Heart Failure

Electrophysiological Consequences of Dyssynchronous Heart Failure and Its Restoration by Resynchronization Therapy

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Background—Cardiac resynchronization therapy (CRT) is widely applied in patients with heart failure and dyssynchronous contraction (DHF), but the electrophysiological consequences of CRT in heart failure remain largely unexplored. Methods and Results—Adult dogs underwent left bundle-branch ablation and either right atrial pacing (190 to 200 bpm) for 6 weeks (DHF) or 3 weeks of right atrial pacing followed by 3 weeks of resynchronization by biventricular pacing at the same pacing rate (CRT). Isolated left ventricular anterior and lateral myocytes from nonfailing (control), DHF, and CRT dogs were studied with the whole-cell patch clamp. Quantitative polymerase chain reaction and Western blots were performed to measure steady state mRNA and protein levels. DHF significantly reduced the inward rectifier K⁺ current (I_{K1}), delayed rectifier K⁺ current (I_{K}) and transient outward K⁺ current (I_{O}) in both anterior and lateral cells. CRT partially restored the DHF-induced reduction of I_{K1} and I_{O} but not I_{O}, consistent with trends in the changes in steady state K⁺ channel mRNA and protein levels. DHF reduced the peak inward Ca²⁺ current (I_{Ca}) density and slowed I_{Ca} decay in lateral compared with anterior cells, whereas CRT restored peak I_{Ca} amplitude but did not hasten decay in lateral cells. Calcium transient amplitudes were depressed and the decay was slowed in DHF, especially in lateral myocytes. CRT hastened the decay in both regions and increased the calcium transient amplitude in lateral but not anterior cells. No difference was found in CaV1.2 (α1C) mRNA or protein expression, but reduced CaV1.2 mRNA was found in DHF cells. DHF reduced phospholamban, ryanodine receptor, and sarcoplasmic reticulum Ca²⁺ ATPase and increased Na⁺-Ca²⁺ exchanger mRNA and protein. CRT did not restore the DHF-induced molecular remodeling, except for sarcoplasmic reticulum Ca²⁺ ATPase. Action potential durations were significantly prolonged in DHF, especially in lateral cells, and CRT abbreviated action potential duration in lateral but not anterior cells. Early afterdepolarizations were more frequent in DHF than in control cells and were reduced with CRT. Conclusions—CRT partially restores DHF-induced ion channel remodeling and abnormal Ca²⁺ homeostasis and attenuates the regional heterogeneity of action potential duration. The electrophysiological changes induced by CRT may suppress ventricular arrhythmias, contribute to the survival benefit of this therapy, and improve the mechanical performance of the heart. (Circulation. 2009;119:1220-1230.)

Key Words: ion channels  remodeling  heart failure  resynchronization  electrophysiology

Nearly 5 million Americans have heart failure (HF), and >250,000 die annually. Up to 50% of deaths in patients with HF are sudden and unexpected, mainly because of lethal ventricular arrhythmias such as ventricular tachycardia and ventricular fibrillation; however, the mechanisms of ventricular tachycardia and ventricular fibrillation in patients with HF remain controversial.¹ ²

Editorial p 1192  
Clinical Perspective p 1230

As HF progresses, the heart adapts to intrinsic and extrinsic stresses through a complex process of chamber remodeling and molecular modifications of myocyte structure and function. The structural remodeling and alteration of cell-to-cell coupling are associated with heterogeneous conduction delays in the ventricular myocardium that can lead to dyssynchronous ventricular contraction.³ Dyssynchronous contraction adversely influences the electrical phenotype of the failing heart and has been associated with regional changes in calcium (Ca²⁺) handling protein expression⁴ and exaggerated heterogeneities in conduction⁵ and repolarization⁶–⁸ which enhances the susceptibility to ventricular arrhythmias in HF.⁹ Predictably, advanced HF with dyssynchronous cardiac contraction (DHF) is associated with a poor prognosis.¹⁰ Cardiac
resynchronization therapy (CRT) with biventricular pacing improves symptoms,11 cardiac function, and mortality,12,13 presumably due to a reduction in stress-strain disparities and improvement in the efficiency of ventricular contraction. We have recently demonstrated that CRT reverses regional molecular remodeling and reduces apoptosis.14

Ion channel remodeling in HF underlies many of the changes in cellular electrophysiology and predisposes to atrial and ventricular arrhythmias in animal models and patients with HF.1,2,15–20 A consistent electrophysiological consequence of HF is a prolongation of ventricular repolarization, which is due in part to functional downregulation of outward potassium currents.2,15–17,19–21 However, little information is available about regional differences in ion channel function and expression or action potential (AP) profiles between early- and late-activated regions of dyssynchronous contracting left ventricles (LVs). Furthermore, the extent to which CRT reverses the DHF-induced electrophysiological remodeling remains unexplored.

Here, we tested the hypothesis that dyssynchronous mechanical contraction in HF contributes significantly to pathological remodeling at the cellular and molecular levels. We studied whether and to what extent remodeling could be reversed by biventricular pacing. We characterized the regional differences of AP, ionic currents, and [Ca2+]transients (CaT) in myocytes isolated from the anterior and lateral LV myocardium of dogs with DHF and CRT and correlated the cellular electrophysiological changes with their subunit mRNA and protein expression in corresponding regions of the LV. These findings suggest distinct mechanisms of altered electrophysiological remodeling in DHF, as well as reverse remodeling by CRT. These data provide new mechanistic understanding of the therapeutic role of CRT and will help to identify new antiarrhythmic targets to prevent sudden death in patients with HF.

Methods
An expanded Methods section is available in the online-only Data Supplement.

Canine Tachypacing-Induced HF Model
All protocols followed 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 described previously.2,14,22 In brief, adult male mongrel dogs underwent left bundle-branch radiofrequency ablation and right atrial pacing (190 to 200 bpm) for 6 weeks (DHF dogs; n=7) or 3 weeks of right atrial pacing (190 to 200 bpm) for 6 weeks (DHF dogs; n=7). These findings suggest distinct mechanisms of altered electrophysiological remodeling in DHF, as well as reverse remodeling by CRT. These data provide new mechanistic understanding of the therapeutic role of CRT and will help to identify new antiarrhythmic targets to prevent sudden death in patients with HF.

