Two Distinct Congenital Arrhythmias Evoked by a Multidysfunctional Na\(^+\) Channel


Abstract—The congenital long-QT syndrome (LQT3) and the Brugada syndrome are distinct, life-threatening rhythm disorders linked to autosomal dominant mutations in SCN5A, the gene encoding the human cardiac Na\(^+\) channel. It is believed that these two syndromes result from opposite molecular effects: LQT3 mutations induce a gain of function, whereas Brugada syndrome mutations reduce Na\(^+\) channel function. Paradoxically, an inherited C-terminal SCN5A mutation causes affected individuals to manifest electrocardiographic features of both syndromes: QT-interval prolongation (LQT3) at slow heart rates and distinctive ST-segment elevations (Brugada syndrome) with exercise. In the present study, we show that the insertion of the amino acid 1795insD has opposite effects on two distinct kinetic components of Na\(^+\) channel gating (fast and slow inactivation) that render unique, simultaneous effects on cardiac excitability. The mutation disrupts fast inactivation, causing sustained Na\(^+\) current throughout the action potential plateau and prolonging cardiac repolarization at slow heart rates. At the same time, 1795insD augments slow inactivation, delaying recovery of Na\(^+\) channel availability between stimuli and reducing the Na\(^+\) current at rapid heart rates. Our findings reveal a novel molecular mechanism for the Brugada syndrome and identify a new dual mechanism whereby single SCN5A mutations may evoke multiple cardiac arrhythmia syndromes by influencing diverse components of Na\(^+\) channel gating function. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2000;86:e91-e97.)

Key Words: Na\(^+\) channel ■ inactivation ■ long-QT syndrome ■ Brugada syndrome

Voltage-gated ion channels play a fundamental role in the emerging understanding of inherited disorders in cardiac rhythm. Mutations in SCN5A, the gene encoding the human cardiac Na\(^+\) channel,\(^1\) underlie two distinct syndromes of abnormal cardiac electrophysiology: the long-QT syndrome (LQT3, linked to chromosome 3)\(^2\) and Brugada syndrome.\(^3\) These disorders produce distinct ECG abnormalities, but both herald an exceptional risk for sudden death due to life-threatening cardiac arrhythmias. Patients afflicted with LQT3 exhibit a delay in cardiac repolarization manifest, as the name implies, as a prolonged QT interval on the ECG. The Brugada syndrome features a distinctive ST-segment elevation in ECG leads that reflect electrical forces in the right ventricle but is not associated with QT-interval prolongation.\(^4\) The peculiar ECG characteristics of these inherited syndromes are manifest in the more common life-threatening cardiac rhythm disorders arising from disease or drug intolerance\(^5\) and thereby serve as valuable models for understanding fundamental molecular mechanisms that underlie arrhythmias.

Na\(^-\) channels initiate excitability in most cardiac cells, opening abruptly to produce an inward depolarizing ionic current (\(I_{\text{Na}}\)). Once activated, the channels rapidly inactivate, extinguishing \(I_{\text{Na}}\) within 10 ms. Nonetheless, the myocyte remains depolarized for several hundred milliseconds, exhibiting the signature plateau that distinguishes the cardiac action potential. LQT3 mutations produce gain-of-function defects by slightly disrupting Na\(^+\) channel inactivation thereby causing a small but persistent \(I_{\text{Na}}\) during the action potential plateau.\(^6–8\) These mutations reside mainly at positions in or near the cytoplasmic linker between domains III and IV or the charged S4 segment in domain IV (Figure 1A), regions that critically influence the rapid inactivation of Na\(^+\) channels.\(^9,10\) In contrast, the SCN5A mutations linked to the Brugada syndrome (Figure 1A) reside at diverse loci that have not been consistently tied to a particular gating process. However, Brugada syndrome mutations have consistently produced loss of function.\(^11\) Although some Brugada-linked mutations encode Na\(^+\) channels that are entirely nonfunctional,\(^3\) others may elicit a functional deficit by further accelerating the rate of rapid inactivation.\(^12\)

