Ionic Mechanisms Responsible for the Electrocardiographic Phenotype of the Brugada Syndrome Are Temperature Dependent

Robert Dumaine, Jeffrey A. Towbin, Pedro Brugada, Matteo Vatta, Dmitri V. Nesterenko, Vladislav V. Nesterenko, Josep Brugada, Ramon Brugada, Charles Antzelevitch

Abstract—The Brugada syndrome is a major cause of sudden death, particularly among young men of Southeast Asian and Japanese origin. The syndrome is characterized electrocardiographically by an ST-segment elevation in V1 through V3 and a rapid polymorphic ventricular tachycardia that can degenerate into ventricular fibrillation. Our group recently linked the disease to mutations in \( \text{SCN5A} \), the gene encoding for the \( \alpha \) subunit of the cardiac sodium channel. When heterologously expressed in frog oocytes, electrophysiological data recorded from the Thr1620Met missense mutant failed to adequately explain the electrocardiographic phenotype. Therefore, we sought to further characterize the electrophysiology of this mutant. We hypothesized that at more physiological temperatures, the missense mutation may change the gating of the sodium channel such that the net outward current is dramatically augmented during the early phases of the right ventricular action potential. In the present study, we test this hypothesis by expressing Thr1620Met in a mammalian cell line, using the patch-clamp technique to study the currents at 32°C. Our results indicate that Thr1620Met current decay kinetics are faster when compared with the wild type at 32°C. Recovery from inactivation was slower for Thr1620Met at 32°C, and steady-state activation was significantly shifted. Our findings explain the features of the ECG of Brugada patients, illustrate for the first time a cardiac sodium channel mutation of which the arrhythmogenicity is revealed only at temperatures approaching the physiological range, and suggest that some patients may be more at risk during febrile states. (Circ Res. 1999;85:803-809.)

Key Words: Brugada syndrome ■ \( \text{Na}^+ \) channel ■ temperature

Polymorphic ventricular tachycardia (VT) and ventricular fibrillation (VF) developing in patients with structurally normal hearts accounts for 5% to 12% of the >300 000 sudden deaths of Americans each year.\(^1,2\) Approximately half of these are attributed to the Brugada syndrome, a familial disease electrocardiographically characterized by a downsloping ST-segment elevation terminating in a negative T wave in the right precordial leads, an apparent right bundle branch block,\(^3,4\) and rapid polymorphic VT capable of degenerating to VF.\(^5,6\) Slightly prolonged H-V intervals are observed in 60% of patients\(^7\) with Brugada syndrome.

Chen et al\(^8\) recently uncovered the first gene defects linked to the Brugada syndrome, identifying different mutations in \( \text{SCN5A} \), the cardiac sodium channel gene, in each of the 3 families studied. A frameshift mutation resulted in an in-frame stop codon in the pore region of domain III in one family. Because the syndrome has an autosomal dominant pattern of inheritance, the frameshift mutation is likely to result in a decrease in the number of functional channels, which can explain the clinical manifestation of the syndrome.\(^3,4\) Missense mutations involving a double substitution of arginine at position 1232 by a tryptophan (Arg1232Trp) and the threonine at position 1620 by a methionine (Thr1620Met) were also found. Heterologous expression of these mutant channels in \( \text{Xenopus} \) oocytes revealed that the Thr1620Met mutation shifts steady-state inactivation of the channels toward more positive potentials and accelerates reactivation, whereas Arg1232Trp did not produce these effects and is thought to be a rare polymorphism. The electrophysiological profile reported for this mutant at room temperature does not adequately explain the ECG signature of the Brugada syndrome, however. We hypothesized that, at more physiological temperatures, the missense mutation may accelerate the decay of the sodium current, thus leaving the large transient outward current normally present in right ventricular epicardium unopposed. To test this hypothesis, we studied the kinetics of the current at 32°C using the patch-clamp technique.
Materials and Methods