ECC, Echocardiography, and Hemodynamic Recordings
To monitor LV function during tachypacing, 2-dimensional echocardiography with tissue Doppler imaging was performed periodically together with recordings of ECGs. Briefly, standard 2-dimensional and color Doppler image data, triggered to the QRS complex, were saved in cine-loop format. LV volumes (end-systolic and end-diastolic) and LV ejection fraction were calculated from the conventional apical 2- and 4-chamber views. LV dyssynchrony was quantified with speckle-tracking radial strain analysis (Figure 1) as described previously.23 The LV dyssynchrony index was defined as the SD of time to peak systolic velocity of 12 segments. Standard 12-lead ECG and hemodynamic data (LV systolic and end-diastolic pressure, dP/dtmax) were recorded 6 weeks after the start of pacing.

Patch Clamp and CaT
All electrophysiological and intracellular Ca2+ measurements were performed at physiological temperature (37°C). All methods for cell isolation, electrophysiological recording, and measurements are routine in our laboratory and have been described previously.2,14,18,16,17,20 Isolated ventricular myocytes from the anterior and lateral mid myocardium were current and voltage clamped by the standard whole-cell patch-clamp technique for measurement of APs, transient outward K+ current (Ito), inward rectifier K+ current (IK1), delayed rectifier K+ currents (IK1 and IKs), and Ca2+ current (ICaL). Borosilicate glass electrodes with tip resistances of approximately 3.0 MΩ when filled with the pipette solution were used.

CaTs were measured by indo-1 fluorescence excited at 365 nm with a xenon arc lamp, and emitted light at 405 and 495 nm was collected with a 2-channel photomultiplier tube assembly. Fluorescence signals were digitized and stored with electrophysiological recordings for offline analysis with custom software.24 The ratio of indo-1 fluorescence (R=I405/I495) was determined after subtraction of cellular autofluorescence. The rate of Ca2+ removal (τCa) was determined by fitting a single exponential to the Ca2+ time course.

Molecular Analysis
Canine Kir2.1, Kcv4.3, KChIP2, K11.1 (either a-go-go related gene [ERG], Kv7.1 (KvLQT1), minK, CaV1.2 (α1C), CaVβ1, CaVβ2, sarcoplasmic reticulum Ca2+ ATPase (SERCA2), phospholamban, ryanodine receptor, and Na+–Ca2+ exchanger steady state mRNA levels were measured by reverse-transcription polymerase chain reaction in tissue isolated from the LV anterior and lateral walls in control, DHF, and CRT dogs (online-only Data Supplement Table I). Kir2.1, Kcv4.3, KChIP2, ERG, KvLQT1, CaV1.2 (α1C), SERCA2, phospholamban, ryanodine receptor, and Na+–Ca2+ exchanger proteins were measured by Western immunoblotting. Detailed methods are provided in the online-only Data Supplement.

Statistical Analysis
Differences among multiple groups were compared by ANOVA with Bonferroni test. Two-group analysis was performed by t test (paired or unpaired as appropriate). Differences in serial studies were assessed by repeated-measures ANOVA. Data are expressed as 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 agree to the manuscript as written.

Results
ECG and Hemodynamic Changes
Figure 1 shows representative standard ECG and LV wall strain plots by tissue Doppler from a control (A), 6-week paced DHF (B), and CRT (C) dog. In the DHF dog, the QRS interval was wider, with a left bundle-branch block pattern compared with the control, and the tissue Doppler image showed dyssynchronous contraction between the septum and the lateral walls. CRT shortened the QRS duration and resynchronized the LV wall strain pattern so that it was similar to the control.

The Table summarizes the ECG, echocardiographic, and hemodynamic changes in control, 6-week paced DHF, and CRT dogs. In sinus rhythm, the R-R interval was shorter and the corrected QT and QRS durations were longer in both DHF and CRT dogs than in control dogs, but no significant difference was observed in these parameters between DHF and CRT dogs. However, during pacing, QRS width was significantly shorter in CRT than DHF dogs, which was...
consistent with the larger dyssynchrony index by tissue Doppler echocardiography in DHF compared with control or CRT dogs. Moreover, CRT significantly decreased the corrected QT interval compared with DHF. Conversely, LV diastolic and systolic volumes, as well as hemodynamic parameters such as LV end-diastolic and end-systolic pressure and dP/dtmax, were not statistically different between DHF and CRT. A trend was observed toward increased stroke volume and ejection fraction in CRT versus DHF dogs.

Cell Capacitance

Cell capacitance was larger in DHF and CRT than in control dogs in cells isolated from both the anterior and lateral walls, but no regional difference was found between anterior and lateral cells. The capacitance of cells isolated from CRT dogs was not different from that of cells isolated from DHF dogs (online-only Data Supplement Table II).

Inward Rectifier K⁺ Current

Figure 2A shows whole-cell \( I_{K1} \) currents in lateral myocytes from control, DHF, and CRT dogs. The densities of \( I_{K1} \) from control and DHF myocytes were similar to data previously reported from our laboratory.\(^{16}\) \( I_{K1} \) density in DHF myocytes was reduced significantly compared with control, whereas CRT partially reversed the DHF-induced reduction of \( I_{K1} \) over a wide voltage range (Figure 2B). On the other hand, no regional difference was found in \( I_{K1} \) between anterior and lateral myocytes isolated from either DHF or CRT hearts (Figure 2C). The relevant component of \( I_{K1} \) for AP repolarization is the outward current “hump” observed at potentials positive to the K⁺ reversal potential. The peak outward component of \( I_{K1} \) at \(-70\) and \(-60\) mV was decreased modestly but significantly (\( P<0.05 \)) in DHF myocytes (1.43±0.52 and 1.07±0.43 pA/pF, respectively) compared with control (1.78±0.54 and 1.26±0.51 pA/pF, respectively) and CRT (1.81±0.81 and 1.18±0.57 pA/pF, respectively). To investigate the molecular basis for changes in \( I_{K1} \), we measured Kir2.1 mRNA and protein levels; these were significantly downregulated in DHF compared with control (\( P<0.05 \)), whereas those in CRT were decreased (although not statistically significant) compared with control (Figures 2D and 2E). No regional differences in Kir2.1 mRNA and protein expression were found among the 3 groups, which is consistent with the cellular electrophysiology.