The opposing nature of the functional effects of LQT3 and Brugada syndrome mutations on inactivation gating suggests
that the two disorders should not coexist in the same patient. Surprisingly, an inherited SCN5A mutation was recently described in which affected individuals exhibited the electrocardiographic manifestations of both syndromes: QT-interval prolongation and distinctive ST-segment elevations. In the present study, we identify the molecular mechanism whereby this single C-terminal aspartic acid insertion (1795insD) present in the Na$^+$ channel subunit (provided by A.L. George, Vanderbilt University). Whole-cell channel currents were recorded at 21°C within 1 to 3 days from cells cotransfected with an equimolar ratio of Na$^+$ channel protein and GFP reporter in HEK 293 cells. Cells were washed with buffer containing (in mmol/L) NaF 10, CsCl 20, EGTA 10, and HEPES 10 (pH 7.35 with NaOH). The bath solution contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 0.1% (0.41 ± 0.02 ms), and TTX 6% (0.79 ± 0.02 ms, and $\tau_d$ = 6.12 ± 0.71 ms. For 1795insD (n = 7), $A_0/A_1$ = 0.09 ± 0.01, $\tau_c$ = 0.81 ± 0.03 ms, and $\tau_d$ = 14.45 ± 1.33 ms. The wild-type and 1795insD $\tau_d$ values differed significantly (P < 0.001).

**Results**

We first examined the phenotypic effect of 1795insD on $I_{Na}$ measured during a sustained depolarization. Figure 1B shows wild-type and 1795insD $I_{Na}$ and reveals a persistent plateau of noninactivating current in the mutant that is nearly absent in wild-type cells. This current plateau is sensitive to 30 μmol/L tetrodotoxin (TTX) in wild-type and 1795insD mutant channels. The sustained component of the wild-type current at 200 ms of depolarization at -20 mV (as a fraction of the peak $I_{peak}$) was 0.41 ± 0.1% for wild type (n = 7) and 1.41 ± 0.2% for 1795insD (n = 7, P < 0.01 vs wild type). The decay phases of both wild-type and mutant $I_{Na}$ at -20 mV were also fitted to a biexponential function of the form $y = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$. For wild type (n = 7), $A_1/A_2$ = 0.12 ± 0.02, $\tau_1$ = 0.79 ± 0.02 ms, and $\tau_2$ = 6.12 ± 0.71 ms. For 1795insD (n = 7), $A_1/A_2$ = 0.09 ± 0.01, $\tau_1$ = 0.81 ± 0.03 ms, and $\tau_2$ = 14.45 ± 1.33 ms. The wild-type and 1795insD $\tau_2$ values differed significantly (P < 0.001).