The coding sequence of hH1a wild type (WT) was cloned in the pGEM-3 (Promega) expression vector.\textsuperscript{9} and the Thr1620Met mutation was produced by site-directed mutagenesis as previously described.\textsuperscript{10,11} The clones were then cut out of pGEM-3 by restriction digest and ligated into pCignal3.1 (Invitrogen) for expression in mammalian cells. Cells were transfected using the Ca\textsuperscript{2+} phosphate precipitation method, as previously described.\textsuperscript{12} The cells were grown in a polystyrene-coated 35-mm culture dish and placed in a heating chamber for electrophysiological measurements (Medical Systems). For patch-clamp experiments, the bath solution contained (in mmol/L) KCl 2, NaCl 150, CaCl\textsubscript{2} 1.5, MgCl\textsubscript{2} 1, glucose 10, and tetraethylammonium 5, at pH 7.2 (NaOH). The pipette solution contained (in mmol/L) NaCl 35, HEPES 10, EGTA 10, and CsF 105, at pH 7.4 (CsOH) and 309 mosm (sucrose). Microelectrodes were drawn from Corning 7052 glass tubing, and microelectrode resistance varied between 0.8 and 1.4 M\textOmega when measured in the extracellular solution. Capacitance and series resistances were adjusted to obtain minimal contribution of the capacitive transients. A 70% to 80% compensation of the series resistance was usually achieved without ringing. Currents were recorded with an Axopatch 200A amplifier (Axon Instruments) and digitally stored on the hard disk of an IBM-compatible computer. Analysis was done with the Axon PCLAMP V.7 suite of software (Axon Instruments). Where indicated, the fit to a Boltzmann distribution function was obtained by fitting the data to $I / [1 + e^{(V - V_{m} - 0.5k)/D}]$, where $V_{m}$ represents the membrane potential, $V_{m}$ is the midpoint of activation, and $k$ the slope factor. The temperature sensitivity coefficient $Q_{10}$ was obtained by expression $\ln(Q_{10}) = 10/\Delta T \times \ln(T_{2}/T_{1})$, where $T_{1}$, $T_{2}$, and $T_{0}$ represent the temperature and the time constant values at the initial and final temperatures, respectively. Statistical significance was determined using ANOVA or paired Student t test.

Cardiac Action Potential (AP) Modeling

We simulated endocardial, left ventricular epicardial, and right ventricular epicardial APs using a modified version of the Luo-Rudy 2 model (see Appendix for the model expressions). Activation of this current is relatively fast, with time to peak to 1.7 ms at +10 mV; the steady-state activation curve is sigmoid, with $V_{0.5} = -4 mV$ (3 activation gates) and a slope of 11.5 mV. The inactivation time constant is 10 ms over the voltage range above -20 mV (threshold = -30 mV), and the reactivation time constant is 67 ms at -80 mV (but 450 ms at -50 mV). Maximal conductance of $I_{Na}$ ($G_{Na}$) was set to 0 endocardial AP, 0.5 for left ventricular epicardial AP, and 1.1 for right ventricular epicardial AP. (1) The model of the transient inward potassium current was incorporated into the Luo-Rudy 2 model (see Appendix for the model expressions). Activation of this current is relatively fast, with time to peak to 1.7 ms at +10 mV; the steady-state activation curve is sigmoid, with $V_{0.5} = -4 mV$ (3 activation gates) and a slope of 11.5 mV. The inactivation time constant is 10 ms over the voltage range above -20 mV (threshold = -30 mV), and the reactivation time constant is 67 ms at -80 mV (but 450 ms at -50 mV). Maximal conductance of $I_{Na}$ ($G_{Na}$) was set to 0 endocardial AP, 0.5 for left ventricular epicardial AP, and 1.1 for right ventricular epicardial AP. (2) The maximal conductance of $I_{Na}$ was decreased by 20% to 50% in epicardial models to obtain realistic durations of simulated APs. (3) To reproduce faster current decay for Thr1620Met mutant as compared with the WT sodium channel, we multiplied both rate constants for inactivation gate h ($\alpha_{h}$ and $\beta_{h}$) by a factor of 2, which leaves steady-state inactivation characteristics unchanged. The faster inactivation of $I_{Na}$ resulted in a 32% smaller peak current during upstroke of AP. Parameters of the model assume that the APs are simulated at the normal body temperature of 37°C. The effects of a faster rate of AP.

Results

Recordings of macroscopic sodium currents at physiological temperatures are technically challenging. To avoid changes in the kinetics of the current or its voltage relationship due to modified solutions and to minimize errors due to uncompensated series resistance, the experiments used for the statistical analysis were done at 22°C and 32°C. We then routinely tested at higher temperatures, on cells transfected with lower amounts of cDNA and expressing low amplitude currents, the predictions from the experiments at lower temperature. Figure 1 shows whole-cell currents recorded 2 days posttransfection using low-resistance patch-clamp electrodes (0.6 to 1.0 M\Omega). The current waveforms and current-voltage relations (IVRs) were similar in WT (n = 5) and Thr1620Met (n = 4) at 22°C. At 32°C, the activation and the decay of $I_{Na}$ was faster for Thr1620Met (Figure 1A) resulting in a steeper IVR (Figure 1B) and a peak of IVR 10 mV more positive when compared with WT (n = 6). In the experiments shown, the voltage errors due to uncompensated series resistance at 32°C were 0.4 and 0.9 mV for WT and Thr1620Met, respectively, as estimated from the capacitive artifacts.