Transient Outward K⁺ Current

Figure 3A shows whole-cell \( I_o \) currents in lateral myocytes from control, DHF, and CRT dogs. The densities of \( I_o \) from control and DHF myocytes in this preparation are similar to those reported previously in our laboratory.\(^{16}\) \( I_o \) in DHF myocytes was reduced significantly compared with control (\( P<0.05 \)), but CRT did not restore the DHF-induced reduction of \( I_o \) (Figure 3B). Interestingly, \( I_o \) current density was not different between

Figure 1. Representative ECGs and changes of LV septal (SEP) and lateral (LTR) strain from control (A), 6-week paced DHF (B), and CRT (C) dogs. ECGs from DHF and CRT dogs are shown during pacing, and control is shown during sinus rhythm. Biventricular pacing synchronized the strain patterns between LV septal and lateral walls and abbreviated the DHF-induced prolongation of QRS duration.
Table. ECG, Echocardiography, and Hemodynamic Parameters

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<th>CRT</th>
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<td>RR</td>
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<td>831 ± 246*</td>
<td>846 ± 133*</td>
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</table>

QTC indicates corrected QT; D1, dysynchrony index; LVEDV, LV end-diastolic volume; LVEVS, LV end-systolic volume; SV, stroke volume; LVEF, LV ejection fraction; HR, heart rate; LVEDP, LV end-diastolic pressure; and LVEP, LV end-systolic pressure.

Values are mean ± SD.

*P<0.05 vs control; †P<0.05 vs DHF.

Figure 5A shows total IK in lateral myocytes from control, DHF, and CRT dogs. As shown in the superimposed currents elicited by a step pulse to +50 mV, the peak and tail IK currents were smaller in DHF than in control myocytes, and CRT partially restored the DHF-induced reduction of IK. Figure 4B displays the current-voltage relationship of the tail current density of IK in the 3 groups, showing that DHF reduced IK density by ≈50% compared with control, whereas CRT partially restored the DHF-induced reduction of IK (P<0.05). In addition, no regional difference was found in the reduction and restoration of IK by DHF and CRT (Figure 4C).

To investigate the molecular basis for changes in IK, we measured the expression of the underlying α-subunit (Kir2.1) and β-subunit (KChIP2) underlying this current (Figures 3D through 3G). Consistent with the IK density, both Kir2.1 and KChIP2 mRNA and protein were significantly downregulated in both DHF and CRT, with no regional differences in each group.

**Delayed Rectifier K+ Currents**

Figure 4A shows total IK in lateral myocytes from control, DHF, and CRT dogs. As shown in the superimposed currents elicited by a step pulse to +50 mV, the peak and tail IK currents were smaller in DHF than in control myocytes, and CRT partially restored the DHF-induced reduction of IK. Figure 4B displays the current-voltage relationship of the tail current density of IK in the 3 groups, showing that DHF reduced IK density by ≈50% compared with control, whereas CRT partially restored the DHF-induced reduction of IK (P<0.05). In addition, no regional difference was found in the reduction and restoration of IK by DHF and CRT (Figure 4C).

Protein were reduced significantly in DHF compared with control myocytes; CRT did not alter KvLQT1 mRNA expression but increased the protein level such that it was not significantly different from control (Figure 4D and 4E). No significant differences were found in minK mRNA expression among the 3 groups (Figure 4F). ERG mRNA expression was reduced in DHF and completely restored by CRT (Figure 4G), but no significant difference was found in ERG protein expression in any of the groups (Figure 4H). In addition, consistent with the physiological data, no regional differences in gene or protein expression were found between anterior and lateral LV tissues from each group.

**Inward Ca2+ Current (I_{Ca}) and CaT**

Figure 5A shows I_{Ca} in LV midmyocardial myocytes isolated from control, DHF, and CRT hearts. In control, no difference was found in the peak I_{Ca} density or current decay between anterior and lateral myocytes; however, DHF reduced the peak I_{Ca} density and slowed the rate of current decay in lateral...
cells compared with anterior cells. Furthermore, in CRT, no regional difference was found in peak $I_{Ca}$ density, but the rate of current decay was still slower in lateral than in anterior cells. The current-voltage relationships (Figure 5B) showed that peak $I_{Ca}$ density was significantly less in lateral cells than in anterior cells in DHF at voltage steps from $-15$ to $+20$ mV ($P<0.05$). In contrast, CRT increased $I_{Ca}$ density in lateral cells but not in anterior cells.

Although the voltage dependence of activation of $I_{Ca}$ (Figure 5C) was not altered significantly in either DHF or CRT compared with control in either anterior or lateral cells, the rate of current decay (Figure 5D) in DHF was significantly slower (41.7±9.5 versus 33.9±5.4 ms, $P<0.05$) in lateral cells and significantly faster (25.6±4.0 versus 32.1±5.3 ms, $P<0.05$) in anterior cells than in control cells, and this produced a robust difference in current decay between anterior and lateral cells in DHF. Moreover, CRT slowed the DHF-induced faster current decay in anterior cells (32.8±7.4 ms, $P<0.05$) but did not affect the rate of $I_{Ca}$ decay in lateral cells (40.4±9.3 ms). Therefore, the total charge carried by $I_{Ca}$ (Figure 5E) was not altered significantly by DHF in either anterior or lateral cells. On the other hand, CRT significantly increased the charge carried by $I_{Ca}$ in lateral (from 0.14±0.04 to 0.19±0.06 pC/pF, $P<0.05$) but not anterior cells compared with control.

