Regional Alterations in Protein Expression in the Dyssynchronous Failing Heart

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Background—Left ventricular (LV) mechanical dyssynchrony induces regional heterogeneity of mechanical load and is an independent predictor of mortality and sudden death in heart failure (HF) patients. We tested whether dyssynchrony also induces localized disparities in the expression of proteins involved with mechanical stress, function, and arrhythmia susceptibility.

Methods and Results—Eleven dogs underwent tachycardia-induced HF pacing, either from the right atrium or high right ventricular free wall. Whereas global LV dysfunction was similar between groups, LV contractile coordination assessed by tagged MRI was markedly dyssynchronous with right ventricular pacing but synchronous with right atrial pacing. In dyssynchronous failing hearts, the lateral LV endocardium displayed a 2-fold increase in phosphorylated erk mitogen-activated protein kinase expression (with no change in phospho-p38 or phospho-jnk), a 30% decline in sarcoplasmic reticulum Ca\(^2\)/H\(^{+}\)-ATPase, an 80% reduction in phospholamban, and a 60% reduction in the gap junction protein connexin43, relative to neighboring myocardial segments. In contrast, hearts from both right atrial–paced HF dogs and an additional 4 noninstrumented control animals showed minimal regional variability in protein expression.

Conclusions—LV dyssynchrony in failing hearts generates myocardial protein dysregulation concentrated in the late-activated, high-stress lateral endocardium. Such molecular polarization within the LV creates transmural and transchamber expression gradients of calcium handling and gap junction proteins that may worsen chamber function and arrhythmia susceptibility. (Circulation. 2003;108:929-932.)

Key Words: heart failure ■ pacing ■ molecular biology ■ sarcoplasmic reticulum

The pathophysiology of heart failure (HF) involves abnormalities of stress response kinase signaling, neurohumoral stimulation, excitation-contraction coupling, gap junction and ion channel function, and chamber remodeling.1,2 In addition, recent studies have shown that left ventricular (LV) mechanical coordination is frequently disturbed in HF, particularly in the setting of interventricular conduction delay. HF patients with interventricular conduction delay and concomitant ventricular dyssynchrony have mortality and sudden cardiac death rates above those predicted from the apparent severity of systolic dysfunction, HF etiology, functional class, or concurrent medical therapy.3,4

Acute mechanical discoordination induced by single-site right ventricular (RV) or LV pacing depresses systolic function, worsens myocardial efficiency, and leads to marked increases in wall stress heterogeneity.5,6 Stress is highest in late-activated myocardial regions because of both exaggerated stretch in early systole (secondary to septal contraction) and late systolic contraction against increased afterload. Chronic discoordination leads to chamber remodeling of both early- and late-activated segments.7 However, its effect on myocardial protein expression remains unknown. In the present study, we tested the hypothesis that stress polarization due to dyssynchrony induces regional expression disparities of key proteins involved with stress response, muscle mechanics, and electrophysiology. Using HF models with or without superimposed LV discoordination, we studied tissue levels of the mitogen-activated protein kinases, sarcoplasmic reticulum Ca\(^2\)-ATPase (SERCA2a) and phospholamban (PLB), and the gap junction protein connexin43 (Cx43).

We reveal marked dyssynchrony-dependent transmural and transchamber protein expression gradients, suggesting a novel mechanism for the enhanced morbidity seen in discoordinate LV failure.

Methods

Model Generation

Adult mongrel dogs (Bruce Rotz Kennels, Shippensburg, Pa) were paced (210 to 250 bpm) from the right atrium (RA; n=5) or RV free...
LV Dysfunction and Coordination Parameters in RA and RV Pacing–Induced HF

<table>
<thead>
<tr>
<th></th>
<th>RV-Paced Dogs</th>
<th>RA-Paced Dogs</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>(Dysynchronous)</td>
<td>(Synchronous)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=5)</td>
<td></td>
</tr>
<tr>
<td>dP/dt\text{max}, mm Hg/s</td>
<td>1295±250</td>
<td>1141±183</td>
<td>NS</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>26±6</td>
<td>32±5</td>
<td>NS</td>
</tr>
<tr>
<td>CURE index</td>
<td>0.52±0.14</td>
<td>0.90±0.04</td>
<td>&lt;0.05</td>
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</tbody>
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Wall (n=6), as described previously,8 or were left uninstrumented (n=4). At terminal study, dogs were anesthetized (10 to 15 mg/kg thiopental, 1% to 2% isoflurane); LV pressures measured by micromanometer (Millar); and the hearts retroperfused with cold cardioplegia, excised, dissected into endocardial and epicardial segments from the interventricular septum and LV lateral wall, and frozen in liquid nitrogen. All procedures followed USDA guidelines and the protocol was approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions.

