Cardiac Resynchronization Therapy Improves Altered Na Channel Gating in Canine Model of Dyssynchronous Heart Failure

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Background—Slowed Na\(^+\) current (\(I_{\text{Na}}\)) decay and enhanced late \(I_{\text{Na-L}}\) (\(I_{\text{Na-L}}\)) prolong the action potential duration (APD) and contribute to early afterdepolarizations. Cardiac resynchronization therapy (CRT) shortens APD compared with dyssynchronous heart failure (DHF); however, the role of altered Na\(^+\) channel gating in CRT remains unexplored.

Methods and Results—Adult dogs underwent left-bundle branch ablation and right atrial pacing (200 beats/min) for 6 weeks (DHF) or 3 weeks followed by 3 weeks of biventricular pacing at the same rate (CRT). \(I_{\text{Na}}\) and \(I_{\text{Na-L}}\) were measured in left ventricular myocytes from nonfailing, DHF, and CRT dogs. DHF shifted voltage-dependence of \(I_{\text{Na}}\) availability by −3 mV compared with nonfailing, enhanced intermediate inactivation, and slowed recovery from inactivation. CRT reversed the DHF-induced voltage shift of availability, partially reversed enhanced intermediate inactivation but did not affect DHF-induced slowed recovery. DHF markedly increased \(I_{\text{Na-L}}\) compared with nonfailing. CRT dramatically reduced DHF-induced enhanced \(I_{\text{Na-L}}\), abbreviated the APD, and suppressed early afterdepolarizations. CRT was associated with a global reduction in phosphorylated Ca\(^{2+}\)/Calmodulin protein kinase II, which has distinct effects on inactivation of cardiac Na\(^+\) channels. In a canine AP model, alterations of \(I_{\text{Na-L}}\) are sufficient to reproduce the effects on APD observed in DHF and CRT myocytes.

Conclusions—CRT improves DHF-induced alterations of Na\(^+\) channel function, especially suppression of \(I_{\text{Na-L}}\); thus, abbreviating the APD and reducing the frequency of early afterdepolarizations. Changes in the levels of phosphorylated Ca\(^{2+}\)/Calmodulin protein kinase II suggest a molecular pathway for regulation of \(I_{\text{Na}}\) by biventricular pacing of the failing heart. (Circ Arrhythm Electrophysiol. 2013;6:546-554.)

Key Words: arrhythmias ■ cardiac resynchronization therapy ■ electrophysiology ■ heart failure ■ Na\(^+\) channels

Congestive heart failure is associated with profound abnormalities in both cardiac rhythm and contractile function. The hallmark of cells and tissues isolated from failing hearts independent of the cause is prolongation of action potential duration (APD) attributable to ion channel remodeling, which is highly arrhythmogenic with frequent aberration of repolarization, such as exaggerated spatial and temporal heterogeneity of repolarization, and early afterdepolarizations (EADs) and delayed afterdepolarizations. Ion channel remodeling, especially K\(^+\) channel downregulation has been consistently demonstrated in human and animal models of heart failure. Slowed Na\(^+\) current (\(I_{\text{Na}}\)) decay and enhanced late \(I_{\text{Na-L}}\) (\(I_{\text{Na-L}}\)) prolong the action potential (AP) and contribute to the high frequency of EADs. Increased \(I_{\text{Na-L}}\) and elevated cytosolic Na\(^+\) ([Na\(^+\)\(^i\)]) in heart failure are linked with cellular Ca\(^{2+}\) overload via the reverse mode of the Na\(^+\)–Ca\(^{2+}\) exchange and with reactive oxygen species (ROS) generation as well as altered mitochondrial biogenesis. However, the role of Na\(^+\) current dysfunction in heart failure progression and arrhythmia predisposition is incompletely understood.

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Dyssynchronous ventricular contraction in heart failure (DHF) alters the electric phenotype and enhances the susceptibility to ventricular tachycardia or ventricular fibrillation. In patients who experience improvement in contractile synchrony, cardiac resynchronization therapy (CRT) improves symptoms, ventricular function and survival in patients with heart failure, and is expected to reduce frequency of arrhythmias and number of appropriate implantable cardioverter defibrillator shocks. We and others have shown that CRT using biventricular pacing in patients with heart failure and intraventricular conduction delays and acutely increases left ventricular (LV) ejection fraction with a reduction in myocardial oxygen consumption. More
recently, we characterized the LV regional remodeling of the cellular electrophysiology in DHF and CRT-induced reverse remodeling.\textsuperscript{15} APs were significantly prolonged in DHF, particularly in cells isolated from the lateral (LTR) LV wall; CRT significantly shortened the AP and reduced the LV regional heterogeneity in AP duration, which is consistent with another more chronic model of CRT in nonischemic cardiomyopathy.\textsuperscript{16}

In this study, we hypothesized that CRT restores the DHF-induced altered Na channel gating and, thus, abbreviates the AP and suppresses EADs. We further explore the role of CaM/ Ca\textsuperscript{2+}/Calmodulin protein kinase II (CaMKII) signaling and oxidant stress, known to alter Na channel inactivation, in the salutary effect of CRT on the cellular electrophysiology of the failing ventricle.