CaTs (Figure 5F) were not different in anterior and lateral cells from control hearts; however, DHF significantly reduced the CaT amplitude and slowed the rate of decay of the CaT most prominently in lateral myocytes. CRT hastened the DHF-induced slowing of the decay of the CaT without changing the amplitude in anterior cells, whereas CaT amplitude was increased and decay hastened significantly in lateral cells. The changes in amplitude and rate of decay of CaT in each group are shown in Figure 5G and 5H, respectively. Notably, CRT almost fully reversed the DHF-induced smaller amplitude and longer decay of CaT.

### Molecular Basis for Abnormal Ca$^{2+}$ Homeostasis

The molecular basis for changes in $I_{Ca}$ and CaT was investigated by determining the steady state levels of CaV1.2 (Ca$_{v1.2}$IC), Ca$_{v1.3}$B1, Ca$_{v1.2}$B2, ryanodine receptor, phospholamban, SERCA2, and Na$^{-}$/Ca$^{2+}$ exchanger mRNA and protein (Figure 6). No significant differences in CaV1.2 mRNA and protein or Ca$_{v1.3}$B1 subunit mRNA expression were found among control, DHF, and CRT hearts (Figure 6A through 6C). On the other hand, Ca$_{v1.2}$B2 mRNA was decreased significantly in DHF but not CRT myocytes compared with control (Figure 6D). Steady state ryanodine receptor and phospholamban mRNA and protein expression were consistently lower in both DHF and CRT myocytes than in control (Figure 6E through 6H), and SERCA2 mRNA and protein levels were reduced significantly in DHF myocytes but were not decreased significantly in CRT (Figure 6I and 6J). On the other hand, Na$^{-}$/Ca$^{2+}$ exchanger mRNA expression (Figure 6K) was increased significantly in CRT ($P<0.05$) compared with control, and protein levels were increased in both DHF and CRT (Figure 6L). However, no regional difference was found in the steady state levels of Ca$^{2+}$ channel or homeostasis-related genes and proteins in each group.

### APs and Early Afterdepolarizations

Figure 7A shows superimposed APs elicited at pacing cycle lengths of 0.5, 1.0, 2.0, and 4.0 seconds in anterior and lateral myocytes from control, DHF, and CRT dogs. Compared with control, DHF prolonged the action potential duration (APD) at all pacing cycle lengths, more prominently in lateral than in anterior cells. In contrast, CRT partially reversed the DHF-induced prolongation of APD in lateral cells but not in anterior cells. The relationship between APD and pacing cycle length of each group (Figure 7B) revealed that APD in both DHF and CRT cells was similarly prolonged in anterior cells, and the slope of the APD–cycle length relationship was increased, whereas in lateral cells, a prominent prolongation of APD in DHF was found, particularly at long pacing cycle lengths, which resulted in a significant difference in APD between anterior and lateral cells ($P<0.05$). By selectively shortening the APD of lateral myocytes, CRT reduced the regional heterogeneity in APD compared with DHF. The resting membrane potential (Figure 7C) was not signifi-
significantly different between control, DHF, and CRT dogs, whereas the phase 1 notch depth (Figure 7D) was significantly attenuated in DHF and CRT dogs, consistent with no restoration of \( I_{\text{K}} \) by CRT.

Early afterdepolarizations (EADs) were observed more frequently in DHF than in control myocytes (\( P < 0.001 \)), and CRT significantly reduced the frequency of EADs compared with DHF (\( P < 0.05 \); Figure 8A); however, no regional differences were found in development of EADs between anterior and lateral cells in each group. Figure 8B shows representative APs with [EAD(+) or without [EAD(−)] EADs in myocytes from DHF hearts. The APD at 90% recovery (APD 90) was markedly shorter in anterior and lateral cells in each group. D through H, KvLQT1, minK, and ERG mRNA and protein expression in mid myocardium of control, DHF, and CRT dogs. A or ANT indicates anterior; L or LTR, lateral; and CSQ, calsequestrin.

\[ p = 0.08 \]

**Discussion**

Our studies reveal the regional cellular electrophysiological consequences of synchronous and dysynchronous ventricular contraction in the failing heart and include several novel insights into electrophysiological remodeling in HF. First, CRT partially reverses DHF-induced \( K^+ \) channel remodeling (\( I_{\text{K}} \) and \( I_{\text{K}} \)) in both the anterior and lateral LV. An interesting divergence in the remodeling of \( K^+ \) currents can be seen: CRT has no effect on DHF-induced downregulation of \( I_{\text{K}} \) or on the expression of Kv4.3 or KChIP2 mRNA or protein. Second, \( Ca^{2+} \) current remodeling and \( Ca^{2+} \) handling were significantly different in the anterior and lateral LV in DHF, and CRT significantly improved \( Ca^{2+} \) homeostasis, especially in the lateral wall. Third, the APD was significantly prolonged in DHF, especially in cells isolated from the lateral LV, and CRT abbreviated the APD in lateral cells and reduced the regional gradient of APD. Finally, EADs were more frequent in DHF, were significantly but not completely reduced to near control levels in CRT, and were associated with a modestly prolonged APD and a markedly reduced APD 90/APD 30 ratio. It is important to recognize that the model that we used corrects dysynchronous contraction but does not affect
tachycardia-induced LV dysfunction. Thus, the reversal of DHF-induced electrical and Ca\(^{2+}\) handling remodeling by CRT is due to the effect of electrical resynchronization by biventricular pacing.

