Ionic mechanisms of cellular electrical and mechanical abnormalities in Brugada syndrome

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THE BRUGADA SYNDROME (BrS) is a right ventricular (RV) arrhythmia that is believed to be responsible for 4–12% of all sudden cardiac death and up to 50% of sudden cardiac death in patients without structural cardiopathy (4, 5). The disease is characterized by ST segment elevation in the right precordial leads and a high incidence of sudden cardiac death, with up to 50% of sudden cardiac death in patients without structural cardiopathy (4, 5). Other genes have been found to be associated with BrS, such as SCN5A (5), accounting for 18–30% of patients with BrS (32, 33). RV motion abnormalities were detected in patients with BrS (22, 24), and such localized morphological changes may contribute to the arrhythmic substrate in BrS. The basis of the motion abnormality in BrS is currently unknown, a question that is of relevance to our knowledge of the arrhythmic substrate of the disease.

Our present study has two goals. The first is to provide a quantitative analysis of the cellular ionic mechanisms of electrical abnormalities in BrS. Our second goal is to examine the influence of cellular electrical alterations under I_{Na} reduction on myocyte mechanical properties. To address these two questions, the selective I_{Na} blocker tetrodotoxin (TTX) was used to mimic the reduced I_{Na} at the myocyte level. A combination of dynamic clamp and computational modeling was used to quantitatively examine the interplay of I_{Na} and I_{to} and its impact on AP repolarization. Myocyte mechanical measurements and modeling were done to examine the impact of I_{Na} reduction on Ca^{2+} dynamics and myocyte contraction.

MATERIALS AND METHODS

Isolation of left ventricular and RV canine cardiomyocytes. All animal usage and handling followed protocols approved by the Institutional Animal Care and Use Committee of University of Cincinnati. Adult male dogs weighing 25–35 kg were used in the study. Ventricular myocytes were isolated using a wedge method, as described previously (11).
Electrophysiology recordings and dynamic clamp simulation. AP recording using the perforated-patch configuration and voltage-clamp recording of \( I_{\text{Na}} \) using whole cell patch clamps were performed, as previously described (11). APs were triggered with just-threshold 2-ms current steps at 1 Hz, and all measurements were performed at steady state, unless stated otherwise. Cardiac \( I_{\text{Na}} \) was recorded at room temperature using the whole cell patch-clamp configuration. The external solution contained the following (in mM): 120 choline-chloride, 5 NaCl, 1.5 MgCl\(_2\), 0.5 CaCl\(_2\), 4 KCl, 5 NaOH, 2.8 sodium-acetate, 5 tetraethylammonium-chloride, 10 HEPES, 10 glucose, 2 4-aminopyridine, 0.1 CdCl\(_2\), and 0.5 BaCl\(_2\) (pH = 7.4). The pipette solution contained the following (in mM): 124 cesium-aspartate, 1 NaCl, 10 HEPES, 10 EGTA, and 2 MgCl\(_2\) (pH = 7.3).

The dynamic clamp combines computer simulation with experimental electrophysiology and allows the introduction of programmable artificial conductance in living cells. To do so, the dynamic clamp software calculates in real time the value of the simulated conductance based on the instantaneous membrane potential and the algorithms describing the conductance and mimics the presence of such a conductance by injecting a corresponding current into the cell. The dynamic clamp was implemented as previously described (11). Voltage sampling of the dynamic clamp software and output of the current injection command were through an Axon Digidata 2100 board (Axon Instruments, Foster City, CA). \( I_{\text{Na}} \), was defined as a rapidly and fully inactivating outward current and was described as previously described (11).

Myocyte mechanics and \( \text{Ca}^{2+} \) transient measurements. APs were elicited by 2-ms depolarizing pulses at 1 Hz. Myocyte shortening was assessed using a video-based edge-detection system (Crescnet Electronics, Sandy, UT). For \( \text{Ca}^{2+} \) kinetics, myocytes were loaded with 10 \( \mu \)M fluo-4 AM (Molecular Probes, Eugene, OR) for 30 min at room temperature. Fluorescent signals were recorded using a photometry electronics, Sandy, UT). For \( \text{Ca}^{2+} \) current measurements, I was defined as a rapidly and fully inactivating outward current and was described as previously described (11).