**Materials and Methods**

**Canine Tachypacing–Induced Heart Failure Model**

All protocols followed the US department of Agriculture and National Institutes of Health guidelines, and were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions. The canine models of DHF or CRT have been previously described and represent models of heart failure with dyssynchronous ventricular contraction (DHF) and synchronous contraction (CRT).\textsuperscript{17–19} In brief, adult male mongrel dogs underwent left bundle–branch–radiofrequency ablation and the right atrial pacing (200 beats/min) for 6 weeks (DHF dogs: n=7), or 3 weeks of right atrial pacing followed by 3 weeks of resynchronization by biventricular pacing at same pacing rate (CRT dogs: n=7). Control (nonfailing [NF]) dogs (n=4) underwent no tachypacing and no ablation.

**Whole-Cell Patch Clamp Recording**

Mid-wall LV anterior (ANT) and LTR myocytes from normal (NF), DHF, and CRT dogs were studied using the whole-cell patch clamp. Ventricular myocytes were current and voltage clamped in the whole-cell configuration of the patch clamp as previously described.\textsuperscript{8,10,20} Voltage and current clamp control and data acquisition were performed using custom-written software. For the measurement of APs, ventricular myocytes were current clamped with borosilicate glass electrodes with tip resistances of 3.0 to 4.0 mol/L\(\Omega\) when filled with pipette solution, and the stimulation frequency was varied over cycle lengths of 2.0, 1.0, 0.5, and 0.25 s. The steady-state APs were recorded and analyzed ≥1 minute after pacing at each cycle length in standard Tyrode’s solution (37°C).

\(I_{\text{s}}\) recording was performed at room temperature with patch pipettes with tip resistances of 1 to 1.5 mol/L\(\Omega\) tip resistance when filled with pipette solution containing (in mmol/L): NaCl 5, CsCl 40, glutamate 80, CsOH 80, Mg-ATP 5, EGTA 5, HEPES 10, CaCl\textsubscript{2} 1.5 (free [Ca\textsubscript{2}]+ =100 mmol/L, pH 7.2 with CsOH, liquid junction potential=+4.5 mV). The bath solution contained (in mmol/L): NaCl 10, MgCl\textsubscript{2} 2, CsCl 5, TEA-Cl 125, HEPES 20 (pH 7.4 with CsOH). In all experiments, recording was begun 10 to 15 minutes after establishment of the whole-cell mode to permit stabilization of current amplitude, voltage dependence, and kinetics of gating. Standard voltage protocols were used for assessment of the voltage-dependence of activation/inactivation, recovery from inactivation, and entry into inactivation. To determine the membrane potential of half inactivation (\(V_{1/2}\)) and the slope factor \(k\), activation or steady-state inactivation data were fit with a Boltzmann function of the form: 

\[
I/I_{\text{max}} = \frac{1}{1 + \exp(-(V-V_{1/2})/k)}
\]

Recovery from inactivation data was fit with a biexponential function of the form: 

\[
G/G_{\text{max}} = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3,\]

using a nonlinear least squares minimization. The decay phase of the current during a voltage step was fit with a biexponential function of the form: 

\[
I(t) = A_{1}\exp(-t/\tau_1) + A_{2}\exp(-t/\tau_2) + A_3,\]

where \(A_1\) and \(A_2\) are the fractions of fast and slow inactivating components, respectively. Persistent (late) \(I_{\text{s}}\), was the tetrodotoxin (30 \(\mu\)mol/L)-sensitive current elicited by 800 ms depolarizations to −20 mV (from −140 mV), from 100 to 500 ms after a depolarizing voltage step normalized to the peak \(I_{\text{s}}\).

**Molecular Analysis**

Canine Na\textsubscript{1.5} steady-state mRNA levels were measured by kinetic real-time polymerase chain reaction of RNA in tissue isolated from the midmyocardial layer of LV ANT and LTR walls in NF, DHF, and CRT dogs. Na\textsubscript{1.5}, and CaMKII proteins were measured by Western immunoblotting. Detailed methods are provided in the online-only Data Supplement.