Assessment of Mechanical Dyssynchrony

A subgroup of 4 dogs was studied by magnetic resonance (MR) imaging to document the effect of activation site on LV coordination. MR 3D tagged images were obtained (GE Signa 1.5T) during either RA or RV free-wall stimulation, and mechanical dyssynchrony indexed by a circumferential uniformity ratio estimate (CURE), as described previously.9 Perfectly synchronous or dyssynchronous contraction patterns yield CURE indices of 1 or 0, respectively.

Western Blot Analysis

Frozen myocardium was homogenized in lysis buffer (Cell Signaling Technology), and equivalent samples (confirmed by coprobing for glyceraldehyde-3-phosphate dehydrogenase or calsequestrin) were loaded for gel electrophoresis. After transfer to nitrocellulose, membranes were blocked and probed overnight at 4°C with primary antibodies for p-p38, p-erk, p-jnk (Cell Signaling Technology; 1:1000 dilution), PLB, SERCA2a (Affinity BioReagents; 1:5000 dilution), or Cx43 (Chemicon Intl; 1:1000 dilution). Membranes were incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology and Upstate Biotechnology; 1:10 000 dilutions) for 1 hour at room temperature. Protein levels were detected by chemiluminescence and autoradiography, quantified using NIH ImageJ software, and blot intensity normalized such that lateral endocardial signal equaled 100 arbitrary units.

Statistical Analysis

Results are expressed as mean±SEM. Statistical significance was assessed by ANOVA with post hoc Tukey tests for multiple comparisons.

Results

Mechanical Dysfunction and Dyssynchrony: RV Versus RA Pacing Models

RA and RV pacing models generated similar degrees of global LV dysfunction, as reflected by depression of dP/dt\text{max} (normal values in anesthetized dog are ≈2000 mm Hg/s) and elevation of LV end-diastolic pressure (Table). However, the two models generated markedly different degrees of LV mechanical coordination. Whereas rapid RA pacing preserved nearly uniform contraction at all LV regional segments, high RV free wall pacing induced substantial LV dyssynchrony, with early interventricular septal contraction and coincident lateral LV stretch followed by delayed lateral LV contraction and septal stretch (CURE index; Table). Cine images based on tagged MRI circumferential strain analysis depicting this dyssynchrony can be viewed in the online-only Data Supplement at http://www.circulationaha.org.

Dyssynchronous HF and Regional Protein Expression

The Figure displays representative Western blots and summary data for regional protein expression of activated MAPKs, SERCA2a, PLB, and Cx43 in failing hearts with preserved (RA pacing) or disturbed (RV pacing) LV coordination. With RV pacing, p-erk increased nearly 100% in the posterolateral LV endocardium relative to the other segments assessed (P<0.009). In contrast, p-erk levels did not vary significantly by region in RA pacing HF models. p-jnk and p-p38 levels did not vary regionally in either RV- or RA-paced models. Total levels for all three MAP kinase proteins were also not significantly different among the four regions (data not shown).

The late-activated LV endocardium also demonstrated significant reductions in SERCA2a and PLB protein expression in dyssynchronous HF. SERCA2a declined ≈30% relative to the other regions (P<0.001), and PLB fell even more (~80%; P<0.001). In contrast, RA pacing did not induce regional disparities in the expression of either protein. Finally, the same posterolateral endocardial region displayed marked downregulation of Cx43 expression (~60% relative to other segments; P<0.001) in RV-paced dogs. Once again, this localized abnormality was not observed in HF with preserved LV coordination, as shown by the RA-paced data.

In noninstrumented control hearts, MAPK and Cx43 levels did not vary significantly by region. However, both SERCA2a and PLB levels were moderately and globally reduced in LV endocardial versus epicardial tissue (by 20% and 30%, respectively; P=0.03), consistent with prior data.12 This transmural gradient was significantly lower than that observed in the lateral endocardium of dyssynchronous HF hearts.