Computational modeling. The computational model was constructed in MATLAB based on the Hund-Rudy dynamic (HRD) model (16). Modifications were made to \( \text{Ca}^{2+} \) dynamics based on recent findings (14, 28, 34). Based on studies by Sher et al. (28), local currents between the T-tubule and the sarcoplasmic reticulum (SR) and a conductance mimics the presence of such a conductance by injecting a corresponding current into the cell. The dynamic clamp was implemented as previously described (11). Voltage sampling of the dynamic clamp software and output of the current injection command were through an Axon Digidata 2100 board (Axon Instruments, Foster City, CA). \( I_{\text{Na}} \), was defined as a rapidly and fully inactivating outward current and was described as previously described (11).

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Based on patch-clamp data, \( I_{\text{Na}} \), in the model RV epicardial cell was set to have a peak current of 30 pA/pF in response to a depolarizing voltage step to +40 mV. All simulation runs were taken to steady state.

Statistical analysis. Group data are presented as means ± SE. Statistical tests of the effects of the dynamic clamp simulation were performed using paired, two-tail Student’s t-tests. Other statistical tests used unpaired Student’s t-tests. Probability values of <0.05 were considered as significant.

\[
\begin{align*}
\frac{d[\text{Ca}^{2+}]}{dt} &= J_{\text{LCC}} + J_{\text{NCX}} + J_{\text{RyR}} - J_D \\
\text{(4)}
\end{align*}
\]

where \( J_d \) is the volume of the dyadic space; \( J_{\text{LCC}}, J_{\text{NCX}}, \) and \( J_{\text{RyR}} \) are the regional fluxes of \( \text{L-type Ca}^{2+} \) channel, NCX, and ryanodine receptor within the dyadic space, respectively; and \( J_D \) represents the currents from the dyadic space to the myoplasm.

\[
[\text{Ca}^{2+}]_{\text{sm}} \text{ is based on the previously described empirical model (34). Two separate equations are used to describe } [\text{Ca}^{2+}]_{\text{sm}} \text{ during the increasing and decreasing phase of the Ca}^{2+} \text{ transient. } I_{\text{offset}} \text{ (in ms) is the time at which the peak Ca}^{2+} \text{ transient occurred. } [\text{Ca}^{2+}]_{\text{sm}} \text{ was found to closely match that described by Sher et al. (28).}
\]

Increasing:
\[
[\text{Ca}^{2+}]_{\text{sm}} = \left[ \text{Ca}^{2+} \right] + \gamma \frac{d[\text{Ca}^{2+}]}{dt}, \quad \gamma = 110 \text{ ms} \quad \text{(5)}
\]

Decreasing:
\[
[\text{Ca}^{2+}]_{\text{sm}} = \left[ \text{Ca}^{2+} \right] + A \cdot \exp \left( -\frac{t - t_0}{\tau} \right),
\]
\[
A = \left[ \text{Ca}^{2+} \right]_{\text{sm}} - \left[ \text{Ca}^{2+} \right], \quad \tau = 92 \text{ ms} \quad \text{(6)}
\]

Fig. 1. Heterogeneity in action potential (AP) and transient outward current (\( I_{\text{Na}} \)) in canine left (LV) and right ventricular (RV) epicardial (Epi) and endocardial (Endo) myocytes. A: representative AP traces recorded from canine RV Epi, Endo, and LV Epi cells. B: \( I_{\text{Na}} \) recorded from RV Epi cells activated by voltage steps from 0 mV to 70 mV in 10-mV increments at 10-s intervals. C: average peak amplitudes of \( I_{\text{Na}} \) at +40 mV in the RV Epi, Endo, and LV Epi cells. Error bars are ±SE.
RESULTS