**Computational Model**

Simulations were performed using a modified version of the canine coupled model incorporating local control of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release.\textsuperscript{21} This model was modified by replacing its description of \(I_{\text{s}}\) with a new Na\textsuperscript{+} channel representation, based on that of Grandi et al\textsuperscript{22} (Figure I in the online-only Data Supplement). The model is further described in the online-only Data Supplement.

**Statistical Analysis**

Differences among the 3 groups were compared by 1-way ANOVA with the Bonferroni test. Bonferroni-corrected comparisons were performed only if the overall comparison was statistically significant. Two-group analysis was performed by unpaired or paired \(t\) test as appropriate (Figures 3D and 4B, respectively). Data were expressed as mean±SD or mean±SEM as indicated in each of the figures. A value of \(P<0.05\) was considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agreed to the article as written.

**Results**

**Changes of \(I_{\text{s}}\) Availability by DHF and CRT**

There is no significant difference in the gating parameters between ANT and LTR cells in \(I_{\text{s}}\), and this study focused on the difference in Na\textsuperscript{+} currents between NF, DHF, and CRT conditions.

Figure 1A shows representative whole-cell \(I_{\text{s}}\) in ventricular myocytes from NF, DHF, and CRT dogs. The current–voltage (I–V) relationship of cardiac \(I_{\text{s}}\) revealed no significant difference in the peak and reversal potential of the \(I_{\text{s}}\) between NF, DHF, and CRT conditions (Figure 1B). Furthermore, no significant difference was found in the voltage-dependence of Na\textsuperscript{+} current activation in each group (Figure 1C; Table 1). On the contrary, DHF produced −3 mV shift in \(I_{\text{s}}\) steady-state inactivation compared with the NF dog (\(V_{1/2};−74.5±3.2\) versus −71.1±3.8 mV; \(P<0.05\); Figure 1D; Table 1), whereas CRT normalized the DHF-mediated hyperpolarizing shift of steady-state inactivation relationship (−70.6±0.2 mV; \(P<0.05\) versus DHF).

In DHF, \(I_{\text{s}}\) had a slower recovery from inactivation, and with a hastened entry into inactivation as compared with NF (Figure 1E; Table 1). The fast time constant of recovery from inactivation (\(\tau_{\text{fast}1}\)) was significantly larger in DHF than in NF (\(\tau_{\text{fast}1}=7.59±2.88\) versus 4.54±0.95 ms; \(P<0.05\)). Overall CRT did not significantly alter \(\tau_{\text{fast}}\) compared with DHF (Table 1). Table 2 and Figure 1F summarizes the effect of DHF or CRT on the entry of cardiac Na\textsuperscript{+} channels into inactivated states. DHF increased the fraction of channels undergoing slow inactivation compared with NF cells (\(y_0\): 0.74±0.08 DHF versus 0.83±0.05 NF; \(P<0.05\)). Myocytes from CRT hearts exhibited intermediate entry into
slow inactivated states with a statistically significantly larger fraction of current entering intermediate inactivated states compared with cells from NF hearts. Consistent with a global rather than regional alteration, CRT-induced changes in $I_{Na}$ kinetics and gating were similar in cells from the ANT and LTR walls.

**Changes of Decay Time Constant by DHF and CRT**

As shown in Figure 2A, DHF slowed $I_{Na}$ decay compared with NF, and CRT normalized the DHF-induced change of $I_{Na}$ decay. The initial ($\tau_{fast}$) and late ($\tau_{slow}$) time constants of $I_{Na}$ decay over a range of voltages are shown in Figure 2B and Table 1. There was no significant difference in the $\tau_{fast}$ between the groups, whereas $\tau_{slow}$ was significantly increased in DHF compared with NF (13.91±6.80 versus 4.44±3.62 ms; $P<0.05$), and CRT normalized the DHF-induced change of $I_{Na}$ decay (5.02±4.37 ms). No significant difference was observed in these time constants between ANT and LTR cells.
Table 1. Functional Effects of DHF and CRT on Na+ Current Recorded From Canine Ventricular Myocytes