Figure 1. Whole-cell current for WT and Brugada syndrome mutant (Thr1620Met) in transiently transfected TSA201 cells at room temperature (22°C) and 32°C. A, Location of the missense mutations Arg1232Trp (R1232W) and Thr1620Met (T1620M) previously described by Chen et al.\textsuperscript{8} Shown are current recordings obtained at different test potentials from -70 to -25 mV (32°C) and -65 to -20 (22°C) in increments of 5 mV from a holding potential of -120 mV for 4 representative cells. B, Normalized peak IVRs for the WT channels (■) and Thr1620Met mutants (●) at 22°C and 32°C (□ and ○) from 4 to 6 different cells at each temperature (see text).
To elucidate the basis for the shift of IVR, we examined the temperature dependence of steady-state activation and inactivation (Figure 2). At 22°C, we observed no significant difference between the mid-activation potential for WT (−243.9 ± 0.8 mV) and Thr1620Met (−239 ± 6 mV) (Boltzmann function fit, Figure 2A). At 32°C, steady-state activation for Thr1620Met was significantly shifted toward more positive potentials when compared with WT (P < 0.001), with mid-potential values of −238.8 ± 0.4 mV and −249.5 ± 0.4 mV, respectively, and was less sensitive than WT to changes of membrane potential with slope factors (k) of 5.4 ± 0.4 mV and 6.8 ± 0.4 mV, respectively (P < 0.05). Steady-state inactivation protocols (Figure 2B) revealed no significant differences between WT and Thr1620Met at 22°C and 32°C with half-inactivation voltages, as follows: −91.5 ± 0.1 mV and −91.9 ± 0.2 mV (22°C) and −87.1 ± 0.2 mV and −86 ± 0.2 mV (32°C) for WT and Thr1620Met, respectively (Figure 2C).

We next looked for changes in the current kinetics of I_{Na} (Figure 3) in a range of potentials encompassing the plateau of the AP and fitted the decay of the current to a sum of 2 exponentials. The fast component accounted for 87 ± 3% and 83 ± 5% (WT) and 88 ± 4% and 90 ± 3% (Thr1620Met) of the total amplitude of the current at 22°C and 32°C, respectively. The time constant of the fast component (τ) of the current decay was similar for WT and Thr1620Met at 22°C between −50 and −20 mV, but significantly faster for Thr1620Met at 32°C (Figure 3B). At a potential of 10 mV, I_{Na} fully inactivated 4 ms sooner in Thr1620Met than in WT (Figure 3A).

To predict the changes in the decay of the sodium current in more physiological conditions, we did a set of experiments at temperatures between 35°C and 42°C and a holding potential of −80 mV. In this set of experiments, the amount of cDNA used for transfection was reduced by 75%, yielding currents with maximal amplitudes between 1 and 2 nA, filtering of the signal was decreased to 10 kHz, and acquisition was increased to 50 kHz to minimize aliasing caused by bandwidth limitations. Figure 4 shows that the decay of the Thr1620Met current is more rapid than WT in physiological conditions, as predicted by the experiments at 32°C. We found Q_{10} values of 1.2 and 2.3 for WT and Thr1620Met, respectively, for the current decay at 0 mV.
We measured the rate of recovery of $I_{Na}$ by varying the interpulse interval of twin pulses to 0 mV from a holding potential of $-110$ mV (Figure 5). At 22°C, recovery was slightly faster for Thr1620Met ($\tau=23.1\pm0.4$ and $17.4\pm0.2$ ms for WT and Thr1620Met, respectively). At 32°C, $\tau$ was much slower for Thr1620Met when compared with WT. A double exponential fit to the data yielded time constants of $1.1\pm0.1$ (84%) and $3.7\pm0.6$ (16%), $4.8\pm0.3$ (90%), and $48\pm5$ (10%) ms for WT and Thr1620Met, respectively. The data of Chen et al. showed a convergence of recovery kinetics at $-110$ mV. Our data suggested that this convergence does not exist at 32°C. We therefore checked to see whether a slowing of recovery at $-80$ mV could be observed at 37°C in 3 cells. Figure 6 shows that recovery of WT current was 93% complete after 60 ms. By contrast, Thr1620Met currents were slower to recover, as expected from the measurements at 32°C, with still 95% of channels available after 100 ms.