AP morphology and \( I_{\text{to}} \) density in canine RV myocytes. Consistent with previous publications (10), APs of RV epicardial myocytes were characterized by a prominent spike-and-dome waveform (Fig. 1A). The phase 1 notch in RV epicardial myocytes was more prominent than that in the left ventricular (LV) epicardial myocytes, and the notch was near-absent in the RV endocardial myocytes. These differences in AP morphology correlate with an \( I_{\text{to}} \) density gradient across the ventricular wall, as well as between the RV and LV (Fig. 1, B and C). RV epicardial myocytes had a robust \( I_{\text{to}} \) with an average density of 28.8 pA/pF at +40 mV (\( n = 14 \) from 4 hearts), while the average \( I_{\text{to}} \) density was 20 pA/pF in LV epicardial cells (\( n = 13 \) from 4 hearts) and 4.8 pA/pF in RV endocardial cells (\( n = 9 \) from 2 hearts).

Effect of \( I_{\text{Na}} \) reduction on RV epicardial AP morphology. TTX was used to examine the influence of selective \( I_{\text{Na}} \) reduction on AP morphology in ventricular myocytes. Consistent with the well-known affinity of TTX for the cardiac \( I_{\text{Na}} \) (25), blockade of canine ventricular \( I_{\text{Na}} \) by TTX had a \( K_d \) of 1.45 \( \mu \)M (Fig. 2, A and B). TTX at 0.3 \( \mu \)M slightly deepened the phase 1 notch and modestly prolonged AP duration (APD) in 90% (17 of 19) RV epicardial myocytes (Fig. 2C). In the rest (10%) of the cells, TTX produced all-or-none repolarization and a triangular AP waveform. Under 1 \( \mu \)M TTX (which produces 40% blockade of \( I_{\text{Na}} \) based on the \( K_d \)), the fraction of RV epicardial myocytes that developed all-or-none repolarization increased to 57% (8 of 14; Fig. 2, D1, D2, and E). TTX of 3 \( \mu \)M resulted in AP collapse in nearly all RV epicardial cells examined (Fig. 2E).

To quantitatively investigate how AP repolarization is influenced by \( I_{\text{Na}} \) and its interplay with \( I_{\text{to}} \), the dynamic clamp was used to modulate \( I_{\text{to}} \) density, while \( I_{\text{Na}} \) was reduced by TTX. The dynamic clamp allowed introduction of various densities

Fig. 2. Influence of Na\(^+\) current (\( I_{\text{Na}} \)) and \( I_{\text{to}} \) on AP repolarization and morphology. A: blockade of canine ventricular \( I_{\text{Na}} \) by tetrodotoxin (TTX). \( I_{\text{Na}} \) was activated by voltage steps from a holding potential of −80 mV in 10-mV increment. B: dose-response curve of TTX blockade of \( I_{\text{Na}} \). Data points are averages from 3 RV myocytes and were fitted using the Hill equation, with \( K_d = 1.45 \, \mu \)M. C and D: representative APs recorded from canine RV endocardial cells under control conditions (dash line) and in the presence of 0.3 \( \mu \)M (C) or 1 \( \mu \)M TTX (solid line; D). E: %RV epicardial myocytes with all-or-none repolarization under various concentrations of TTX. F: APs recorded from an RV endocardial cell with the simulation of various densities of artificial \( I_{\text{Na}} \) using the dynamic clamp under control (left) or with 3 \( \mu \)M TTX (right). G: AP duration (APD) at 90% repolarization (APD90; expressed as ratio over APD90 in the absence of simulated \( I_{\text{Na}} \)) vs. \( I_{\text{to}} \) density relationships for the same cell as in F under control and in the presence of 1 or 3 \( \mu \)M TTX. Average \( I_{\text{to}} \) density in canine RV epicardial cells is expressed as ±SD.