<table>
<thead>
<tr>
<th></th>
<th>Steady-State Inactivation, mV</th>
<th>Na-L</th>
<th>Decay Time Constant, ms (@–20 mV)</th>
<th>Recovery From Inactivation, ms</th>
<th>Activation, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$V_{1/2}$</td>
<td>$n$</td>
<td>$\tau_{fast}$</td>
<td>$\tau_{slow}$</td>
<td>$n$</td>
</tr>
<tr>
<td>Nonfailing</td>
<td>–71.1±3.8</td>
<td>12</td>
<td>1.29±0.32</td>
<td>4.44±3.62</td>
<td>12</td>
</tr>
<tr>
<td>ANT</td>
<td>–70.5±3.2</td>
<td>6</td>
<td>1.38±0.14</td>
<td>4.04±2.81</td>
<td>6</td>
</tr>
<tr>
<td>LTR</td>
<td>–71.7±4.5</td>
<td>6</td>
<td>1.18±0.42</td>
<td>4.84±4.52</td>
<td>6</td>
</tr>
<tr>
<td>DHF</td>
<td>–74.5±3.2†</td>
<td>12</td>
<td>1.41±0.18</td>
<td>13.91±6.80†</td>
<td>13</td>
</tr>
<tr>
<td>ANT</td>
<td>–74.0±3.6</td>
<td>6</td>
<td>1.43±0.18</td>
<td>17.65±8.80</td>
<td>5</td>
</tr>
<tr>
<td>LTR</td>
<td>–75.6±3.4</td>
<td>6</td>
<td>1.46±0.13</td>
<td>11.24±4.18</td>
<td>8</td>
</tr>
<tr>
<td>CRT</td>
<td>–70.6±2.9#</td>
<td>7</td>
<td>1.46±0.24</td>
<td>5.02±4.37#</td>
<td>8</td>
</tr>
<tr>
<td>ANT</td>
<td>–69.9±3.5</td>
<td>4</td>
<td>1.53±0.30</td>
<td>3.07±1.39</td>
<td>5</td>
</tr>
<tr>
<td>LTR</td>
<td>–71.8±0.6</td>
<td>3</td>
<td>1.34±0.03</td>
<td>8.27±6.14</td>
<td>3</td>
</tr>
</tbody>
</table>

Mean±SD. ANT indicates anterior; CRT, Cardiac resynchronization therapy; DHF, dysynchronous heart failure; and LTR, lateral.
†$P<0.05$ vs NF, ‡$P<0.05$ vs DHF by ANOVA with Bonferroni test.

Change of Late $I_{Na}$ ($I_{Na,L}$) by DHF and CRT

Figure 3A shows the superimposed persistent $I_{Na,L}$ in myocytes from NF, DHF, and CRT canine ventricles ([$Na^+]_o=10$ mmol/L). $I_{Na,L}$ was elicited by 800 ms depolarizations to −20 mV (from −140 mV), and tetrodotoxin-sensitive currents were normalized to peak $I_{Na}$. The current integral was calculated between 100 and 500 ms after the depolarizing pulse. As shown in Figure 3B and Table 3, DHF significantly increased $I_{Na,L}$ compared with NF (1.33±0.66% versus 0.09±0.10%; $P<0.05$), but this increase was virtually eliminated in CRT myocytes (0.42±0.21%; $P<0.05$ versus DHF).

We then studied $I_{Na,L}$ in physiological bath solution ([$Na^+]_o=140$ mmol/L). $I_{Na,L}$ was elicited by 2000 ms depolarizations to −40 mV (from −140 mV). $I_{Na,L}$ was measured as the average amplitude of the current between 200 and 220 ms. DHF significantly increased $I_{Na,L}$ in physiological Na+ conditions (0.16±0.08–0.82±0.26 pA/pF; $P<0.05$ versus NF), and CRT dramatically reduced the DHF-induced increase of $I_{Na,L}$ (0.23±0.16 pA/pF; $P<0.05$ versus DHF). To demonstrate the $I_{Na,L}$ increase in heart failure, we measured $I_{Na}$ in [$Na^+]_o=140$ mmol/L at baseline and after ranolazine, which is known to block $I_{Na,L}$. Ranolazine (1 μmol/L) reduced $I_{Na,L}$ in myocytes from DHF (0.82±0.25–0.37±0.15 pA/pF; $P<0.05$) but not in myocytes isolated from NF or CRT hearts (Figure 3C and 3D). There were no regional differences in $I_{Na,L}$ in ANT and LTR cells in either [$Na^+]_o=10$ mmol/L and 140 mmol/L conditions.