**Materials and Methods**

Site-directed mutagenesis was performed on SCN5A cDNA cloned in pSP64T, as previously described. The 1795insD cDNA was then subcloned into the HindIII–XhoI sites of the expression vector pCGI (kindly provided by David Johns and Eduardo Marbán, Johns Hopkins University, Baltimore, Md) for bicistronic expression of the channel protein and GFP reporter in HEK 293 cells. Cells were cotransfected with an equimolar ratio of Na$^+$ channel human β1 subunit (provided by A.L. George, Vanderbilt University). Whole-cell Na$^+$ currents were recorded at 21°C within 1 to 3 days from HEK cells exhibiting green fluorescence using pipette resistances of 1 to 3 MΩ. (Axopatch 200B, Axon Instruments). The pipette solution contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 10, and HEPES 10 (pH 7.35 with NaOH). The bath solution contained (in mmol/L) NaCl 145, KCl 1, CaCl2, 1.8, MgCl2 1, HEPES 10, and glucose 10 (pH 7.35). Currents were sampled at 20 kHz (Digidata 1200 A/D board, Axon Instruments) and low pass–filtered at 5 kHz. The data were acquired using pClamp 8.0 (Axon Instruments) and analyzed using Clampfit (Axon Instruments). Voltage-clamp protocols are described with each figure (provided as insets). The results are expressed as mean ± SEM, and statistical comparisons were made using one-way ANOVA (Microcal Origin), with P < 0.05 indicating significance. Multiexponential functions were fitted to the data using nonlinear least-squares methods (Microcal Origin).
Figure 2. Voltage-dependent properties of 1795insD channel gating. A, Voltage dependence of activation (□, ■) and inactivation (○, ●) for wild-type (filled symbols) and 1795insD channels (open symbols). The voltage-clamp protocols are shown, and the data were fitted to a Boltzmann function (\(v = 1 + \exp((-V - V_1/2)/k)) \), where \(V_1/2\) is the half-maximal voltage and \(k\) is the slope factor. For activation, wild-type (n = 8) \(V_1/2 = -37.3\pm 2.3\) mV and \(k = -6.9\pm 0.64\) for 1795insD (n = 10), \(V_1/2 = -38.3\pm 2.05\) mV (\(P < 0.001\) vs wild type) and \(k = 7.9\pm 0.58\) (P = 0.23 vs wild type). For inactivation, wild-type (n = 7) \(V_1/2 = -88.5\pm 1.61\) mV and \(k = 6.2\pm 0.36\). For 1795insD (n = 8), \(V_1/2 = -98.2\pm 2.1\) mV (\(P < 0.001\) vs wild type) and \(k = 6.8\pm 0.24\) (P = 0.15 vs wild type). The mutation caused a negative shift in the voltage dependence of inactivation but did not alter activation. B, Separation of steady-state inactivation into fast (□, ■) and slow (○, ●) inactivation using the voltage-clamp protocols inset (see Results). The filled and open symbols indicate wild-type and 1795insD data, respectively. A larger fraction of channels underwent slow inactivation when depolarized. Solid lines indicate Boltzmann fits to the data. For slow inactivation, wild-type (n = 3) \(V_1/2 = -80.3\pm 5.4\) mV and \(k = 8.2\pm 1.1\) (n = 3), whereas 1795insD (n = 6) \(V_1/2 = -101.1\pm 2.2\) mV (\(P < 0.001\) vs wild type) and \(k = 8.4\pm 0.2\) (P = 0.78 vs wild type). For fast inactivation, wild-type (n = 6) \(V_1/2 = -55.7\pm 1.6\) mV and \(k = 6.5\pm 0.56\), whereas for 1795insD (n = 6) \(V_1/2 = -75.1\pm 2.62\) mV and \(k = 13.4\pm 0.43\) (both \(P < 0.001\) vs wild type).

phase of the current decay, and this component was slowed from 6.1±0.7 to 14.4±1.3 ms by the mutation (\(P < 0.001\)).

Nonetheless, these gain-of-function effects would not explain the coexistent ST-segment changes in 1795insD carriers. The effect of 1795insD on Na⁺ channel gating over a range of membrane potentials is examined in Figure 2A, which plots the voltage dependence of activation (squares) and inactivation (circles) for wild-type and mutant channels. The solid lines show Boltzmann curves fit to the data (see Figure 2A legend for parameters). Over a range of depolarized membrane potentials (clamp protocols, Figure 2A, inset), activation was not changed by the mutation. In contrast, over a range of holding potentials, inactivation of the mutant channel was altered: \(V_1/2\) was shifted negative by nearly 10 mV, from \(-88.5\pm 1.61\) to \(-98.2\pm 2.1\) mV (\(P < 0.003\)).