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of artificial \(I_{\text{to}}\) conductance (\(G_{\text{to}}\)) in RV endocardial cells, where the native \(I_{\text{to}}\) is small. Consistent with our laboratory’s previous studies (11, 31), we found that \(I_{\text{to}}\) had a biphasic effect on AP repolarization: increasing \(I_{\text{to}}\) density first moderately prolonged AP and then resulted in all-or-none repolarization once \(I_{\text{to}}\) density was beyond a certain threshold (Fig. 2F, left). TTX shifted the \(I_{\text{to}}\) density-APD relationship curve to the left, significantly reducing the threshold for \(I_{\text{to}}\)-induced all-or-none repolarization (Fig. 3C). Fractional shortening was markedly attenuated (Fig. 3C). Maximum rates of contraction and relaxation were also reduced (Fig. 3). The average \(I_{\text{to}}\) threshold for all-or-none repolarization was decreased from 42.4 pA/pF in control to 24.3 pA/pF with 3 \(\mu\)M TTX (\(n = 3\) from 3 hearts).

**Effects of \(I_{\text{Na}}\) reduction on RV epicardial cell mechanics and \(Ca^{2+}\) transient.** We then studied how repolarization alterations produced by \(I_{\text{Na}}\) reduction affect the mechanical properties of RV epicardial cells. AP was simultaneously measured with myocyte contraction or intracellular \(Ca^{2+}\). TTX of 1 \(\mu\)M, while deepening the phase 1 notch and prolonging APD, attenuated the magnitude of cell shortening and delayed the relengthening (Fig. 3A, left), and suppressed peak amplitude and delayed the rise of the \(Ca^{2+}\) transient (Fig. 3A, right). In those cells in which 1 \(\mu\)M TTX resulted in an all-or-none repolarization, both cell shortening and \(Ca^{2+}\) transient were substantially suppressed (Fig. 3B). Quantitatively, under 1 \(\mu\)M TTX and in RV epicardial myocytes in which a spike-and-dome AP morphology was preserved, the fractional shortening and relengthening (Fig. 3A, left) were all-or-none, and the APD was nearly identical to the relationship between increasing \(G_{\text{Na}}\) and APD (Fig. 5, A and C; also see Fig. 2G). Decreasing \(G_{\text{Na}}\) progressively shifted the \(G_{\text{to}}\)-APD relationship curve to the left and lowered the threshold for \(I_{\text{to}}\)-induced all-or-none repolarization (Fig. 5C), reproducing our experimental observations shown in Fig. 2G. This intriguing interplay between \(G_{\text{Na}}\) and \(G_{\text{to}}\) is visually illustrated by the relationship between \(G_{\text{to}}, G_{\text{Na}},\) and the APD (Fig. 5D). There exists a distinct threshold (or a

\[I_{\text{to}}\] is critical for TTX-induced repolarization and contractile alterations. In contrast to the findings in RV epicardial myocytes, up to 3 \(\mu\)M TTX only slightly affected AP morphology and did not cause all-or-none repolarization in LV epicardial and RV endocardial cells, where the native \(I_{\text{to}}\) is smaller (Fig. 4A, top). TTX had only a minor effect on contraction in these cells (Fig. 4A, bottom).

We used 5 \(\mu\)M TTX to produce a high degree of \(I_{\text{Na}}\) reduction in RV epicardial cells. As expected, TTX collapsed the AP and resulted in marked suppression of cell shortening (Fig. 4B, left). We next produced a partial “blockade” of the native \(I_{\text{to}}\) by introducing an artificial inward \(G_{\text{to}}\) that has the opposite polarity as the native \(I_{\text{to}}\) using the dynamic clamp. This partial “blockade” of \(I_{\text{to}}\) restored the spike-and-dome AP waveform, even under such significant \(I_{\text{to}}\) reduction. Moreover, it also restored cell contraction to near the control level (Fig. 4B, right).