APD Prolongation and Late $I_{Na}$

Normal impulse formation and conduction depend on the fast inward $I_{Na}$. Also, an increase in $I_{Na,L}$ can markedly prolong APD and promote polymorphic ventricular tachycardia. AP prolongation in DHF is highly arrhythmogenic with frequent EADs that were not observed in myocytes isolated from NF hearts. CRT partially abbreviated the DHF-induced APD prolongation and reduced the frequency of EADs in cells isolated from both the ANT and LTR LV.4,15 To understand the contribution of $I_{Na,L}$ to prolongation of the AP in DHF and CRT, we added ranolazine to the bath solution during continuous AP recording. Shown in Figure 4A, ranolazine (1 μmol/L) did not change the AP in myocytes from NF; however, it significantly shortened APD in cells isolated from DHF hearts (Figure 4B and 4C), consistent with an increase of $I_{Na,L}$, having a substantial role in APD prolongation in DHF. Myocytes from CRT hearts had a reduced $I_{Na,L}$ density compared with DHF; thus, ranolazine had less effect on APD and EADs in cells from these hearts compared with DHF (Figure 4D).

CRT has a number of effects on electric remodeling in the failing heart, including reversal of Na+ channel downregulation and improvement of SR Ca handling.5 To determine whether the changes in $I_{Na,L}$ could explain the changes in APD, we used a mathematical model of the canine AP.21,22 Increasing normalized $I_{Na,L}$ from 0.11% to 0.88% of the peak $I_{Na}$ reproduced APD prolongation similar to that observed in myocytes from DHF hearts. An intermediate normalized $I_{Na,L}$ of 0.35% of peak current shortened the AP compared with DHF, similar to that observed in CRT. The simulations are consistent with changes of $I_{Na,L}$ in heart failure contributing to APD prolongation in DHF and its abbreviation by CRT (Figure 4E).

Ca2+/Calmodulin Protein Kinase II

Heart failure is associated with global23 and regional19,24 increases in CaMKII activity that are partially reversed...
by CRT. CaMKII modulates the function of Na currents, producing an increase in \( I_{\text{Na-L}} \); therefore, we propose that changes in CaMKII expression in myocytes are linked to alteration in the Na current phenotype in NF, DHF, and CRT myocytes. As shown in Figure 5, DHF increased total and phosphorylated (pThr287) CaMKII compared with NF. Notably, CRT significantly reduced the DHF-induced increase of CaMKII pThr287 with a trend in a reduction in total CaMKII. No significant regional differences between ANT and LTR regions of the LV were found in any group.

Discussion

The failing heart is characterized by a number of changes in its electrophysiological function, prominently prolongation of the AP and delayed repolarization. This is attributed to a number of changes in ionic and transporter currents described in heart failure, and these prominently include changes in the density and gating of the Na\(^+\) current. We previously reported that resynchronization of mechanical contraction by biventricular pacing of the failing heart, despite persistence of global LV dysfunction, reverses
Cardiac Resynchronization and Na Channel Function

Table 3. Effect of DHF and CRT on the Peak and Late Na+ Current

<table>
<thead>
<tr>
<th>Current</th>
<th>[Na+]o=10 mmol/L</th>
<th>[Na+]o=140 mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak I(<em>{Na}) PA/pF n Late I(</em>{Na}) %</td>
<td>Peak I(<em>{Na}) PA/pF n Late I(</em>{Na}) %</td>
</tr>
<tr>
<td>Nonfailing</td>
<td>-60.3±9.7 14 0.09±0.10</td>
<td>10 0.17±0.16 8</td>
</tr>
<tr>
<td>ANT</td>
<td>-60.0±11.7 7 0.07±0.04</td>
<td>5 0.17±0.09 4</td>
</tr>
<tr>
<td>LTR</td>
<td>-60.6±8.2 7 0.10±0.14</td>
<td>5 0.16±0.13 4</td>
</tr>
<tr>
<td>DHF</td>
<td>-51.5±16.9 16 1.32±0.66†</td>
<td>10 0.82±0.25† 8</td>
</tr>
<tr>
<td>ANT</td>
<td>-47.5±21.3 8 1.34±0.91</td>
<td>4 0.78±0.18 4</td>
</tr>
<tr>
<td>LTR</td>
<td>-54.3±13.4 8 1.31±0.53</td>
<td>6 0.83±0.29 4</td>
</tr>
<tr>
<td>CRT</td>
<td>-52.8±15.9 8 0.42±0.21†#</td>
<td>8 0.29±0.14†# 8</td>
</tr>
<tr>
<td>ANT</td>
<td>-53.1±20.4 4 0.54±0.19</td>
<td>4 0.27±0.20 4</td>
</tr>
<tr>
<td>LTR</td>
<td>-52.4±14.6 4 0.26±0.09</td>
<td>4 0.31±0.16 4</td>
</tr>
</tbody>
</table>

Mean±SD. ANT indicates anterior; CRT, Cardiac resynchronization therapy; DHF, dyssynchronous heart failure; and LTR, lateral.
†P<0.05 vs NF, ‡P<0.05 vs DHF by ANOVA with Bonferroni test.