To gain more insight into the arrhythmogenic effects of a faster decay of the sodium current ($I_{Na}$), we simulated APs from the endocardium, epicardium of the left ventricle, and epicardium of the right ventricle, which is known to exhibit a prominent transient outward current $I_{to}$ in several species including human. In our simulation (Figure 7), the faster inactivation of $I_{Na}$ increased the net outward current and lowered the voltage level at the end of phase 1 (Figure 7B and 7C, notches) with little effect on the configuration of the other phases of the endocardial and left epicardial APs. In the right ventricular epicardium, however, the acceleration of the $I_{Na}$ decay brings this voltage below the calcium current ($I_{CaL}$) threshold, thus eliminating the dome and triggering all-or-none repolarization (Figure 7C).

### Discussion

Our data suggest that the threonine at position 1620 in the coding sequence of SCN5A is an important determinant of the temperature sensitivity of the human cardiac sodium channel. Taking excitation, the inward sodium current is opposed by the transient outward $I_{to}$. As $I_{to}$ decays, the relative contribution of $I_{to}$ to the net current gradually increases and creates a notch resulting in the spike-and-dome morphology of the right epicardial AP plateau. At 32°C, the missense mutation Thr1620Met leads to faster decay and slower reactivation of the current. The more rapid decay of $I_{Na}$ would be expected to leave $I_{to}$ unopposed, thus accentuating phase 1 of the AP. The outward shift of current flowing during phase 1 can lead to loss of the AP dome (plateau) as a result of an all-or-none repolarization at the end of phase 1. This phenomenon is observed in cells and tissues displaying a prominent $I_{to}$ such as right ventricular epicardium, but not those exhibiting a small $I_{to}$ such as ventricular endocardium. Our AP simulation confirmed this mechanism. Loss of the dome in right ventricular epicardium but not in endocardium creates a transmural voltage gradient that may serve as a basis for the ST-segment elevation observed clinically and for aberrations in the J wave of the ECG. The resulting transepicardial and transmural dispersion of repolarization can also give rise to phase 2 reentrant extrasystoles capable of precipitating a rapid polymorphic VT/VF, the reentrant arrhythmias responsible for sudden death in the Brugada syndrome.

Our simulation also led to another interesting prediction. Because we wanted to alter the model minimally, we only increased the inactivation rate of $I_{Na}$ with no modifications of the activation kinetics. This resulted in a 32% decrease in the amplitude of the peak sodium current. We initially expected this decrease to reduce the upstroke velocity of the AP and the activation of $I_{to}$ and, ultimately, limit the AP plateau depression. Our simulation suggests that the amplitude of $I_{Na}$ is not very sensitive to small variation in the AP upstroke rate. Therefore, reduction of $I_{Na}$ density or acceleration of the inactivation is likely to be very effective in modulating phase 1 by leaving the fully activated $I_{to}$ unopposed.

We observed a 10-mV shift in steady-state activation. Our measurements of the maximal current are accurate within 1 mV of the voltage imposed in the range 6 to 9 nA at 32°C. Therefore, the voltage shift cannot be attributed to uncompensated series resistance. Clinically, this 10-mV positive shift of steady-state activation would be expected to raise the activation threshold and thus lead to a mild slowing of conduction. This change in excitability may be responsible for the small H-V interval prolongation and right bundle branch block observed in many patients with the Brugada syndrome.

WT and Thr1620Met channels displayed similar steady-state inactivation curves at all temperatures studied. This finding contrasts with the results of Chen et al., possibly because of differences in expression systems (Xenopus oocytes versus HEK cells) and/or specific protocols. It is noteworthy that whole-cell measurements of $I_{Na}$ produce a time-dependent shift of the steady-state inactivation parameters, which is not observed with the agar cushioned 2-electrode voltage-clamp technique applied on frog oocytes. We routinely checked for time-dependent shifts in the mid-
potential of steady-state inactivation ($V_{0.5}$) and obtained rates of $20.42 \pm 0.05$ mV/min ($n = 3$) and $20.27 \pm 0.02$ mV/min ($n = 4$) for WT and Thr1620Met, respectively, at 32°C and $20.13 \pm 0.08$ mV/min ($n = 3$) and $20.11 \pm 0.15$ mV/min ($n = 3$), respectively, at room temperature. The rates were more rapid during the first 8 to 10 minutes and then remained stable below 0.05 mV/min in all the experiments. Most of the data were obtained after 20 minutes in whole-cell configuration. Therefore, our measurements of $V_{0.5}$ are off by 6 to 8 and 2 to 3 mV at 32°C and room temperature, respectively, when compared with the initial midpotential. This may have contributed to the disparate results of the 2 studies.