**Computational model of the RV cardiac myocyte.** A modified Hund-Rudy canine ventricular myocyte model (16) was used to further examine the influence of \(I_{\text{Na}}\) and \(I_{\text{to}}\) on AP morphology. The modeled AP showed slight to moderate increase in duration as the \(Na^{+}\) conductance (\(G_{\text{Na}}\)) was decreased until the AP collapsed at \(\sim 50\%\) \(G_{\text{Na}}\) (Fig. 5, A and B). Interestingly, this biphasic effect of \(G_{\text{Na}}\) reduction on APD is nearly identical to the relationship between increasing \(G_{\text{Na}}\) and APD (Fig. 5, B and C; also see Fig. 2G). Decreasing \(G_{\text{Na}}\) progressively shifted the \(G_{\text{to}}\)-APD relationship curve to the left and lowered the threshold for \(I_{\text{to}}\)-induced all-or-none repolarization (Fig. 5C), reproducing our experimental observations shown in Fig. 2G. This intriguing interplay between \(G_{\text{Na}}\) and \(G_{\text{to}}\) is visually illustrated by the relationship between \(G_{\text{to}}, G_{\text{Na}},\) and the APD (Fig. 5D). There exists a distinct threshold (or a

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**Fig. 3.** A and B: effect of \(I_{\text{to}}\) reduction on contractility and \(Ca^{2+}\) transient in RV epicardial cells. Representative cell shortening (left bottom) and \(Ca^{2+}\) transient (right bottom) simultaneously recorded with AP (top) as 1 \(\mu\)M TTX (shaded lines) produced spike-and-dome (S&D; A) or triangular (B) AP waveforms in canine RV epicardial cells. C: average fractional shortening and maximum rates of cell contraction (+dL/dt) and relaxation (−dL/dt) under control and in the presence of TTX. Values are means ± SE; \(N = 11\) myocytes from 4 hearts. *\(P < 0.05\), **\(P < 0.01\) vs. control in a paired t-test.

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shallow “cliff”), where APD no longer increases as a function of decreasing G_Na or increasing I_to, but where the AP collapses. The degree of G_Na reduction that a myocyte can “tolerate” without AP collapse is critically determined by I_to; this threshold is $\approx 50\%$ G_Na reduction with 1.0 × G_to (i.e., I_to level in RV epicardium) and is much higher with lower levels of I_to found in other regions of the ventricles.

BrS also results from SCN5A mutations that affect various aspects of I_to gating properties (1). We evaluated the impact of two commonly seen BrS-related kinetic alterations, accelerated I_Na inactivation and shift in I_Na steady-state inactivation (SSI) (37), on the modeled AP repolarization. Increases in I_Na inactivation rate ranging from 30 to 80% and negative shifts in the SSI ranging from $\approx 5$ to 13 mV have been reported for BrS-linked mutations (37). A 25% increase in I_Na inactivation rate resulted in a prolonged APD, and a 50% increase resulted in all-or-none repolarization (Fig. 6A). The I_Na inactivation vs. APD relationship has a biphasic shape similar to the G_Na-APD relationship, with a threshold for AP collapse around 1.5× wild-type inactivation rate (Fig. 6B). Figure 6C shows how I_Na inactivation rate impacts AP under various I_to levels. Negative shifts of I_Na SSI impact AP repolarization, presumably through reduction of the effective G_Na. As expected, this shift either slightly prolonged or collapsed the AP (Fig. 6, D and E). Because the resting potential of the model ($\approx 85$ mV) sits in the steep part of the SSI curve, small negative shifts in SSI have a fairly minor effect on the AP; threshold for all-or-none repolarization occurs at around $-11$-mV shift of SSI (Fig. 6F).

As the SSI shift increases and the resting potential enters the shallow part of the SSI curve, large negative shifts in SSI have a fairly major effect on the AP. Threshold for all-or-none repolarization occurs at around $-11$-mV shift of SSI (Fig. 6F).