I\(_{Na}\) Remodeling in DHF and CRT

Normal impulse formation and conduction as well APD depend on the inward I\(_{Na}\). Studies of I\(_{Na}\) regulation in heart failure have been somewhat variable and model dependent. In this study, we found peak current was not significantly altered, although others have reported downregulation in a similar model. However, we did observe slowing of the decay and rise in late current, and this has been observed in a canine myocardial infarction model of heart failure and in failing human hearts. An increase in the late component of Na+ current (I\(_{Na-L}\)) can markedly prolong APD and facilitate the generation of EADs and promote polymorphic ventricular tachycardia. Therefore, changes in I\(_{Na-L}\) density and kinetics may predispose to arrhythmias either by disrupting conduction and prolonging repolarization. In this study, the increase of I\(_{Na-L}\) by DHF prolonged APD, although CRT partially normalized I\(_{Na-L}\), and abbreviated the APD.

Ranolazine is an inhibitor of a number of ion channels with relative selectivity for I\(_{Na-L}\) and antiarrhythmic effects in vitro. In an ischemic canine model of HF, ranolazine improved abnormal repolarization and contraction in LV myocytes. Consistent with CRT modifying I\(_{Na-L}\) as key mechanism for electric reverse remodeling, we found that ranolazine suppresses I\(_{Na-L}\) and, at a low dose, completely normalized the APD and prevented EADs in acutely isolated DHF myocytes. In cells from NF and CRT hearts with significantly less I\(_{Na-L}\), the drug had little effect on the APD. Simulations of the experimentally

Figure 4. Cardiac resynchronization therapy (CRT) abbreviates dyssynchronous heart failure (DHF)-induced prolongation of action potential duration (APD) by reducing late Na+ current. A, Representative APs recorded from myocytes isolated from the lateral wall of the LV in nonfailing (NF), DHF, and CRT canine ventricles at baseline and after ranolazine (1 μmol/L) at a paced cycle length of 2000 ms. B, Action potential duration at 90% repolarization (APD\(_{90}\)) at baseline and after ranolazine at a paced CL of 2000 ms in NF, DHF, and CRT, and (C) fractional change in APD\(_{90}\) by ranolazine in each group. D, Development of early afterdepolarizations (EADs) at baseline and after ranolazine in ventricular myocytes from NF, DHF, and CRT dogs. E, Alteration of I\(_{Na-L}\) in a canine mathematical AP model mimicking DHF and CRT reproduced the experimental effects of DHF and CRT on the APD. ¶P<0.01, †P<0.05 vs NF, ‡P<0.01 vs DHF by ANOVA with Bonferroni test. **P<0.01, *P<0.05 vs baseline by paired t test.
determined changes in the magnitude of $I_{\text{Na,L}}$ in a canine AP model for each condition predicted prolongation of the APD in DHF, albeit larger than that observed experimentally. The quantitative contribution of the increase in $I_{\text{Na,L}}$ to APD is complex, but reproduces the AP prolongation in DHF and the partial reversal of the APD prolongation in CRT myocytes. The data, which are consistent with a global reduction in APD and hastening of repolarization by CRT, suggest a mechanism for the antiarrhythmic properties of this pacing therapy. The data do not allow us to speak to the role of ranolazine as an antiarrhythmic therapy in this model because we used a concentration of the drug designed to affect only $I_{\text{Na,L}}$ and not other changes in the Na current or other currents that are remodeled in heart failure.

**CaMKII and Oxidant Stress**

Among the many signaling changes in the failing heart, an increase in CaMKII protein levels and activity has been regularly observed. A mechanistic link between the increase in CaMKII activity and $I_{\text{Na,L}}$ that is observed in human and canine heart failure is suggested by recent studies showing that intracellular CaMKII signaling increases $I_{\text{Na,L}}$ and Na$^+$ influx in failing myocytes by slowing inactivation kinetics and shifting steady-state inactivation. In our study, consistent with these previous results, DHF is associated with an increase CaMKII activity; moreover, CRT partially normalizes CaMKII activity indexed by a reduction in the autophosphorylated forms of the enzyme (Figure 5), at the same time reducing the amplitude of $I_{\text{Na,L}}$. The data support a critical role of CaMKII and its regulation in the remodeling and reverse electric remodeling of ventricular myocyte electrophysiology by DHF and CRT, respectively. Oxidative stress is linked to the progression of heart failure, and mitochondria are a critical source of ROS in failing myocardium. Blocking $I_{\text{Na,L}}$ reduces hydrogen peroxide–induced arrhythmogenic activity and contractile dysfunction. Oxidative stress–induced afterdepolarizations are directly linked to CaMKII activity. Furthermore, Kohlhaas et al suggest that increased [Na$^+$] in heart failure promotes ROS formation by reducing mitochondrial Ca$^{2+}$ uptake. Cardiac resynchronization of the failing heart potently alters the mitochondrial proteome with an associated improvement in mitochondrial function reflected in an increase in the respiratory control index. Moreover, DHF is associated with global and region activation of stress-related proteins, and CRT reduces stress kinase activation. We did not directly measure ROS in these studies; however, our findings suggest a mechanism of remodeling in DHF characterized by an ROS- and CaMKII-mediated increase of $I_{\text{Na,L}}$, and that is reversed by a reduction in CaMKII activity decreasing $I_{\text{Na,L}}$ and suppressing EADs in CRT.