Discussion

The present study provides novel evidence that mechanical dyssynchrony spatially polarizes ventricular protein expression, particularly within the late-activated lateral wall, generating transmural and transventricular gradients in p-erk, SERCA2a, PLB, and Cx43 levels. Similar expression gradients were not seen in HF with equivalent global dysfunction but preserved LV coordination, indicating the importance of dyssynchrony to this process. We propose that such regionally specific molecular polarization may contribute to heterogeneous electromechanics and underlie enhanced arrhythmia susceptibility in HF patients with LV dyssynchrony.

Mechanical load heterogeneity in the setting of LV discoordination was examined by Prinzen et al5 using MR tagged imaging and stress modeling. The authors predicted that late-activated ventricular segments were subjected to greatest stress because of locally enhanced preload (secondary to early systolic stretch) and afterload (due to late systolic contraction against high LV cavity pressures), and correlated...
increased wall stress to increased regional blood flow, nutrient consumption, and hypertrophy. The endocardium is particularly subject to stress redistribution because of myocardial fiber orientation, direct cavity pressure load, and a greater compromise in blood flow. Biomechanical stress on the posterolateral endocardium in dyssynchronous HF is uniquely elevated, therefore, and may explain the distribution of molecular abnormalities in our study.

Several investigations have examined regional changes in protein expression in HF. Using a canine model of LV hypertrophy, Dosch et al reported higher atrial natriuretic peptide levels in LV basal and midwall segments, with peak expression in basal endocardium. Prestle et al described SERCA2a and PLB transmural expression gradients in failing human hearts, with \( \approx 25\% \) lower levels in the LV free wall endocardium. The present study supports the latter findings, but by contrasting models of LV failure differing only in degree of mechanical LV coordination, firmly identifies local biomechanical input (rather than systemic hemodynamics or neurohumoral signaling) as a key regulator of protein expression. We believe this is the first study providing a possible molecular mechanism for the enhanced arrhythmia susceptibility seen in dyssynchronous HF.

We did not test the functional impact of the molecular abnormalities observed, as this will require extensive isolated myocyte analysis from different layers and regions of the heart. However, prior data support such changes as potentially important. For example, increased SERCA2a/PLB levels have been associated with improved SERCA2a-mediated calcium sequestration and enhanced systolic function. In this regard, the increase in the lateral endocardium SERCA2a/PLB ratio (\( \geq 3 \)-fold) may reflect a regionally targeted adaptive mechanism to counter higher stress. Elements of the PLB promoter (e.g., GATA box) are responsive to biomechanical stretch and might explain the disparate changes seen in septal (stretched) versus lateral (higher-stress) SERCA2a/PLB expression. To our knowledge, however, specific regulators of SERCA2a and PLB that might drive reduced expression in response to mechanical stress have not been identified. Cx43 downregulation has been linked with arrhythmia susceptibility in a variety of models, likely because of unidirectional conduction delay and...
facilitated reentry.\textsuperscript{16,17} Furthermore, MAPK activation and Cx43 downregulation may be connected, as constitutively activated p-jnk potently reduces Cx43 levels in mouse myocardium, and p-erk has been implicated in Cx43 downregulation in liver epithelial cells.\textsuperscript{18,19} Ongoing studies are addressing the physiological importance of the expression changes revealed in the present study, as well as expanding the analysis by means of subproteomic and transcriptome analysis.

In conclusion, LV mechanical dyssynchrony superimposed with tachycardia-induced HF induces marked regional heterogeneity of protein expression at the site of greatest hemodynamic load. These observations raise the possibility that cardiac resynchronization therapy may not only improve ventricular mechanics, but also modulate regional myocardial protein expression. Further studies with more chronic HF models are needed to determine the extent to which cardiac resynchronization can reverse molecular polarization and impact net cardiac function and arrhythmia susceptibility.

Acknowledgments
This work was supported by grants from the National Institutes of Health (P50-HL52307) and from Guidant, Inc.

References
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Circulation. 2003;108:929-932; originally published online August 18, 2003;
doi: 10.1161/01.CIR.0000088782.99568.CA
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/108/8/929

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