**Effect of G_Na reduction on Ca²⁺ influx and dynamics.** To understand the basis of the suppression of myocyte mechanics under I_to reduction, we next examined the influence of G_Na on Ca²⁺ dynamics. Before AP collapse, G_Na reduction in the RV epicardial cell model is associated with minor depression of Ca²⁺ transient amplitude and a delay in the rise of Ca²⁺ transient (Fig. 7, A and B). At 50% G_Na, there is a precipitous drop in peak Ca²⁺ transient level, coinciding with the collapse of the AP (Fig. 7B). The depression of Ca²⁺ transient amplitude reflects a progressive reduction in SR Ca²⁺ release (I_Ca_L), which is almost completely abolished at 50% G_Na reduction (Fig. 7C). The decline in I_Ca_L closely follows the decline seen in peak L-type calcium current (I_Ca_Ca). On collapse of the AP, decreased SR load becomes another contributor to the depressed myocyte mechanics, as shown in the AP voltage-clamp experiments (Fig. 7, D and E). Either normal spike-and-dome RV epicardial AP or triangular AP induced by TTX was used as AP clamp waveform. Myocytes were repeatedly activated until Ca²⁺ transients reached steady state, then caffeine-induced Ca²⁺ transient was measured as an indication of SR load (Fig. 7D). Triangular AP waveform was associated with significantly reduced Ca²⁺ transient amplitude (consistent with data shown in Fig. 3), as well as suppressed caffeine-induced Ca²⁺ transient amplitude (ΔF/F₀ = 1.39 for spike-and-dome AP vs. 0.62 for triangular AP, n = 6 and 4, respectively).

**DISCUSSION**

In the present study, we examined the ionic basis of the cellular electrical abnormalities in BrS using a combination of patch clamp, dynamic clamp simulation, and computational modeling. We demonstrate quantitatively how AP morphology is influenced by an intriguing interplay between G_Na and I_to. We also examined how cellular electrical abnormalities induced by I_to reduction influence myocyte mechanical properties. Our results indicate that cellular electrical alterations under reduced I_Na lead to significant alterations of myocyte excitation-contraction coupling and mechanics. These altered myocyte mechanics are a possible contributing factor for the wall motion abnormalities observed in the disease.

Our results illustrate quantitatively how myocyte cellular electrical properties are affected by selective I_Na reduction. An earlier modeling study suggests that 50% I_to reduction only has a minor influence on I_to-mediated AP repolarization and APD in canine ventricular cells, causing a slight shift in the I_to-APD curve (15). Our experimental and modeling results, however, show that the AP morphology in canine ventricular cells is much more sensitive to I_to reduction and is strongly influenced by the counterbalance between I_Na and I_to. In fact, the threshold for all-or-none repolarization has a near linear relationship with the ratio of G_Na over G_to (Fig. 5D). This counterbalancing of I_Na and I_to is manifested as a leftward shift in the I_to density vs. APD relationship and reduction of the threshold for I_to-induced all-or-none repolarization when I_Na is reduced. About 35% of all identified BrS-linked SCN5A mu-

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**Fig. 4.** A: representative AP (top) and cell shortening (bottom) recorded with AP under control condition (solid line) or with 3 μM TTX (shaded line) in canine RV endocardial (left) or LV epicardial cells (right). B: left: AP (top) and cell shortening (bottom) recorded from a canine RV epicardial cell under control conditions and with 5 μM TTX. B, right: effect of I_to “blockade” using the dynamic clamp in the same cell in the presence of 5 μM TTX on AP (top) and cell shortening (bottom). Inset: dynamic clamp current output.
tations result in nonfunctional channels and thus in haplo-insufficiency (37). For those mutations that reduce \( I_{\text{Na}} \) density, a 50–80% reduction in heterologously expressed current density is typically seen (2, 17, 20, 30). Therefore, \( I_{\text{Na}} \) reduction in heterozygous carriers of these mutations should be 50%. We show that, under various degree of \( I_{\text{Na}} \) reduction, \( I_{\text{to}} \) in a certain fraction of RV epicardial myocytes falls in the range that produces AP all-or-none repolarization. This fraction is predicted to be 23% of RV epicardial cells under 25% \( I_{\text{Na}} \) reduction and ~50% of cells under 50% \( I_{\text{Na}} \) reduction, calculated based on our modeling result (Fig. 5D) and a normally distributed RV epicardial \( I_{\text{to}} \) density. This prediction generally agrees with our experimental finding that AP collapse occurred in ~10 and 50% of RV epicardial cells under 0.3 and 1 μM TTX, respectively (which should produce 18 and 41% \( I_{\text{Na}} \) blockaded based on the \( K_d \)). By comparison, even under a high degree of \( I_{\text{Na}} \) reduction, \( I_{\text{to}} \) in other regions of the ventricles is still within the range where a spike-and-dome AP is maintained. Based on the proposed repolarization abnormality model for BrS (5), epicardial and transmural dispersions of repolarization occur when AP collapses at some RV epicardial sites, but not at others, and at RV epicardium but not at other transmural layers of the myocardium. Such dispersion of repolarization creates the substrate of arrhythmogenesis, leading to phase 2 reentry and fatal ventricular arrhythmias.