**Limitations**

One limitation in this study is the absence of a direct measurement of oxidant levels in the tissue; however, our recent study demonstrating that resynchronization improved mitochondrial oxidative phosphorylation coupling suggests that CRT may suppress ROS level in the heart failure. Second, as we have shown previously, CRT improves multiple ion channel and Ca$^{2+}$ handling abnormalities in DHF, and the functional effects on cardiac electrophysiology are complex and interrelated. Therefore, we used computer simulations to quantify the effect of reverse remodeling of Na$^+$ current in the context of the changes in other ionic currents and transporters in our models of DHF and CRT. An isolated change in $I_{\text{Na,L}}$ in the simulated AP is sufficient to dramatically alter the duration consistent with a significant contribution of $I_{\text{Na,L}}$ to

![Figure 5. Cardiac resynchronization therapy (CRT) partially normalizes the dyssynchronous heart failure (DHF)-induced increase of Ca$^{2+}$/Calmodulin protein kinase II (CaMKII). A, Total CaMKII protein expression in nonfailing (NF), DHF, and CRT canine ventricle. B, Phosphorylation of CaMKII (p-Thr 287) in NF, DHF, and CRT ventricular myocardium. ANT indicates anterior; and LTR, lateral myocardium. †P<0.05 vs NF, #P<0.05 vs DHF by ANOVA with Bonferroni test.](http://circep.ahajournals.org/)

![Graph A](http://circep.ahajournals.org/)

![Graph B](http://circep.ahajournals.org/)
prolongation of AP in the failing heart but is certainly not the only change that influences the APD in DHF and CRT.

Clinical Implications

CRT is a remarkable therapy that improves symptoms and survival in a subset of patients with heart failure associated with dyssynchronous mechanical contraction. The mechanical act of resynchingronization contraction by pacing has significant effects on cellular signaling in the failing heart that are associated with not only improved mechanical performance but also enhanced energetic efficiency. This provides an explanation for the mechanism of action of CRT in heart failure and identified pathways that are central to improving cardiac function and which could be approached pharmacologically in patients that do not respond to CRT. In addition, a role for $I_{Na}$ in the AP prolongation associated with DHF and its reversal by CRT in the setting of improved mechanical and electric performance of the heart suggest a pathogenic role of the late Na$^+$ current in arrhythmias in heart failure that could be addressed not only by CRT, but also by pharmacological agents that target this current component.

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Disclosures

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References

29. Pu J, Boyden PA. Alterations of Na$^+$ currents in myocytes from epicardial border zone of the infarcted heart. A possible ionic mechanism

**CLINICAL PERSPECTIVE**

Cardiac resynchronization therapy using biventricular pacing improves symptoms, cardiac function, exercise capacity and, when combined with defibrillator therapy, reduces mortality in patients with heart failure who have dyssynchronous contraction (diastolic heart failure). The mechanical act of resynchronizing contraction by pacing has significant effects on cellular signaling in the failing heart that are associated with not only improved mechanical performance but also enhanced energetic efficiency. Our previous studies demonstrated altered expression and function of ionic currents and calcium transients in dyssynchronous heart failure and restoration by cardiac resynchronization therapy in a canine pacing–induced dyssynchronous heart failure model. An increase in the late component of Na+ current (I(NaL)) prolongs action potential duration and is associated with arrhythmias in long-QT syndrome or heart failure. This study demonstrated an important role for I(NaL) in the action potential prolongation in dyssynchronous heart failure and its reversal by cardiac resynchronization therapy, associated with a reduction of Ca2+/Calmodulin protein kinase II activity in the setting of improved mechanical and electric performance of the heart. The data support a pathogenic role for the late Na+ current in arrhythmias in heart failure that could be addressed not only by cardiac resynchronization therapy, but also by pharmacological agents that target this current component.
Cardiac Resynchronization Therapy Improves Altered Na Channel Gating in Canine Model of Dyssynchronous Heart Failure