**K\(^{+}\) Channel Remodeling**

Downregulation of K\(^{+}\) currents is the most consistent ionic current change in animal models\(^1,16,20,21\) and human HF\(^15\). K\(^{+}\) current downregulation may promote ventricular tachycardia and ventricular fibrillation,\(^21\) either by direct prolongation of AP\(^19\) in the voltage range at which \(I_{\text{Ca,L}}\) reactivation occurs, which predisposes to the development of EADs,\(^25\) or by heterogeneous reduction of the repolarization reserve and the promotion of functional reentry. Although expressed cardiac K\(^{+}\) channels vary in different species, \(I_{\text{o}}\) downregulation is the most consistent ionic current change in failing mammalian hearts.\(^15,16,20,21\) In the present study, \(I_{\text{o}}\) and its related genes, Kv4.3 and KChIP2, were downregulated homogeneously by DHF and were not affected by CRT. The data suggest that tachycardia, HF, or altered ventricular activation is more important in downregulation of \(I_{\text{o}}\) than mechanical synchrony. Reduced \(I_{\text{k1}}\) density in HF\(^16,18,20\) may contribute to prolongation of APD and enhanced susceptibility to spontaneous membrane depolarization.\(^26\) Although small but significant changes in \(I_{\text{k1}}\) density were observed in the outward current component, and the largest changes were observed at very negative voltages (Figure 2B and 2C), the resting membrane potential of myocytes did not differ in any of the groups (Figure 7C), because the major voltage range of \(I_{\text{k1}}\) was beyond physiological potentials. On the other hand, in tachypacing-induced HF models, \(I_{\text{k1}}\) is downregulated, but \(I_{\text{k2}}\) is less consistent.\(^18,21\) In the present study, both \(I_{\text{k1}}\) and \(I_{\text{k2}}\) densities were reduced in a regionally homogenous fashion by DHF in spite of the higher wall stress and myocyte stretch in the late-activated lateral wall than in the early-activated anterior wall\(^27\); this was partially but significantly restored by CRT without a change in global LV function, which suggests that K\(^{+}\) channel remodeling was not directly associated with the mechanical stress caused by dyssynchronous ventricular contraction.

The dichotomy in the regulation of \(I_{\text{o}}\) compared with \(I_{\text{k1}}\) and \(I_{\text{k2}}\) in CRT is remarkable. These currents share regulatory
mechanisms that are altered in the failing heart and are differentially remodeled by CRT. The detailed regulation by the autonomic nervous system, renin-angiotensin-aldosterone signaling, and reactive oxygen species are distinct for each of the K⁺ currents studied and may explain the differences in response to CRT. In addition, biventricular tachypacing in the present study improved the synchrony of mechanical contraction, but global LV function as assessed by LV ejection fraction or end-diastolic pressure was still significantly depressed. It is likely that the molecular mechanisms for the altered functional expression of each current are mixed. The steady state levels of Kv4.3 and KChIP2 mRNA are consistently downregulated by tachypacing in the presence and absence of mechanical synchrony; thus, the balance between transcription and RNA degradation is altered. Furthermore, altered ventricular activation by CRT may suppress transcription and RNA degradation is altered. Furthermore, altered ventricular activation by CRT may suppress functional potassium current magnitude of the current reduction, which suggests an additional posttranslational mechanism of functional downregulation. However, the reduction in Kv4.3 and KChIP2 proteins in particular is not as pronounced as the magnitude of the current reduction, which suggests an additional posttranslational mechanism of functional downregulation.

Figure 6. Ca⁺⁺ handling–related mRNA and protein expression in control, DHF, and CRT myocytes. A and B, Cav1.2 (α1c) mRNA and protein. C and D, Cavαβ1 and Cavαβ2 mRNA. E and F, Ryanodine receptor (RyR2) mRNA and protein. G and H, Phospholamban (PLN) mRNA and protein. I and J, SERCA2 mRNA and protein. K and L, Na⁺/Ca⁺⁺ exchanger (NCX) mRNA and protein. A or ANT indicates anterior; L or LTR, lateral; and CSQ, calsequestrin. †P<0.05 vs control.

Figure 7. APD in LV myocytes from control, DHF, and CRT hearts. A, Representative superimposed APs recorded at pacing cycle lengths (CL) of 0.5, 1.0, 2.0, and 4.0 seconds. B, Relationship between pacing CL and APD at 90% recovery (APD₉₀) from anterior and lateral myocytes in each group. C and D, Bar plot of resting membrane potential (RMP; C) and phase 1 notch amplitude (D) at pacing CL of 2.0 seconds. †P<0.05 vs control; †P<0.05 vs DHF; *P<0.05 vs anterior. ANT or A indicates anterior; LTR or L, lateral.
control, DHF, and CRT dogs, consistent with our previous data in human myocardium.17

Ca\textsubscript{\beta} subunit expression has been positively correlated with peak Ca\textsubscript{\alpha,1.2} current density.35 The present data showed that Ca\textsubscript{\alpha,1.2} mRNA levels were unchanged in DHF and CRT, whereas steady state Ca\textsubscript{\alpha,1.2} protein levels were reduced in DHF and partially restored by CRT. In DHF, ryanodine receptor, phospholamban, and SERCA2a mRNA and protein levels were downregulated, whereas Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger was upregulated, consistent with previous studies.4,31,36,37 No regional differences in mRNA and protein expression were found in any of these mediators of Ca\textsuperscript{2+} handling in DHF and CRT, which suggests that the regional differences of Ca\textsuperscript{2+} handling in DHF and its restoration by CRT are posttranscriptional.

The mechanisms underlying the differences in regional remodeling of K\textsuperscript{+} currents and Ca\textsuperscript{2+} handling in DHF remain obscure. Plotnikov et al4 reported that cardiac dyssynchrony by LV pacing (120 to 150 bpm for 3 weeks) produced a slower decay of \(I_{Ca}\) inactivation, consistent with the present results. Moreover, this phenomenon was suppressed by \(\beta\)-adrenergic blockade. These findings suggest that DHF-induced changes of \(I_{Ca}\) inactivation kinetics might be mediated by regionally heterogeneous \(\beta\)-adrenergic receptor stimulation. Furthermore, the Ca\textsuperscript{2+}-handling proteins are functionally regulated by phosphorylation, prominently by the intracellular enzymes protein kinase A and Ca\textsuperscript{2+}-calmodulin–dependent protein kinase II (CaMKII),38–40 as well as a variety of phosphatases that may be regionally regulated.14

Figure 8. EADs in myocytes from control, DHF, and CRT hearts. A, Bar plot of frequency of EADs (%EADs indicates fraction of APs with EADs). B, Representative superimposed APs recorded in myocytes isolated from lateral wall of DHF hearts with [EAD(±)] or without [EAD(−)] EADs. C, Bar plots of APD\textsubscript{20}, APD\textsubscript{90}, and ratio of APD\textsubscript{20} to APD\textsubscript{90} (APD\textsubscript{20}/APD\textsubscript{90}) in failing myocytes. All data were obtained during pacing at 0.25 Hz.