Our results also demonstrate that a moderate (~50%) increase in \( I_{\text{to}} \) density can push RV epicardial AP beyond the threshold for all-or-none repolarization, even under full \( I_{\text{Na}} \) (Fig. 5D), which is consistent with the finding that a \( KCNE3 \) mutation that increases \( I_{\text{to}} \) density is linked to BrS (9).

It is clear that BrS is a cardiac electrical disorder without any global contractile dysfunction of the RV. Nevertheless, RV wall-motion abnormalities were detected in patients with BrS, and the origin of the RV motion abnormality is less than clear. It has been proposed that the wall-motion abnormalities indicate the existence of structural changes of the RV, and such structural changes may contribute to the arrhythmogenic substrate in BrS (22, 24). Our results suggest that the contractile abnormality in BrS is not necessarily an indication of RV structural changes, but may be secondary to the cellular electrical abnormalities, as proposed previously (6). We demonstrate that cellular electrical changes in RV epicardial cells under reduced \( I_{\text{Na}} \) are associated with changes in myocyte mechanics. In particular, all-or-none repolarization results in pronounced suppression of both \( Ca^{2+} \) transient amplitude and myocyte contraction. It is plausible that, under \( I_{\text{Na}} \) reduction, localized occurrence of AP collapse and suppression of myocyte contraction in RV epicardium are manifested as subtle wall-motion abnormalities of the myocardium. These myocyte-level mechanical changes, however, are not likely to result in

Fig. 5. Computational model of the influence of Na \(^+\) (\( G_{\text{Na}} \)) and \( I_{\text{to}} \) conductance (\( G_{\text{to}} \)) on RV epicardial AP morphology. A: modeled RV epicardial APs at 100, 70, and 30% \( G_{\text{Na}} \). B: modeled RV epicardial APD as a function of \( G_{\text{Na}} \). C: modeled RV epicardial APD as a function of \( G_{\text{to}} \) under various fractions of \( G_{\text{Na}} \). D: modeled APD as a function of both \( G_{\text{Na}} \) and \( G_{\text{to}} \). Left: side view. Right: top view. Bar indicates ±SD of \( G_{\text{to}} \) found in RV epicardial myocytes.
any dramatic contractility loss of the RV. Under moderate $I_{Na}$ reduction, only a fraction of the RV epicardial cells develop all-or-none repolarization and significant loss of contractility. Other regions of the RV myocardium, due to their smaller $I_{to}$ density, are presumably spared of any dramatic contractile changes (see Fig. 4). Therefore, changes in RV mechanics in BrS as a consequence of myocyte electrical abnormalities are likely to be subtle, as observed clinically. Localized suppression of myocyte contraction may also alter the stress-strain relationship of the myocardium, contributing to the development of subtle structural abnormalities described in BrS patients (13). Interestingly, no correlation has been found between $SCN5A$ mutation and RV wall motion abnormalities in BrS in a recent study, pointing to the complexity of the underlying mechanisms of the wall motion abnormalities in the syndrome (7).