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SUPPLEMENTAL MATERIAL

The action potential simulations were performed using a modified version of the canine coupled model incorporating local control of Ca\(^{2+}\)-induced Ca\(^{2+}\) release\(^1\). This model was modified by replacing its description of \(I_{Na}\) with a new Na\(^+\) channel representation, based on that of Grandi et al (see Figure S1)\(^2\). The Na\(^+\) channel model has 13 states, and transition rates between these states were constrained by the steady state activation, steady state inactivation, time course of intermediate inactivation, time course of recovery from inactivation, late \(I_{Na}\) amplitude and \(I_{Na}\) kinetics (time to peak and current decay). The model is formulated at 37°C and accounts for experimentally-observed temperature-dependent shifts in \(I_{Na}\) kinetics.\(^3,4\) In order to isolate the effect of late \(I_{Na}\) in CRT, the NF transition rate into the burst mode (transition rate \(a_8\) in Figure S1) was varied. In CRT, \(a_8\) is 2 times larger than \(a_8\) in NF; in DHF, \(a_8\) is 7 times larger than \(a_8\) in NF. Model parameters are listed below:

\[
\begin{align*}
G_{Na} &= 8.5 \\
I_{Na} &= G_{Na} \cdot (O + LO) \cdot (V - E_{Na}) \\
\end{align*}
\]

\[
\begin{align*}
P_{1a1} &= 3.9926 \\
P_{2a1} &= 0.0204 \\
P_{1a4} &= 5.758 \\
P_{2a4} &= 107.6672 \\
P_{1a5} &= 1.62 \times 10^{-8} \\
P_{2a5} &= 6.2134 \\
P_{1b1} &= 0.003 \\
P_{2b1} &= 9.3532 \\
P_{1b2} &= 0.0367 \\
P_{2b2} &= 6.6636 \\
P_{1b3} &= 0.0017 \\
P_{2b3} &= 17.3338 \\
P_{1b5} &= 0.0133 \\
P_{2b5} &= -7.1875 \times 10^{-6} \\
P_{1a6} &= 27.2731 \\
P_{1b6} &= 2.445 \times 10^{-6} \\
P_{2b6} &= 11.7989 \\
P_{1a7} &= 0.0047
\end{align*}
\]
\[ P_{2a7} = 25.9073 \]
\[ P_{1b7} = 0.003 \]
\[ P_{2b7} = 53.443 \]
\[ P_{1a8} = 2.4222 \times 10^{-6} \text{ (NF)}, \ 4.8444 \times 10^{-6} \text{ (CRT)}, \ 1.6955 \times 10^{-5} \text{ (DHF)} \]
\[ P_{1b8} = 0.0015 \]
\[ P_{2a4_2} = 1.2109 \]

\[ a_1 = \frac{P_{1a1}}{(P_{2a1} \times \exp(-V/17) + 0.2 \times \exp(-V/150))}; \]
\[ a_2 = \frac{P_{1a1}}{(P_{2a1} \times \exp(-V/15) + 0.23 \times \exp(-V/150))}; \]
\[ a_3 = \frac{P_{1a1}}{(P_{2a1} \times \exp(-V/12) + 0.25 \times \exp(-V/150))}; \]
\[ b_1 = P_{1b1} \times \exp(-V/P_{2b1}); \]
\[ b_2 = P_{1b2} \times \exp(-(V-P_{2b2})/(P_{2b1})); \]
\[ b_3 = (P_{1b3} \times \exp(-(V-P_{2b3})/(P_{2b1}))); \]
\[ a_5 = P_{1a5} \times \exp(-V/P_{2a5}); \]
\[ b_5 = (P_{1b5} + P_{2b5} \times V); \]
\[ a_4 = (P_{1a4} \times \exp(V/P_{2a4})); \]
\[ b_4 = (a_3 \times a_4 \times a_5)/(b_3 \times b_5); \]
\[ a_6 = (P_{1a6} \times \exp(V/(P_{2a4} \times P_{2a4_2})))\times P_{1a6}; \]
\[ b_6 = P_{1b6} \times \exp(-V/P_{2b6}); \]
\[ a_7 = (P_{1a7} \times \exp(V/P_{2a7})); \]
\[ b_7 = P_{1b7} \times \exp(-V/P_{2b7}); \]
\[ a_8 = P_{1a8}; \]
\[ b_8 = P_{1b8}; \]
**Supplemental Figure 1**

Thirteen state model used by Grandi et al.\(^2\) for the Na current incorporated into the coupled canine AP model of Greenstein et al.\(^1\)

**References**