Altered \(I_{Ca}\) and Ca\textsuperscript{2+} Handling

Changes in \(I_{Ca}\) functional expression in HF are variable.30 Depending on the model and stage of HF, some studies showed a decrease in \(I_{Ca}\) density, whereas others reported no change.2,16,31 In the present study, the peak \(I_{Ca}\) density and current decay in DHF were regionally different between myocytes isolated from the lower-stress anterior and higher-stress lateral walls. Furthermore, CRT regionally restored the DHF-induced changes in \(I_{Ca}\), increasing the peak \(I_{Ca}\) density in lateral cells and slowing the decay in anterior cells; CRT thus served to mitigate the DHF-induced regional heterogeneities in \(I_{Ca}\) density and gating.

HF causes significant changes in Ca\textsuperscript{2+}-handling proteins.2,32 In failing human hearts, the steady state level of the Ca\textsubscript{\alpha,1.2}(\alpha1C) mRNA was reported to be decreased33 or unchanged.17 Moreover, Vanderheyden et al34 recently suggested the possibility of reverse molecular remodeling of SERCA2 in CRT responders. In the present study, Ca\textsubscript{\alpha,1.2}(\alpha1C) mRNA and protein were not different between
than in control myocytes, and CRT reduced the occurrence of EADs in both anterior and lateral cells. 

Prolongation of APD<sub>90</sub> was associated with an increased frequency of EADs, but notably, a smaller APD<sub>95</sub>/APD<sub>90</sub> ratio was even more strongly correlated with the appearance of EADs (Figure 8C). Thus, APD prolongation alone may not be sufficient to generate EADs in failing myocytes. The APD<sub>95</sub>/APD<sub>90</sub> ratio is an empiric metric but suggests that long APs with a reduced plateau voltage are the most likely to exhibit EADs. This type of AP profile that is generated by the reduction in K<sup>+</sup> current density and reduced I<sub>K<sup>r</sup></sub> density with slowed kinetics is highly susceptible to EADs that result from reactivation of I<sub>K<sup>r</sup></sub>.

Study Limitations
The model of DHF and CRT used in the present study is a limitation. Six weeks of tachypacing (200 bpm) reproducibly induces dilated cardiomyopathy with LV enlargement, increased LV end-diastolic pressure, and decreased dP/dt<sub>max</sub>; however, the study was designed to examine the effects of CRT with ongoing HF, and thus, tachypacing was maintained. This differs from CRT in patients, which is performed at lower heart rates. Therefore, the processes of both remodeling and the reversal of remodeling in this circumstance are likely to be different from those in human DHF.

The second limitation is that the present study focused on the cellular and molecular bases of electrophysiological remodeling in DHF and restoration by CRT. It did not evaluate the susceptibility to and frequent development of arrhythmia in vivo or in a whole-heart model.

Clinical Implications
CRT has emerged as an effective pacing/mechanical therapy for patients with HF and a prolonged QRS duration. CRT has been associated with improved cardiac function, symptomatology, and exercise capacity and, when combined with defibrillator therapy, reduced mortality. The role of CRT in preventing arrhythmias or reversing adverse electrical remodeling remains controversial. The present study provides novel information on the altered expression and function of ionic currents and CaTs in DHF and after CRT. Understanding the fundamental mechanisms of the altered ion channel function and Ca<sup>2+</sup> homeostasis, as well as the electrophysiological remodeling in DHF, and the capacity for restoration by CRT will not only help define the therapeutic role of CRT but will help to identify antiarrhythmic targets that can be exploited by other therapeutic strategies designed to prevent sudden death.

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Disclosures
Dr Tomaselli has received a research grant from Boston Scientific. The remaining authors report no conflicts.

References


CLINICAL PERSPECTIVE

Cardiac resynchronization therapy (CRT) with biventricular pacing improves symptoms, cardiac function, and exercise capacity, and when combined with defibrillator therapy, it reduces mortality in patients with heart failure who have dysynchronous contraction (DHF). CRT reduces stress-strain disparities and thus improves the efficiency of contraction of the ventricle. However, the role of CRT in preventing arrhythmias or reversing adverse electrical remodeling remains controversial. The present study provides novel information on the altered regional expression and function of ionic currents and calcium (Ca2+) transients in DHF and partial restoration by CRT in a canine pacing-induced DHF model. CRT partially restores the DHF-induced reduction of selected K+ currents and significantly improves Ca2+ homeostasis, especially in the lateral wall of the left ventricle. This overall effect of CRT is a combination of the DHF-induced prolongation of action potential duration in cells isolated from the lateral left ventricle, thus reducing the regional action potential duration gradient and the frequency of potentially arrhythmogenic early afterdepolarizations compared with DHF. Thus, CRT partially reverses both the cellular triggers and substrate for arrhythmias in DHF.
Electrophysiological Consequences of Dyssynchronous Heart Failure and Its Restoration by Resynchronization Therapy
Takeshi Aiba, Geoffrey G. Hesketh, Andreas S. Barth, Ting Liu, Samantapudi Daya, Khalid Chakir, Veronica Lea Dimaano, Theodore P. Abraham, Brian O'Rourke, Fadi G. Akar, David A. Kass and Gordon F. Tomaselli

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Supplemental Methods

Canine Tachypacing-Induced Heart Failure Model

Adult male mongrel dogs (n=14) underwent left bundle branch (LBB) radiofrequency ablation, and were followed for one week to ensure that there was no recovery and shortening of QRS duration. Then dogs were anesthetized and intubated and a left lateral thoractomy was performed to place bipolar leads (Medtronic) on the epicardial surfaces of the RA, right ventricular free wall and the postero-lateral LV wall. The atrial and both ventricular leads were connected to a pulse generator (Medtronic) and rapid RA pacing was initiated to produce DHF. After 3 weeks of RA tachy-pacing (190-200 bpm) to achieve LV dysfunction, dogs were divided into two groups; one group underwent continued RA tachy-pacing with progression to HF with LV dyssynchrony (DHF dogs; n=7), while another group underwent bi-ventricular pacing at the same pacing rate producing HF with synchronous ventricular contraction (CRT dogs; n=7).