Our data demonstrate a faster decay of the sodium channel but slower recovery from inactivation for the Thr1620Met mutant at physiological temperature(s). Therefore, the channels are likely to spend more time in the inactivated states, suggesting that the mutation has a stabilizing effect on inactivation of the channel. This is opposite to the effects of mutations observed in the LQT3 form of long QT syndrome, in which a late sodium current is amplified because of destabilized (less absorbent) inactivated states. Given the location of the Thr1620Met mutation in a region strongly involved in the activation-inactivation coupling, we speculate that the effects may be the result of changes in activation and/or deactivation altering this coupling. The exact mechanism for the acceleration of the current decay remains to be established by an in-depth biophysical study of the single-channel behavior of the Thr1620Met mutant.

To our knowledge, this is the first report of a cardiac sodium channel mutation involved in a genetic disease that reveals its arrhythmogenic potential primarily at temperatures approaching the physiological range. The results suggest caution in the interpretation of data obtained from heterologous expression systems at room temperature.
temperature therefore cannot fully compensate for the influence of
potassium ions

Figure 7. Effect of accelerated inactivation of \( I_{th} \) on the simu-
lated propagating endocardial (A), left ventricular epicardial (B),
and right ventricular epicardial (C) APs at 37°C (see Materials
and Methods). Each panel shows 2 APs simulated with normal
kinetic of \( I_{th} \) inactivation (solid lines) and with inactivation rates
increased 2 times (dashed lines), as predicted from the \( Q_{10} \)
of the Thr1620Met channel.

On the basis of our \( Q_{10} \) measurements, the decay of the
Thr1620Met current is 2.4, 3, and 3.4 times faster than WT at 37°C,
39°C, and 40°C, respectively. Furthermore, Li et al\(^{15}\) showed that
the inactivation kinetics of \( I_{th} \) was 2 times faster at 36°C than at
room temperature. The speeding of the inactivation of \( I_{th} \) at a higher
temperature therefore cannot fully compensate for the influence of
\( I_{th} \) on the balance of currents during the early phase 1 repolarization.
These results suggest that the increased temperature sensitivity of
the Thr1620Met current decay may predispose some Brugada
patients to arrhythmias during a febrile state (fever).

Appendix

Model of the Transient Outward Current, \( I_{to} \)

\[ I_{to} = G_{to} \times z \times y \times R(V) \times (V - E_{K}), \]

where \( E_{K} \) is the reversal potential for potassium ions

\[ \alpha_{i}(V) = \frac{10 \times \exp \left( \frac{V - 40}{25} \right)}{1 + \exp \left( \frac{V - 40}{25} \right)} \]

\[ \beta_{i}(V) = \frac{10 \times \exp \left( \frac{V + 90}{25} \right)}{1 + \exp \left( \frac{V + 90}{25} \right)} \]

\[ R(V) = \exp \left( \frac{V}{100} \right) \]

\[ \alpha_{f}(V) = \frac{0.015}{1 + \exp \left( \frac{V + 60}{5} \right)} \]

\[ G_{m} = 0.5 \text{ mS/\mu F for the left ventricular epicardium; } G_{m} = 1.1 \text{ mS/\mu F for the right ventricular epicardium. } V, \text{ transmembrane} \]

\[ \text{voltage (mV); } G_{m}, \text{ maximum conductance of the channel (mS/\mu F); } z \text{ and } y, \text{ activation gate (3 gates per channel) and inactivation gate,} \]

\[ \text{respectively; } R(V), \text{ outward rectification factor for the channel; } \alpha_{i} \text{ and } \beta_{i}, \text{ voltage-dependent opening and closing rate constants of} \]

\[ \text{activation gate (msec}^{-1}); \alpha_{f} \text{ and } \beta_{f}, \text{ voltage-dependent opening and} \]

\[ \text{closing rate constants of inactivation gate (msec}^{-1}). \]

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