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 cation flux (fast inactivation) and render the channel refractory for sustained periods to restimulation (intermediate and slow inactivation). Mutations in SCN5A modify inactivation in distinctive ways to elicit either the congenital long-QT syndrome (LQT3) or the Brugada syndrome.1,2 In LQT3, cardiac repolarization is delayed, often through partial disruption of fast inactivation that permits a persistent inward current during the action potential plateau3 (other mechanisms are described).4 In contrast, Brugada syndrome is characterized by distinctive ST-segment elevations and is typically associated with mutations that enhance Na channel inactivation.5–7

Despite their proarrhythmic potential,8 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 disorders9 through blockade of the sustained inward current through the mutant channels.10–12 In contrast, Na channel blockade exacerbates the ECG pattern in Brugada syndrome.13 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,14 suggesting that Na channel blockade may have proarrhythmic potential in LQT3.

Recognizing the mechanistic role of cardiac Na channel inactivation in antiarrhythmic drug action,15,16 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)16,17 and worsening ST-segment elevation with Na channel blockade.17 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-
3.0% for WT, increased compared with WT (Figure 2, inset: 1795insD was transfected tsA-201 cells after a sustained (500-ms) holding period at 21°C as described previously. The pipette solution ratio of Na channel human reporter in tsA-201 cells. Cells were cotransfected with an equimolar subunit. Whole-cell Na currents were recorded 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, CaCl 1.8, MgCl 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 are 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, CaCl 1.8, MgCl 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 are 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.20 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 Vrest 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^{-x/\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 (τ 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 (τ) 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), τ 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 $V_{rest}$. In contrast, the duration of subthreshold depolarization had little effect on the magnitude of the 1795insD sustained-current component ($I_{sus}$), and flecainide also had no effect on $I_{sus}$ after either brief or prolonged prepulses (Figure 3B, inset).

$I_{sus}$ (n=8, paired, % predrug peak $I_{Na}$) 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=NS versus control). These results suggest that unlike peak $I_{Na}$, 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 ($\tau=6.5\pm1$ ms) was faster than WT ($\tau=42.4\pm5.4$ 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 $I_{Na}$ 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 $V_{rest}$.

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

In addition to the predominant, rapid inactivation gating process that terminates $I_{Na}$, 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>
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<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_{Na} 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_{Na} or flecainide block was assessed (Figure 5B). A fixed-duration pulse (P1) of 1 second to induce I_{Na} was followed by a variable-length hyperpolarization and a test pulse (P2). 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,6 the amplitude of the slow component of recovery (A2) was greater in 1795insD than WT (Table 3). Moreover, on exposure to flecainide, the time constant for slow recovery (τ2) 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, τ2 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_{Na} 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_{Na} gating effects on development and recovery from flecainide block yielded a marked use-dependent reduction in 1795insD peak I_{Na}. At 0.95 Hz, flecainide reduced P2/P1 peak I_{Na} 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_{Na}-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 loci22 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

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**Table 2. Depolarization-Dependent Block**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>τ, ms</th>
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<tbody>
<tr>
<td>WT (n=7)</td>
<td>0.08±0.008</td>
<td>364±41</td>
</tr>
<tr>
<td>WT + flecainide (n=4)</td>
<td>0.15±0.02*</td>
<td>283±73.2</td>
</tr>
<tr>
<td>1795insD (n=7)</td>
<td>0.15±0.01</td>
<td>406±43.6</td>
</tr>
<tr>
<td>1795insD + flecainide (n=4)</td>
<td>0.18±0.03</td>
<td>163.8±30†</td>
</tr>
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</table>

A indicates amplitude of the I_{Na} component.

*P<0.01 vs WT.
†P<0.01 vs 1795insD.
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 İNa and may precipitate ECG changes that recapitulate the Brugada syndrome.25,26 While providing a mechanistic rationale for the ECG changes evoked by flecainide in LQT3 patients, our findings also support the possibility that flecainide use in LQT3 patients may have proarrhythmic risks.

Previous studies of the sustained, noninactivating current (İNa) in LQT3 have found that lidocaine and its analogues preferentially reduce İNa over peak İNa.10,11,25 In contrast, 1795insD İNa was resistant to subthreshold depolarizations (unlike peak İNa), and 1 μmol/L flecainide had no effect on İNa even after a sustained prepulse (Figure 3B, inset). The findings are consistent with the clinical observation of absence QTc shortening during flecainide treatment of 1795insD patients (Figure 1). At a mechanistic level, the results also suggest that 1795insD channels opening in a “bursting” mode are resistant to closed-state inactivation and are thereby insensitive to flecainide blockade. The finding that open, noninactivating channels are insensitive to drug was unexpected, given previous results from use of higher (200 μmol/L) concentrations of flecainide with cloned neuronal Na channels that identify a component of open-channel block.26 To explore this issue in more detail, we used the WT channel and rapid trains of brief pulses at 10 Hz (20 successive 50-ms steps from −120 to 0 mV) to minimize the contribution of İNa. Peak İNa was reduced in flecainide by only 4.1 ± 2% under drug-free condition (n = 4, not shown) and did not differ from the effect of a train of subthreshold pulses to −50 mV (3.3 ± 0.6%, n = 4), suggesting that open-state inactivation plays only a minor role under these conditions (ie, 1 μmol/L flecainide, the cardiac isoform).

**Use-Dependent Block, İM Inactivation, and Brugada Syndrome**

The 1795insD mutation enhances occupancy of a slow, inactivated state with intermediate kinetic characteristics (İM).6,27 Here, we show that this gating defect increases the rate at which flecainide block develops during sustained depolarization (Figure 5A, Table 2), delays recovery from flecainide block (Figure 5B, Table 3), and has a net effect to increase use-dependent block (inset, Figure 5B). Hence, in addition to enhanced closed-state fast inactivation, the İM gating defect induced by 1795insD may doubly sensitize carriers to a Brugada phenotype during flecainide therapy (Figure 1). Proarrhythmic flecainide blockade may also be a risk in patients with other Brugada mutations that enhance İNa (ie, T1620M).7 Conversely, additional studies in the LQT3 channels ΔKPQ (n = 6), R1644H (n = 4), and N1325S (n = 3) revealed no İNa inactivation defects (data not shown), suggesting that İNa inactivation is not a primary mechanism of flecainide-induced ST-segment elevation in LQT3.14 Figure 6 proposes a framework for future studies, linking the distinct Na channel inactivation gating defects of LQT3 and Brugada syndrome to the flecainide effects seen in Figures 2 to 5.

Although flecainide caused a marked slowing of 1795insD recovery, this effect was modest in WT (Figure 5B; 1 μmol/L flecainide). A previous report using cardiac myocytes described both rapid and slow components of recovery from use-dependent flecainide block with 3 μmol/L concentrations,27 and a marked component of slow recovery was identified in heterologously expressed WT and ΔKPQ channels exposed to much higher flecainide concentrations (10 to 100 μmol/L).28 The therapeutic plasma concentration of flecainide is 0.2 to 1 μg/mL (0.4 to 2 μmol/L),19 however, suggesting that the principal block mechanism at therapeutically relevant concentrations for WT channels is tonic suppression of peak İNa (Figures 3 and 4). Although previous studies28 found an IC50 of 80 μmol/L for tonic flecainide block of ΔKPQ channels, we find a 39.4 ± 8% (n = 4) reduction of peak İNa with only 1 μmol/L flecainide (holding potential −100 mV, see legend to Figure 4). Nonetheless, slow recovery from use-dependent flecainide block may become more significant under conditions that promote İM inactivation (eg, Figure 5B). It is noteworthy that studies of

**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.8 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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