Isolation of Ventricular Myocytes

Midmyocardial myocytes were isolated from the anterior and lateral wall by perfusion of the left anterior descending (LAD) artery and a branch of the left circumflex (LCx) artery, respectively with a nominally Ca\textsuperscript{2+} free solution containing collagenase and protease. Tissues were minced and cells were isolated by gentle trituration in a modified KB solution, and then myocytes were stored in Tyrode solution containing (in mmol/l) 140 NaCl, 5 KCl, 1 MgCl\textsubscript{2}, 2 CaCl\textsubscript{2}, 10 HEPES and 10 glucose, adjusted to pH 7.4 with NaOH at room temperature (22-25°C). Only Ca\textsuperscript{2+}-tolerant rod-shaped myocytes with cross striations and without spontaneous
contractions or significant granulation were selected for experiments.

**Ionic current and calcium transient (CaT) measurements**

Isolated ventricular myocytes were current and voltage clamped using the standard whole-cell patch-clamp technique with an Axopatch 200A patch-clamp amplifier (Axon Instruments) interfaced with a personal computer as previously described.\(^1,2\) Voltage and current control, and data collection were performed using custom-written software. Borosilicate glass electrodes with tip resistances of \(\approx 3.0 \, \text{M} \Omega\) when filled with the pipette solution were used.

For the measurement of APs, standard Tyrode’s was used as the external solution. The pipette solution contained (in mmol/l): 120 K\(^+\)-glutamate, 10 KCl, 10 HEPES, 5 EGTA and 5 Mg-ATP adjusted to pH 7.2 with KOH. The stimulation frequency was varied over the cycle lengths (CLs) of 4.0, 2.0, 1.0 and 0.5 sec, and the steady-state APs were recorded and analyzed at least 1 min after initiating pacing at each CL.

All currents were recorded in the whole-cell configuration of the patch clamp. Transient outward K\(^+\) current (\(I_{\text{to}}\)) was recorded in Tyrode solution containing 5\(\mu\)mol/l nisoldipine to block \(I_{\text{Ca}}\) and 3 \(\mu\)mol/l E-4031 (Wako, Japan) and HMR1556 (Sanofi-Aventis Deutschland GmbH) to block the rapid (\(I_{\text{Kr}}\)) and slow (\(I_{\text{Ks}}\)) components of delayed rectifier K\(^+\) currents (\(I_{\text{K}}\)). Inward rectifier K\(^+\) current (\(I_{\text{K1}}\)) was quantified as the Ba\(^{2+}\)-sensitive (0.5 mmol/l) current in the normal Tyrode solution elicited by hyperpolarizing pulse from a holding potential of -40 mV. \(I_{\text{K}}\) was recorded as a composite current, and voltage dependence of \(I_{\text{K}}\) activation was evaluated by fitting the I-V relationship of tail currents to the Boltzmann equation:

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

where \(I_{\text{K,\text{tail}}}\) was the tail current density. Ca\(^{2+}\) current (\(I_{\text{Ca}}\)) was recorded in Tyrode’s solution with equimolar replacement of KCl by CsCl, and a pipette solution containing (in mmol/l): 80 Cs-glutamate, 40 CsCl, 10 HEPES, 5 EGTA and 5 Mg-ATP adjusted to pH 7.2 with CsOH. Cell
capacitance was estimated by integrating the area under an uncompensated depolarizing step of 10 mV from a holding potential of -80 mV.

CaTs were measured by indo-1 fluorescence exited at 365 nm with a Xenon arc lamp, emitted light at 405 and 495 nm was collected with a two-channel photomultiplier tube assembly. Fluorescence signals were digitized and stored with electrophysiological recordings for off-line analysis with custom-written software. The ratio of indo-1 fluorescence \( (R = \frac{F_{405\text{nm}}}{F_{495\text{nm}}}) \) was determined after subtraction of cellular autofluorescence. The rate of \( \text{Ca}^{2+} \) removal \( (\tau_{\text{ca}}) \) was determined by fitting a single exponential to the \( \text{Ca}^{2+} \) time course. All the electrophysiological and intracellular \( \text{Ca}^{2+} \) measurements were performed at the physiological temperature (37ºC).

### Real Time-PCR

Real-time quantitative PCR (RT-PCR) was performed using an ABI Prism 7900 Sequence-Detection System (Applied Biosystems). One step RT-PCR for the desired amplicon was conducted with total RNA isolated from cardiac tissue using the Qiagen RNeasy Midi Kit with an on column DNase digestion according to the manufacturer’s instructions. Primers and probes were designed using the Primer Express software (Supplement Table 1). A trio of oligonucleotides with appropriate melting temperatures and structural features were selected and synthesized. At the 5’ end, the probe was covalently linked to a fluorescent reporter dye 6-carboxyfluorescein (FAM or VIC) while the 3’ end was linked to the quenching dye 6-carboxy-N,N,N’,N’-tetramethylrhodamine (TAMRA). During PCR, the 5’ nuclease activity of the AmpliTaq Gold DNA polymerase cleaves the reporter dye from the quenching dye; hence, releases a fluorescent signal that is proportional to the amount of starting target template. Reverse transcription and amplification reactions were conducted in one step under the following conditions: 48ºC for 38 min (reverse transcription), 95ºC for 10 min (initial denaturation), and
the next two steps (amplification) were repeated 40 times: 95°C for 15 sec and 60°C for 1 min. Optimization of both the primer (between 50-900 nM) and probe (between 50-250 nM) concentrations giving the lowest threshold cycle (C_T) and maximum change in the normalized reporter signal (ΔR_n) were determined. ROX served as the internal passive reference dye for normalization. No amplification (i.e. no reverse transcriptase) and no template samples served as negative controls. The efficiency of the RT-PCR reaction per sample was evaluated based on the same sample RT-PCR results with 18S rRNA control reagents. Each amplicon C_T was normalized against its 18S rRNA C_T.