Two factors appear to be the main contributor to suppressed Ca$^{2+}$ dynamics under $I_{Na}$ reduction: decreased $I_{CaL}$ influx and reduced SR load. It has been shown that the amplitude of $I_{CaL}$ elicited by an AP is determined by the voltage of the early phase of AP, with the highest $I_{CaL}$ activation occurring at around +10 mV, a voltage that coincides with the peak of the $I_{CaL}$ current-voltage curve (8). RV epicardial myocytes, with their large density of $I_{to}$, have a prominent early repolarization and an average phase 1 notch voltage of $-23.3 \text{ mV} \ (n = 9)$. Under $I_{Na}$ reduction, accentuation of phase 1 repolarization moves the AP trajectory further away from the voltage for maximum $I_{CaL}$ activation, decreasing excitation-contraction coupling and contractility. We also show that on collapse of AP, decreased SR load becomes a second contributor to the suppressed RV epicardial mechanics. Decreased SR load is likely the result of reduced total Ca$^{2+}$ entry, as well as abbreviated APD that has been shown to favor Ca$^{2+}$ extrusion by NCX (26) and inhibit SR Ca$^{2+}$ reloading (3).

In the Luo-Rudy model, membrane excitability is not compromised unless the $G_{Na}$ falls below 20% of its normal value (27). We show that the AP waveform and myocyte contraction in RV endocardial and LV epicardial myocytes were not significantly affected by 3 μM TTX. [Decrease in peak AP amplitude was observed under TTX, agreeing with previous findings (18, 29).] Even under significant $I_{Na}$ blockade with 5 μM TTX, partial $I_{to}$ “blockade” using the dynamic clamp was able to fully restore AP dome and contraction in RV epicardial cells. These results suggest that the myocyte-level AP morphological or mechanical change we observed under $I_{Na}$ reduction was not due to suppressed AP upstroke, but was mainly a result of alteration of subsequent AP voltage trajectory. However, it should be noted that a reduction of $I_{Na}$ is likely to result in conduction delay, as proposed in the depolarization disorder model of BrS (22). This is supported by the observation that BrS patients with RV wall motion abnormalities had a wider QRS complex than those without wall motion abnormalities (32). The impact of $I_{Na}$ reduction on conduction is not tested in the present myocyte-level study. Another limitation of our experiments and simulations is that they were performed at the single-myocyte level, which allows the dissection of the un-

Fig. 6. Computational model of the influence of $I_{Na}$ kinetics on RV epicardial AP morphology. A: modeled RV epicardial APs at 100, 125, and 150% of $I_{Na}$ inactivation rate. B: modeled RV epicardial APD$_{90}$ as a function of $I_{Na}$ inactivation rate. C: modeled APD$_{90}$ as a function of $G_{Na}$ and $I_{Na}$ inactivation rate. D: modeled RV epicardial APs at control and 6- and 12-mV negative shifts in $I_{Na}$ steady-state inactivation (SSI). E: modeled RV epicardial APD$_{90}$ as a function of shifts in $I_{Na}$ SSI. F: modeled APD$_{90}$ as a function of $G_{Na}$ and negative shifts in $I_{Na}$ SSI curve. WT, wild type.
derlying ionic mechanisms of BrS abnormalities. It is recognized that intercellular coupling may average out localized AP abnormalities and alter the amount of \( I_{Na} \) and \( I_{Na} \) available to load the membrane capacitance, which may affect the extrapolation of our quantitative results (e.g., the threshold of \( I_{Na} \) reduction for all-or-none repolarization) to the tissue and organ levels.

In conclusion, using a combination of dynamic clamp simulation and computational modeling, we demonstrate the quantitative influence of selective \( I_{Na} \) reduction on AP morphology in canine ventricular myocytes and show that \( I_{Na} \) reduction alters repolarization by reducing the threshold for \( I_{Na} \)-induced all-or-none repolarization. These repolarization changes are associated with altered \( Ca^{2+} \) dynamics and suppressed myocyte contraction and may be a contributor to the contractile abnormality of the RV wall in BrS.

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DISCLOSURES

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