A negative shift in the voltage dependence of inactivation implies that the mutation stabilized inactivation, an observation in apparent conflict with the results in Figure 1B, which shows that at least a portion of the mutant channels exhibit impaired inactivation properties. We therefore considered the possibility that distinct kinetic components of inactivation gating, fast and slow inactivation, were affected differently by the mutation. The voltage dependence of fast and slow inactivation was examined individually using the protocols shown in Figure 2B (inset). To assess fast inactivation, brief test pulses (10 ms) were used to avoid slow inactivation. Because channels may undergo both fast and slow inactivation during a sustained depolarization, slow inactivation was assessed using longer test pulses, but a brief recovery period at \(-120\) mV was inserted to allow recovery from only fast inactivation. Figure 2B (squares) shows that nearly all of the wild-type and mutant channels were fast-inactivated (unavailable) at membrane potentials positive to \(-40\) mV (voltages near the action potential plateau), whereas only a portion of the channels slow-inactivated over the same membrane potential range (Figure 2B, circles). Moreover, nearly 50% of the mutant channels (open circles) were induced to slow-inactivate at membrane potentials positive to \(-40\) mV versus only \(\approx 20\%\) of the wild-type channels (filled circles). In addition, \(V_1/2\) for slow inactivation of the mutant channels shifted to more negative membrane potentials than wild type (\(-101.1\pm 2.2\) versus \(-80.3\pm 5.4\) mV, \(P < 0.003\)), suggesting that the mutant enhanced slow-inactivated state stability.

The \(V_1/2\) for fast inactivation was also shifted negatively by the mutation (\(-75.1\pm 2.6\) versus \(-55.7\pm 1.6\) mV, \(P < 0.001\), Figure 2B), but the magnitude of the shift was distorted by the much slower rate of fast inactivation at more hyperpolarized potentials. In additional experiments that examined \(I_{Na}\) availability after varying depolarization periods at \(-100\) mV, the time constant for development of fast inactivation was 35 ms for wild type but only 15 ms for 1795insD (data not shown). Hence, the measured \(I_{Na}\) availability after 10 ms (Figure 2B) underestimates the full extent of fast inactivation at these hyperpolarized membrane potentials. This discrepancy is greater for wild type than for 1795insD, explaining the rather marked shift between the 10-ms wild-type and mutant voltage-dependent availability curves, as well as the apparent change in the slope (Figure 2B). At the same time, prolonging the depolarization period to 20 to 30 ms would partly engage the slow inactivation process (see Figure 3A, \(\tau \approx 70\) ms) and if used in Figure 2B would tend to overestimate the extent of fast inactivation. Hence, the data in Figure 2B do not estimate steady-state fast inactivation. Rather, the data provide a snapshot that compares the extent of fast inactivation for the wild-type and mutant channels at 10 ms and suggest that fast inactivation of 1795insD over a range of hyperpolarized membrane potentials was hastened.

This enhancement in inactivation at membrane potentials negative to the channel-opening threshold (\(\approx -50\) mV)
would suggest that fast inactivation from closed states may have been augmented by the mutation. At the same time, Figure 1B shows clearly that the fast inactivation of open channels at $-20$ mV was partly disabled. In fact, the analysis of Figure 2B is insensitive to effects on fast inactivation of open channels, because only peak $I_{Na}$ after a period of depolarization is plotted. It is noteworthy that the destabilizing effect on fast inactivation of the open channel would predominate at the depolarized membrane potentials associated with the action potential plateau. Analogous opposing effects on fast inactivation from open and closed states were recently described for skeletal muscle Na$^+$ channel mutations linked to paramyotonia congenita.$^{15}$

Figure 3. Enhanced slow kinetic component of inactivation for 1795insD. A, Development of slow inactivation for wild type (filled symbols) and mutant (open symbols) was evaluated using the voltage-clamp protocol inset. The solid lines show least-squares fits to a single exponential function $y = Ae^{-t/\tau}$. The time constant $\tau$ for the development of slow inactivation was 69.5±25.0 ms for wild type ($n=4$) and 116.3±50.6 ms for mutant ($n=4$, $P=0.43$ versus wild type). However, the mutation increased the magnitude of the slow inactivation component ($A$) from 0.06±0.008 (wild type) to 0.19±0.01 (1795insD, $P<0.001$). B, Recovery from inactivation was also examined in the same cells using the protocol inset (same symbol convention). The recovery time course was well characterized by two kinetic components, representing fast and slow inactivation (shown in inset, on a logarithmic time axis), and was therefore fitted by a two-exponential function ($y=A_1e^{-t/\tau_1} + A_2e^{-t/\tau_2}$). For wild type and 1795insD, $\tau_1$ was 3.8±0.47 ms and 7.7±1.3 ms, respectively ($P=0.06$), and $\tau_2$ was 72.4±6.54 ms and 92.7±12.2 ms, respectively ($P=0.19$). $A_1$ was 0.57±0.04 for wild type and 0.66±0.03 for 1795insD ($P=0.17$), whereas $A_2$ was 0.13±0.005 for wild type and 0.2±0.006 for 1795insD ($P<0.001$).

To evaluate the kinetic features of enhanced 1795insD slow inactivation, we used a two-pulse protocol (Figure 3A). The duration of the first depolarization (P1) was varied, and the extent of slow inactivation was indicated by the fractional reduction in peak $I_{Na}$ during the P2 pulse relative to that recorded in the P1 pulse. A 10-ms repolarization to $-120$ mV was interposed between P1 and P2 to allow recovery from fast inactivation. Figure 3A shows that with increasing P1 duration, the current elicited by P2 decreases as a result of slow inactivation. The solid line shows a single exponential function ($y=Ae^{-t/\tau}$) fitted to the data. The amplitude ($A$) of the slowly inactivating component was significantly greater in the mutant (0.19±0.01) than wild type (0.06±0.01, $P<0.001$), a result consistent with Figure 2B. The time constant $\tau$ was $<200$ ms in both cases (see Figure 3A legend), consistent with the intermediate component of inactivation induced by relatively short depolarizations (hundreds of milliseconds), termed "$I_{Na}^{slow}$" to distinguish it from the classic slow-inactivated state induced by much longer periods of depolarization.$^{19}$

Recovery from inactivation was also evaluated with a paired-pulse protocol (Figure 3B) by depolarizing the cells for 1 second (P1) and varying the hyperpolarization interval before the P2 pulse. Both wild-type and mutant data followed a biphasic time course, reflecting components of recovery attributable to fast and slow inactivation, and were therefore fitted with a biexponential function (solid lines). The time constants for recovery from fast and slow inactivation did not differ significantly for the mutant and wild-type channels (see Figure 3B legend). The time constants for recovery from slow inactivation approached 100 ms and were similar to values reported previously for recovery from the $I_{Na}$ state.$^{17}$ The amplitude of the slow recovery component was greater for the mutant (0.2±0.01) than the wild-type channel (0.13±0.01, $P<0.001$), consistent with increased entry into the slow-inactivated state during the preceding 1-second depolarizing (P1) pulse, as demonstrated in Figure 3A.

The 1795insD mutation substantially increases the likelihood that channels will slow-inactivate during a depolarization lasting 100 to 200 ms (Figure 3A), well within the period of a cardiac action potential. By increasing the fraction of slow-inactivated channels, the mutation slows the overall rate of recovery of availability after a sustained depolarization (Figure 3B). These findings suggest that 1795insD should reduce Na$^+$ channel availability to a greater extent at rapid stimulation rates or during a premature contraction, where the hyperpolarized diastolic intervals between pulses are brief. To examine this prediction, we repeatedly depolarized wild-type or mutant channels for 0.5 seconds (0 mV) at cycle lengths of either 0.52 or 2.5 seconds (Figure 4A). These values were selected on the basis of the observed differences in wild-type and mutant recovery kinetics (Figure 3B) and also lie within clinically observed cycle lengths and diastolic intervals$^{20}$ (Figure 4E). Wild-type and mutant $I_{Na}$ elicited during the 1st, 2nd, and the 20th depolarizations are shown. At the slower cycle lengths (Figure 4A, top), the magnitude of $I_{Na}$ remained constant throughout the train of stimuli for both channels. However, at short cycle lengths, both wild-type and mutant $I_{Na}$ decreased during successive stimuli
This effect was more pronounced for the mutant, and the marked difference from wild type was apparent by the second beat. Data for a number of cells at both stimulation rates are summarized in Figure 4B. By the 20th stimulus at the rapid rate, $I_{Na}$ was decreased by 74.5% (n=5), whereas wild-type $I_{Na}$ was decreased by only 51.6% (n=6, $P=0.006$).