**Western Immunoblotting**

LV tissue sections were carefully dissected from epicardium, endocardium and mid-myocardial from anterior and lateral regions in normal, DHF and CRT dogs, which were rapidly frozen in liquid nitrogen after organ harvest. Samples were run in duplicate or triplicate on 10%, 12.5%, or 15% Tris HCl precast gels (Bio-Rad, Hercules, CA) in 25 mM Tris, 192 mM glycine, and 0.1% (wt/vol) SDS running buffer. A standard control sample was also run on all gels to allow for comparisons across gels. Primary antibody incubations were performed overnight at 4°C with the appropriate antibodies. Antibodies to Cav1.2 (AB5412), Kir2.1 (AB5374,), Kv4.3 (AB5194), and ERG (AB5908) were purchased from Millipore. Antibodies to KChIP2 (PA1927), RyR2 (MA3-916), PLN (MA3-922), SERCA2 (MA3-910), and NCX (MA3-926) were purchased from Affinity Bioreagents. The antibody to KvLQT1 was made for our laboratory. Secondary horseradish peroxidase-conjugated antibodies were obtained from Jackson Immuno Research (West Grove, PA). Membranes were exposed and developed with enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s instructions.
<table>
<thead>
<tr>
<th>Current</th>
<th>Clone</th>
<th>Primer Pair</th>
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| I<sub>K<sub>1 | cKir2.1 | F: GTCACAGACGAATGCCAGTT  
R: TTCTTTGCTGGCCACTCTT  
T: CTTCTCATCATGGGGGCTCA T | AF277647   |
| I<sub>K<sub>0 | cKv4.3  | F: AAGTCTCTCCTGTCACAGTCGA  
R: TGGAGGGCTGATGCTTGT  
T: AGCAACACTGTCATGGGGCTTC | AF049887   |
|         | cKChIP   | F: GCTGTTTTGTCGTTATCTTCTC  
R: AGCTACCATTTGAGATCATCAC  
T: AAACATAGATGACAGACAGACTAACTGGGCTTCAAG | AF458385   |
| I<sub>K<sub>1 | cKv11.1(ERG) | F: CAGGGTGGGGTCATTCCAA T  
R: CGAAGAT CTGGGTGTA AT  
T: TCTCATCTGTGTCATGCTCATCGGCCTTC | AF017429   |
| I<sub>K<sub>s | cKv7.1(KVLQT1) | F: CACAGACGAAGCAGACAGAAG  
R: CCTCACCAGCTGTAATGGA  
T: CAGATCCGGCGGCGCAGCTC | XM_540790  |
|         | cminK    | F: ACCCGTTTACGAATACATCGA  
R: AACAGGCGCCCTGGCAATA  
T: CCTGTCTTCTCTGTGGAGGTCAGA | XP_544868  |
| I<sub>G<sub>a,L | cCav1.2 (α1c) | F: CGATCTCTACAACCTGACTCTT  
R: GCAGGTGGCCAGCCGACTGTCT  
T: TCTGAGCGACATGGACACCAAG | AF458389   |
|         | cCACNB1  | F: GGCGAGGGCTGGCCAGAATGCA  
R: GCTTGTGTCGTTCTTCCCTGCAGCCACTCATTGTC | ENSCAT00000026162* |
|         | cCACNB2  | F: CATAGCTGGGAAGGACAGAAAGAACAGA  
R: CTCAGGGCACTGGAACATCATCT  
T: TCGGTTGATTCAGCTGTCGGGAC | ENSCAT00000007219* |
|         | NCX      | F: TCCTCATGATGGGTCATCTGA  
R: TTCAACAGCCGAGATGGTGCAT  
T: ATGGTGAGTGGCCATGGTGCTCTTGTC | M57523     |
|         | SR       | F: CTGAGTCTGGCACTCTCTCACTCAG  
R: CCATAGCCAGATGAACTGCAA  
T: CAAATGGCCACATGTAATGGAAATGCTAATA | AF440217   |
|         | cPLN     | F: GCAATCA TTTGATGTCATGTCAGT  
R: TTCATGGGTGAGAGATTGAATGTA  
T: TTCCTGCGCAATCTCCAGTGCATC | M35393     |
|         | cSERCa2  | F: GATTGAAGTGGACCTCCCTCTGTAA  
R: GCCGTG CCCCTTGTGCTTCT  
T: CAAGCAGGGATTCAGTCTTAT | U94345     |

Primers labeled T represent the Taqman fluorescent probes. They are labeled on the 5’ end with FAM and the 3’ end with Tamra. Mink (KCNE1) and KvLQT1 (KCNQ1) sequences are from genomic DNA sequence which is available at http://www.ensembl.org/index.html.

Primer set shown for KChIP = cKChIPE6dd which recognizes KChIP26, KChIP24, KChIP2, KChIP2T, and KChIP2S but not KChIP25 splice variants.

*Ensemble transcript ID
### Supplemental Table S2. Cell Capacitance

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<td>266 ±101 *</td>
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Mean ±SD. * p<0.05 vs. control

### Supplemental Table S3. Action Potential Duration and Early After Depolarizations

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Mean ± SD

A: anterior, L: lateral, APD<sub>20</sub> = action potential duration at 20% recovery APD<sub>90</sub> = action potential duration at 90% recovery, EAD = early after depolarization

† p<0.05 vs. EAD(-), ‡ p<0.01 vs. EAD(-)
Supplemental References:

