Gating-Dependent Mechanisms for Flecainide Action in SCN5A-Linked Arrhythmia Syndromes

Prakash C. Viswanathan, PhD; Connie R. Bezzina, PhD; Alfred L. George, Jr, MD; Dan M. Roden, MD; Arthur A.M. Wilde, MD, PhD; Jeffrey R. Balser, MD, PhD

Background—Mutations in the cardiac sodium (Na) channel gene (SCN5A) give rise to the congenital long-QT syndrome (LQT3) and the Brugada syndrome. Na channel blockade by antiarrhythmic drugs improves the QT interval prolongation in LQT3 but worsens the Brugada syndrome ST-segment elevation. Although Na channel blockade has been proposed as a treatment for LQT3, flecainide also evokes “Brugada-like” ST-segment elevation in LQT3 patients. Here, we examine how Na channel inactivation gating defects in LQT3 and Brugada syndrome elicit proarrhythmic sensitivity to flecainide.

Methods and Results—We measured whole-cell Na current (I_{Na}) from tsA-201 cells transfected with ΔKPQ, a LQT3 mutation, and 1795insD, a mutation that provokes both the LQT3 and Brugada syndromes. The 1795insD and ΔKPQ channels both exhibited modified inactivation gating (from the closed state), thus potentiating tonic I_{Na} block. Flecainide (1 μmol/L) tonic block was only 16.8±3.0% for wild type but was 58.0±6.0% for 1795insD (P<0.01) and 39.4±8.0% (P<0.05) for ΔKPQ. In addition, the 1795insD mutation delayed recovery from inactivation by enhancing intermediate inactivation, with a 4-fold delay in recovery from use-dependent flecainide block.

Conclusions—We have linked 2 inactivation gating defects (“closed-state” fast inactivation and intermediate inactivation) to flecainide sensitivity in patients carrying LQT3 and Brugada syndrome mutations. These results provide a mechanistic rationale for predicting proarrhythmic sensitivity to flecainide based on the identification of specific SCN5A inactivation gating defects. (Circulation. 2001;104:1200-1205.)

Key Words: flecainide ■ long-QT syndrome ■ ion channels ■ pharmacology

Cardiac Na channels continuously undergo voltage-dependent structural rearrangements: “activation” allows Na permeation, whereas “inactivation” processes terminate current during the action potential plateau (other mechanisms are described). In contrast, Brugada syndrome is characterized by distinctive ST-segment elevations and is typically associated with mutations that enhance Na channel inactivation.

Despite their proarrhythmic potential, antiarrhythmic agents with Na channel blocking (class I) activity have been widely used to treat life-threatening cardiac arrhythmias. Class I agents may be particularly useful in managing patients with LQT3 disorders through blockade of the sustained inward current through the mutant channels. In contrast, Na channel blockade exacerbates the ECG pattern in Brugada syndrome. Despite this apparent divergence between the clinical manifestations of Na channel blockade in the LQT3 and Brugada syndromes, it was recently shown that flecainide, a potent Na channel blocker, produced ST-segment elevation characteristic of Brugada syndrome in a number of LQT3 patients, suggesting that Na channel blockade may have proarrhythmic potential in LQT3.

Recognizing the mechanistic role of cardiac Na channel inactivation in antiarrhythmic drug action, these observations suggest that LQT3 channels may possess multiple, distinct inactivation defects that evoke both antiarrhythmic and proarrhythmic flecainide sensitivity. Recently, an SCN5A mutation was identified that provokes the ECG features of both LQT3 and Brugada syndrome (1795insD) and worsening ST-segment elevation with Na channel blockade. We exploited the unique characteristics of this channel to probe the linkage between Na channel inactivation and flecainide blockade. Our findings provide a framework for understand-
Increased compared with WT (Figure 2, inset: 1795insD was transfected tsA-201 cells after a sustained (500-ms) holding depolarization to 21°C as described previously. The pipette solution contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 10, and HEPES 10 (pH 7.35 with CsOH); the bath solution contained NaCl 145, KCl 4, CaCl₂ 1.8, MgCl₂ 1, and HEPES 10 (pH 7.35). Flecainide (acetate salt) was made up in a 10-mmol/L stock solution and diluted in the bath solution (1 μmol/L), and data were recorded after a 3-minute period for drug equilibration. Voltage-clamp protocols were described with each figure, results are expressed as mean±SEM, and statistical comparisons and curve fitting were performed with Microcal Origin.

Methods

The 1795insD and ΔKPQ mutant channels were prepared as previously described and were subcloned into the expression vector pCGI (provided by David Johns, Johns Hopkins University, Baltimore, MD) for bicistronic expression of the channel protein and GFP reporter in tsA-201 cells. Cells were cotransfected with an equimolar ratio of Na channel human β₁ subunit. Whole-cell Na currents were recorded at 21°C as described previously. The pipette solution contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 10, and HEPES 10 (pH 7.35 with CsOH); the bath solution contained NaCl 145, KCl 4, CaCl₂ 1.8, MgCl₂ 1, and HEPES 10 (pH 7.35). Flecainide (acetate salt) was made up in a 10-mmol/L stock solution and diluted in the bath solution (1 μmol/L), and data were recorded after a 3-minute period for drug equilibration. Voltage-clamp protocols were described with each figure, results are expressed as mean±SEM, and statistical comparisons and curve fitting were performed with Microcal Origin.

Results

Mutations Augment Closed-State Inactivation and Tonic Block

Figure 1 shows the precordial leads from a 1795insD carrier who exhibited minor baseline ST-segment elevation at baseline (left). After intravenous flecainide (2 mg/kg over 10 minutes), a marked increase in ST-segment elevation (Figure 1, right) was observed, and the rate-corrected QT interval did not shorten (QTc remained ≈470 ms). We therefore examined the sensitivity of 1795insD channels to flecainide. Figure 2 shows wild-type (WT) and mutant $I_{Na}$ recorded from transfected tsA-201 cells after a sustained (500-ms) holding period at −100 mV; currents recorded from the same cell before and after exposure to a clinically relevant concentration of flecainide (1 μmol/L) are superimposed. This “tonic block” of peak $I_{Na}$ for 1795insD channels was markedly increased compared with WT (Figure 2, inset: 1795insD was 58.0±6.0% versus 16.8±3.0% for WT, $P<0.01$). Previous studies indicate that antiarrhythmic drugs may bind avidly to the inactivated conformational state of the cardiac Na channel. Although cardiac Na channels typically inactivate from open states when depolarized, the channels also inactivate directly from closed (rested) states while hyperpolarized. Given the observed increase in 1795insD tonic block at a relatively hyperpolarized holding potential (Figure 2), we tested the hypothesis that LQT3 mutations may enhance inactivation from closed states at membrane potentials near $V_{Na}$ and thereby confer higher flecainide block sensitivity. Previous studies have demonstrated that the 1795insD mutation induces a hyperpolarizing shift in the voltage-dependence of steady-state availability, a finding that could be explained by enhanced closed-state inactivation gating. To examine closed-state inactivation directly, we measured the peak $I_{Na}$ elicited by a test pulse to −20 mV after subthreshold depolarizations (−90 or −100 mV) of incremental duration (protocol inset, Figure 3A). Peak $I_{Na}$ gradually decreased as the period of mild prepulse depolarization increased. The solid lines indicate least-squares fits of a single exponential function ($y=y_0+Ae^{-t/\tau}$) to the data at each voltage (fitted parameters at −100 mV in Table 1). Relative to the peak $I_{Na}$ available at −120 mV, the extent of closed-state inactivation (A) was increased in 1795insD, and the rate of development of closed-state inactivation was also accelerated ($\tau$ for WT was 42.4±5.4 ms versus 12.0±0.9 ms for 1795insD, $P<0.001$). Figure 3B examines the effects of flecainide at −100 mV in the same group of cells as studied in panel A. Representative 1795insD $I_{Na}$ recorded before (from panel A) and after flecainide exposure are also shown superimposed for the shortest (1 ms) and longest (500 ms) prepulse to −100 mV (Figure 3B, inset). Flecainide substantially increased the extent to which subthreshold depolarizations reduced peak $I_{Na}$, and this effect was more pronounced in the mutant channel. Notably, the time constant ($\tau$) for reduction in peak $I_{Na}$ during drug exposure mirrored the values measured for closed-state inactivation in drug-free conditions (Table 1). At −100 mV (Figure 3B), $\tau$ in flecainide was 33.8±3.6 ms for WT ($P=NS$ versus drug-free) and 10.9±1.7 ms for 1795insD ($P=NS$ versus drug-free). Hence, drug-exposed channels became unavailable, with a kinetic signature that recapitulated closed-state inactivation in drug-free conditions, sug-
gesting that the gating process potentiates tonic block at membrane potentials near $V_{\text{rest}}$. In contrast, the duration of subthreshold depolarization had little effect on the magnitude of the 1795insD sustained-current component ($I_{\text{sus}}$), and flecainide also had no effect on $I_{\text{sus}}$ after either brief or prolonged prepulses (Figure 3B, inset). $I_{\text{sus}}$ (n = 8, paired, % predrug peak $I_{\text{Na}}$) was: control 3.3 ± 0.5 and 2.8 ± 0.6 for 1- and 500-ms prepulses, and with flecainide 2.5 ± 0.6 and 2.3 ± 0.5 for 1- and 500-ms prepulses ($P < 0.05$ versus control). These results suggest that unlike peak $I_{\text{Na}}$, channels occupying a mode that produces sustained current are less susceptible to closed-state inactivation, and consistent with this feature, are also resistant to flecainide block.

Like 1795insD patients, carriers of the LQT3 ΔKPQ mutation have exhibited “Brugada-like” ECG changes during exposure to IV flecainide. Using the same approach as shown in Figure 3, we examined closed-state inactivation and tonic flecainide block of ΔKPQ channels (fitted parameters, Table 1). Figure 4A indicates that the rate of closed-state inactivation of the ΔKPQ channel ($\tau=6.5$ ms) was faster than WT ($\tau=24.5$ ms, $P<0.001$ versus ΔKPQ). Although the extent of closed-state inactivation for ΔKPQ was somewhat less than 1795insD (although greater than WT), the time constant of ΔKPQ closed-state inactivation was even faster than 1795insD ($P<0.001$). Also like 1795insD, the rate at which peak $I_{\text{Na}}$ is suppressed at $-100$ mV during exposure to flecainide mirrored the rate of closed-state inactivation (Figure 4B, Table 1). The findings indicate that like 1795insD, the ΔKPQ channel is susceptible to enhanced tonic flecainide block as a result of increased closed-state inactivation gating near $V_{\text{rest}}$.

### Intermediate Inactivation, the Brugada Syndrome, and Drug Sensitivity

In addition to the predominant, rapid inactivation gating process that terminates $I_{\text{Na}}$, depolarized Na channels may occupy one or more inactivated states that recover slowly (and incompletely) during the diastolic interval between stimuli. Recent studies have shown that Brugada syndrome mutations (both 1795insD and T1620M) exhibit excessive “intermediate” inactivation during depolarization periods rel-

### Table 1. Closed-State Inactivation (A) (-100 mV)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>$\tau$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n=11)</td>
<td>6±1</td>
<td>42.4±5.4</td>
</tr>
<tr>
<td>WT + flecainide (n=5)</td>
<td>19±2</td>
<td>33.8±3.6</td>
</tr>
<tr>
<td>1795insD (n=12)</td>
<td>27±2*</td>
<td>12±0.9†</td>
</tr>
<tr>
<td>1795insD + flecainide (n=6)</td>
<td>52±2</td>
<td>10.9±1.7†</td>
</tr>
<tr>
<td>ΔKPQ (n=9)</td>
<td>14±1*</td>
<td>6.5±1††</td>
</tr>
<tr>
<td>ΔKPQ + flecainide (n=4)</td>
<td>45±3</td>
<td>7.7±1</td>
</tr>
</tbody>
</table>

$P<0.01$ vs WT.
†$P<0.001$ vs WT.
‡$P<0.001$ vs 1795insD.
I$_m$ component (A) was significantly greater in the mutant (0.15±0.01) than the WT (0.08±0.008, P<0.01). Flecainide (1 μmol/L) exposure accelerated the rate (τ) of depolarization-dependent block (Table 2) in 1795insD but not in WT.

Even greater differences were apparent when the time-dependent recovery from I$_m$ or flecainide block was assessed (Figure 5B). A fixed-duration pulse (P$_1$) of 1 second to induce I$_m$ was followed by a variable-length hyperpolarization and a test pulse (P$_2$). In drug-free conditions, both WT and 1795insD exhibited 2 clear recovery components, reflecting fast and intermediate inactivation (fitted parameters, Table 3). As shown previously, the amplitude of the slow component of recovery (A$_2$) was greater in 1795insD than WT (Table 3). Moreover, on exposure to flecainide, the time constant for slow recovery (τ$_2$) was slightly delayed for WT (~2-fold) but was markedly delayed for 1795insD (~4-fold, Table 3, arrow in Figure 5B). In 1 μmol/L flecainide, τ$_2$ was 282.4±43 ms for 1795insD, but it was only 75.0±7.1 ms for WT (P<0.05). These results suggest that excessive I$_m$ occupancy has a small effect on flecainide block development (Table 2) but substantially slows the recovery from pulse-dependent Na channel blockade (Figure 5B, Table 3). During trains of ten 1-second depolarizations (Figure 5B, inset), the combined 1795insD I$_m$ gating effects on development and recovery from flecainide block yielded a marked use-dependent reduction in 1795insD peak I$_m$. At 0.95 Hz, flecainide reduced P$_2$/P$_1$ peak I$_m$ over the drug-free value by 22.4±4.0% in 1795insD but only by 12.8±1.0% in WT (P<0.05). At a slower pulse rate (0.33 Hz), flecainide block for 1795insD still exceeded WT (19.6±3.1% in 1795insD versus 5.4±1.1% in WT, P<0.01). This I$_m$-related pharmacological potentiation could further compromise Na channel availability in Brugada syndrome.

### Discussion

Mechanistic overlap between the LQT3 and Brugada syndromes has been foreshadowed by recent studies linking both phenotypes to the same (1795insD)$^{17}$ or nearly adjacent SCN5A loci$^{22}$ and clinical evidence that patients harboring only LQT3 mutations develop Brugada-like ST-segment elevations when treated with flecainide.$^{14}$ Our findings identify a common component of inactivation gating dysfunction in LQT3 and Brugada syndrome (enhanced closed-state inactivation). Cardiac Na channels exhibit substantial closed-state inactivation at or below the resting membrane potential (V$_{rest}$=−90 mV),$^{20}$ and we find that both the 1795insD and ΔKPQ mutations further enhance fast inactivation from closed states (Figures 3A and 4A). In both mutants, flecainide...
block increased with mild depolarization (below the activation threshold), with kinetic characteristics that recapitulated closed-state inactivation (Figures 3B and 4B), suggesting that the excess tonic block observed for ΔKPQ or 1795insD at Vrest is attributable to increased closed-state inactivation. In the myocardium, a number of conditions (mutations, drugs, ischemia, etc) substantially reduce peak \( I_{Na} \) and may precipitate ECG changes that recapitulate the Brugada syndrome. 26 To explore this issue in more detail, we used the WT ECG changes evoked by flecainide in LQT3 patients, 14 our findings also support the possibility that flecainide use in LQT3 patients may have proarrhythmic risks.

Previous studies of the sustained, noninactivating current (\( I_{sus} \)) in LQT3 have found that lidocaine and its analogues \( I_{Na} \) and may precipitate ECG changes that recapitulate the Brugada syndrome. 23,24 While providing a mechanistic rationale for the ECG changes evoked by flecainide in LQT3 patients, 14 our findings support the possibility that flecainide use in LQT3 patients may have proarrhythmic risks.

Table 3. Recovery From Inactivation and Drug Block

<table>
<thead>
<tr>
<th>Condition</th>
<th>( A_1 )</th>
<th>( A_2 )</th>
<th>( \tau_1 )</th>
<th>( \tau_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n=8)</td>
<td>0.83±0.003</td>
<td>0.15±0.003</td>
<td>4.7±0.16</td>
<td>40±3.4</td>
</tr>
<tr>
<td>WT + flecainide (n=3)</td>
<td>0.82±0.02</td>
<td>0.18±0.01</td>
<td>6.1±0.2</td>
<td>75±7.1†</td>
</tr>
<tr>
<td>1795insD (n=8)</td>
<td>0.78±0.007</td>
<td>0.2±0.007*</td>
<td>6.7±0.6</td>
<td>72±11.7</td>
</tr>
<tr>
<td>1795insD + flecainide (n=4)</td>
<td>0.81±0.01</td>
<td>0.16±0.02</td>
<td>6.7±0.9</td>
<td>282±4.43‡§</td>
</tr>
</tbody>
</table>

\( A_1 \) indicates amplitude of fast component of recovery; \( A_2 \) amplitude of slow component.

*P<0.001 vs WT \( A_2 \).
†P<0.01 vs WT \( \tau_2 \).
‡P<0.01 vs 1795insD \( \tau_2 \).
§P<0.05 vs WT + flecainide \( \tau_2 \).

Figure 6. Hypothetical framework summarizing how SCN5A inactivation gating defects may influence flecainide block in LQT3 and Brugada syndromes.
Na channel function in cells isolated from the epicardial border zone of the 5-day infarcted canine heart reveal an inactivation defect consistent with enhancement of \( I_{Na} \), as well as enhanced use-dependent Na channel blockade. Hence, inactivation lesions associated with the Brugada and LQT3 syndromes that augment Na channel blockade may serve as models for understanding the acquired proarrhythmic sensitivity of patients with cardiac ischemia to class I agents. Our findings provide a mechanistic rationale for the proarrhythmic effects of flecainide in patients with specific cardiac Na channelopathies and may also provide a general framework for predicting the pharmacological sensitivities of new SCN5A mutations based on their functional gating defects.

Acknowledgments
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