The observed time-dependent effect of stimulus rate on $I_{Na}$ availability (Figures 4A and 4B) was manifest in the ECG ST-segment changes of family members carrying the 1795insD mutation. Figure 4C shows the ECG tracing during rest (top) and with exercise (bottom). Although the ST-segment elevation at rest was moderate, it was profoundly increased during exercise. Figure 4D (top) shows that exercise nearly tripled the ST-segment elevation, from $\approx 1.2$ to 3.3 mm, and this trend mirrored the change in heart rate (Figure 4D, bottom). Conversely, Figure 4E plots the QT interval measured in the same patient (and a control patient) at various heart rates during the exercise test. The QT interval of the 1795insD carrier was markedly prolonged, only at slow heart rates; at an R-R interval of 1150 ms, the QT interval was 540 ms (504 ms corrected for heart rate, see Figure 4E legend). Consistent with this clinical effect, we observed a significant reduction in the maintained, noninactivating inward current when 1795insD channels were depolarized at high frequencies that did not allow full recovery from slow inactivation between pulses. For paired 500-ms depolarizations to $-20$ mV separated by a 50-ms interval at $-120$ mV, the second pulse-peak $I_{Na}$ was $90.5\pm1.4\%$ of the first pulse, but the maintained current of the second pulse was only $48.3\pm8.5\%$ of the first pulse (n=5, $P=0.0012$, data not shown). Hence, the mutation prolongs cardiac repolarization with a rate dependence opposite to that of ST-segment elevation but consistent with the classic long-QT phenotype.
Discussion

Fast-inactivated $\text{Na}^+$ channels normally remain quiescent during the action potential plateau and rapidly recover from inactivation during the hyperpolarized diastolic interval between stimuli. However, during sustained depolarization, $\text{Na}^+$ channels may enter stable, nonconducting conformational states that require a prolonged period of hyperpolarization to recover (slow inactivation). The classic slow-inactivated state has recovery time constants $>1$ second and is induced by much longer depolarizations ($\approx 60$ seconds) than considered in the present study. Although cardiac $\text{Na}^+$ channels are less prone to occupy this ultrastable state than their skeletal muscle counterparts, the cardiac channels do exhibit a more prominent intermediate kinetic component of slow inactivation ($I_{S\delta}$) that is induced by shorter depolarization periods, well within the length of the cardiac action potential. Our results (Figures 2 and 3) show that 1795insD channels have an even greater propensity to slow-inactivate over depolarization periods typical for the cardiac action potential, consistent with a functional effect on $I_{S\delta}$. This facilitated slow inactivation reduced the availability of $\text{Na}^+$ channels at rapid stimulation rates (Figures 4A and 4B). Conversely, at slow stimulation rates where the recovery interval is prolonged, slow-inactivated channels can recover fully, so peak $I_{S\delta}$ was not affected by the mutation (Figures 4A and 4B).

The anticipated proarrhythmic manifestation of the small, persistent inward $\text{Na}^+$ current caused by disrupted fast inactivation (Figure 1B) is prolonged action potential duration. This delay in myocyte repolarization, apparent on the ECG as QT-interval prolongation, was recently validated in an elegant quantitative model that incorporated the LQT3 fast inactivation gating defect. In that study, the maintained current and other LQT3 gating characteristics were reproduced by incorporating a low-frequency gating mode. This gating mode permitted bursts of openings throughout the action potential plateau due to a destabilized fast inactivation process. The bursting mode did not allow slow inactivation, in contrast to the higher frequency background gating mode that did allow channels to both fast- and slow-inactivate. Our finding that high-frequency depolarization suppressed maintained current even more than peak $I_{S\delta}$ may be explained by this model, because frequent depolarization may pull the gating equilibrium toward the background mode, permitting slow inactivation but preventing bursting. The frequency-dependent effects of membrane potential on slow inactivation and maintained current in disease-linked $\text{Na}^+$ channel mutants deserve further investigation.

