same frequency as I1768V channels (Fig. 2C; also, see Supplementary Fig. 2\textsuperscript{1} published online at the \textit{Physiological Genomics} web site). At \(-20\, \text{mV},\) WT bursting frequency was \(0.0029 \pm 0.0006\%\) (\(n = 3\)) and I1768V bursting frequency was \(0.0027 \pm 0.0010\%\) (\(n = 4\)) (not significant). We also characterized the onset of inactivation kinetics (computed as \(\tau_{1/2}\), the time to decay to half of peak current) (Fig. 3) of the I1768V mutant and WT and observed no significant difference. However, we did find that the mutation affected the kinetics of the recovery from inactivation, which we measured by

\begin{footnote}
\textsuperscript{1}Supplementary materials (APPENDIX and Supplementary Figs. 1 and 2) to this article are available online at \url{http://physiolgenomics.physiology.org/cgi/content/full/10/3/191/DC1}.
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repeated application of a two-pulse protocol. Channels were inactivated by conditioning pulses (500 ms, -10 mV) and allowed to recover from inactivation at the holding potential (-100 mV) for variable times before $I_{Na}$ was measured in response to test pulses (-10 mV). Normalized test pulse current was plotted vs. recovery intervals (Fig. 4A). We observed a significant mutation-induced speeding of this process. Time to half recovery was 3.0 ± 0.1 ms ($n = 8$) for WT and 1.3 ± 0.1 ms for I1768V channels ($n = 8$) ($P < 0.05$). We reasoned that a twofold increase the rate of recovery from inactivation might increase channel availability and con-

![Repeated application of a two-pulse protocol](image)

**Fig. 1.** The I1768V mutation does not alter the voltage dependence of activation. A: averaged currents recorded in response to a series of voltage pulses from -80 mV to +30 mV (5-mV increments) for wild-type (WT, $n = 5$) and I1768V ($n = 8$) channels. B: current-voltage relationship of peak inward current in HEK 293 cells expressing and I1768V channels (measured in 10 mM Na+, see METHODS) (for WT $n = 5$, and for I1768V $n = 8$).

![Voltage dependence of activation](image)

**Fig. 2.** The I1768V mutation does not increase sustained macroscopic Na+ channel activity or single channel bursting. A: background currents were recorded in the presence of tetrodotoxin (TTX, 30 µM) as described in METHODS. TTX-sensitive and averaged current traces recorded WT ($n = 8$) and I1768V ($n = 5$) channels during sustained (150 ms) depolarization (-10 mV). B: average TTX-sensitive current measured at 150 ms; WT 0.06 ± 0.02% of the peak current ($n = 8$); I1768V 0.06 ± 0.02% ($n = 5$) ($P < 0.05$). C: bursting probability determined as described in METHODS at -20 mV for WT and I1768V channels.

![TTX-sensitive current and bursting probability](image)

*A NOVEL SCN5A MUTATION ASSOCIATED WITH LONG QT-3*
Boltzmann parameters
(open symbols) and I1768V (solid symbols) channels. Fits to experimental data (smooth curves) yielded the resulting availability data were determined using 500-ms conditioning pulses to the voltages indicated along the abscissa for WT (8), P (I1768V, n = 8). Mean values are not significantly different comparing WT vs. I1768V data points.

Fig. 3. The I1768V mutation does not alter the time course of the onset of inactivation. A: families of current traces recorded from cells expressing WT or I1768V channels in response to a series of brief (25 ms) test pulses (–60, –25, –10, 0, and 10 mV). B: time to half inactivation (\(\tau_{1/2}\), measured as time to 50% decay of transient inward current) during pulse plotted vs. test pulse voltage (WT, n = 5; I1768V, n = 8). Mean values are not significantly different comparing WT vs. I1768V data points.

Fig. 4. The I1768V mutation speeds the recovery from inactivation. A: the recovery from inactivation induced by conditioning pulse (500 ms, –10 mV) was measured using a paired pulse protocol (see text and METHODS). Plots show amplitude of peak inward current, normalized to fully recovered current, as a function of time after imposition of the conditioning pulse for WT (open symbols) and I1768V (solid symbols) channels. There is a significant mutation-induced speeding of the process; the time to half recovery for WT is 3.01 ± 0.06 ms (n = 8) and for I1768V is 1.81 ± 0.11 ms (n = 5), and 30°C = 0.86 ± 0.19 (n = 4), corresponding to a Q10 ≈ 3, for both WT and I1768V channels. The differences between WT and I1768V at both 22°C and 32°C were significant [\(P < 0.01\) and \(P < 0.05\), respectively (see Supplementary Fig. 1)].

To determine whether this increase in the rate of channel recovery from inactivation might underlie the 7.6-mV shift in inactivation gating, we applied the same rate increase to recovery transitions in a computer simulated Markov model of the cardiac Na+ channel (9). Our simulation demonstrated that a doubling of the rate of recovery from inactivation results in a leftward shift in the time to half recovery from inactivation (Fig. 5B), a +7-mV shift in the voltage dependence of channel availability (Fig. 5C), and no effect on current density or activation (Fig. 5A). These results, which are remarkably similar to the experimental data of Fig. 1, demonstrate that the I1768V mutation-induced speeding of the rate of recovery from inactivation might underlie the 7.6-mV shift in inactivation.
mutation acts to make recovery from inactivation more energetically favorable, and this singular alteration in kinetics is sufficient to account for all of the experimentally observed gating changes.

We have demonstrated previously that mutation-induced shifts in the voltage dependence of activation and/or inactivation will affect the window of overlap between the activation and availability curves (1), an effect subsequently reported in a de novo SCN5A mutation linked to SIDS (29). The resulting current can be measured directly by using a slow positive ramp pulse protocol. We used this positive ramp protocol (25) (slow voltage ramp from $-100$ mV to $+50$ mV in 2 s) to investigate the effect of the I1768V mutation on the window current (Fig. 3). We measured a small, but significant, mutation-induced change in the voltage dependence of the window current $([-44.6 \pm 1.1 \text{ mV}])$ (n = 9), WT; $[-39.6 \pm 0.6 \text{ mV}]$ (n = 15), I1768V $P < 0.01$ (Fig. 6). Our theoretical analysis indicates that this change in window current is a consequence of the I1768V mutation-induced change in the kinetics of the recovery from inactivation.

**DISCUSSION**

The I1768V mutation in the α-subunit of the cardiac Na$^+$ channel results in an amino acid substitution (I1768V) in the transmembrane segment S6 of domain IV proximal to the start of the COOH terminus. Interestingly, although isoleucine and valine have similar structural properties [both are aliphatic and have non-reactive side chain groups ($\text{CH}_3$)], the substitution mutation results in abnormalities in channel gating. Notably, isoleucine in position 1768 is very conserved between channel isoforms and species, suggesting an important structural role. The I1768V mutation results in two primary effects on channel biophysics: an increase in steady-state channel availability and an increase in the rate of recovery from inactivation. Importantly, although residue 1768 is within the S6 segment of domain IV of the channel, it is very close to the proximal end of the carboxy tail of the channel, a structure that has recently gained more attention as key modulator of Na channel inactivation in both brain and heart (10, 16). It is quite possible that the effects of the mutation on channel gating are a consequence of allosteric changes in structure that alter COOH-terminal interactions with the channel.

The I1768V mutation is unusual in that very few LQT-3-related mutations have been reported to reside in one of the transmembrane spanning segments. The three other mutations that have been reported in
transmembrane regions are located in S4DIII (T1304M) (28) or in S4DI (R1623Q) (11, 14). The overwhelming majority of LQT-3-linked mutations are in cytoplasmic linkers (21). Interestingly, other mutations have been identified in S6 of the skeletal isoform of the Na⁺ channel and are associated with hypokalemic periodic paralysis (HypoPP) (7, 22).

The I1768V mutation is also unusual, but clearly not unique, in that it is an LQT-3-linked mutation that does not result in an obvious gain of function from failure of channel inactivation (bursting). Instead, one of the functional consequences of this mutation is alteration in “overlap” or “window” that is critical to maintenance of the plateau phase of the cardiac action potential, similar to one other previously described LQT-3 (1) and SIDS (29) mutation. Thus this mechanism of channel dysfunction may be more common than previously considered (1).

Here we demonstrate that altered channel availability, which underlies this overlap current, may be caused by mutation-induced changes in the kinetics of recovery from inactivation. We show that I1768V channel expressed in HEK 293 cells produces an increase in the rate of channel recovery from inactivation and increase in channel availability. We also use a computational model to demonstrate that the observed increase in the rate of channel recovery from inactivation is fully sufficient to account for the shift in inactivation gating. The I1768V mutation acts to make recovery from inactivation more energetically favorable, and this causes a change in recovery kinetics. This alteration in kinetics is sufficient to account for all of the experimentally observed gating changes. The increase in channel availability, stemming from faster channel recovery, acts to increase the window of overlap between the inactivation and activation curve. This more extensive overlap occurs at voltages corresponding to the delicate plateau phase of the cardiac action potential.

It has been demonstrated and is now well-accepted that LQT-3 mutations that act to diminish transitions into a nonconducting inactivated state during the plateau phase of the cardiac action potential promote sustained whole cell current activity and underlie the primary disease phenotype: delayed repolarization of the ventricle (8). Not all SCN5A mutations linked to repolarization dysfunction disrupt inactivation in this manner and promote sustained current during the plateau (1, 2, 29). Here we show that mutation-induced changes in the energetics of transitions out of the inactivated state can also play an important role in key channel properties that underlie control of repolarization in the ventricle. As we learn more about the mechanisms underlying disorders of repolarization, we also gain insight into possible novel therapeutic strategies to treat them. A key implication of the analysis presented in this study is that compounds that act to slow transitions from inactivated to rested channels may prove uniquely useful in the treatment of repolarization dysfunction due to the I1768V and related SCN5A mutations.

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