Less is known about the mechanisms linking $\text{Na}^+$ channel gating to the ECG manifestations of the Brugada syndrome. However, repolarization in the epicardial cell layer is known to be more sensitive to untoward suppression of peak $I_{S\delta}$ (eg, by ischemia or drugs) due to a more prominent repolarizing $K^+$ current in this layer ($I_{K\alpha}$). It is therefore postulated that mutations that reduce peak $I_{S\delta}$ could selectively hasten epicardial repolarization, creating a transmural gradient for repolarization in the right ventricular outflow tract, consistent with the observed right precordial ST-segment changes in Brugada syndrome.

Studies of 1795insD with whole-cell recordings from *Xenopus* oocytes did not identify a persistent inward current, nor did they reveal slow gating defects that could explain the dual clinical phenotype. Using expressed $\text{Na}^+$ channels in cultured mammalian cells to allow improved voltage-clamp conditions, we show that 1795insD enhances $\text{Na}^+$ channel slow inactivation and thereby reduces peak $I_{S\delta}$ predominantly at rapid stimulation rates. This rate-dependent effect on $I_{S\delta}$ availability mimics the exercise-dependent ST-segment changes in the affected carrier, suggesting that augmented slow inactivation is the molecular mechanism underlying the observed ECG changes during exercise. At the same time, we detected a plateau of persistent $\text{Na}^+$ current during sustained depolarization, indicative of impaired fast inactivation. This gating effect readily explains the QT prolongation observed at slow heart rates, typical for the LQT3 phenotype.

These results suggest that patients with both LQT3 and Brugada syndrome would benefit from heart rate control, and, indeed, pacemaker therapy has proved to be beneficial in this particular family. In the on-demand mode (VVIR or even AAIR), pacing only occurs when the intrinsic heart rate drops (generally below 50 bpm). As such, higher pacing rates are not induced, and the problem of pacing-induced ST-segment elevation has not occurred. Given the detrimental effect of exercise on the ST segments of 1795insD carriers (Figure 4D), $\beta$-adrenergic stimulation may also exacerbate the ST-segment elevation of 1795insD carriers, although this may be less predictable. Studies of patients with other Brugada mutations have found that unopposed $\beta$-adrenergic stimulation may improve right precordial ST-segment elevation, presumably by increasing the L-type $\text{Ca}^{2+}$ current in a manner that restores the epicardial action potential dome and thereby reducing electrical heterogeneity.

The fact that a single mutation in the C-terminus alters both the fast and slow components of inactivation suggests these kinetically distinct gating processes may interact functionally and even share structural characteristics. Although previous studies have shown that a nearby mutation in the carboxy terminus (E1784K) disrupts fast inactivation and provokes the long-QT syndrome, our findings suggest that the C-terminus may have a mechanistic role in the intermediate ($I_{M\alpha}$) component of slow inactivation. Recent studies have postulated a mechanistic link between the $I_{M\alpha}$ gating component and use-dependent local anesthetic action. Hence, an increased propensity for $\text{Na}^+$ channels to enter the $I_{S\delta}$ state may not only explain the heart rate-dependent ECG phenotype (ST elevation) but also could underlie the unusual sensitivity of Brugada syndrome patients to potent $\text{Na}^+$ channel blocking agents. Our findings reveal a novel molecular mechanism for the Brugada ECG phenotype and clarify how *SCN5A* mutations at a single locus may engender more than one arrhythmia syndrome by influencing kinetically distinct components of $\text{Na}^+$ channel gating function.

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References


Two Distinct Congenital Arrhythmias Evoked by a Multidysfunctional Na⁺ Channel
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