Mechanical alternans and restitution in failing SHHF rat left ventricles

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1The Ohio State University Biophysics Program and Dorothy M. Davis Heart and Lung Research Institute, Columbus 43210; 2The Cleveland Clinic Foundation, Department of Cardiology, Cleveland 44195; 3Department of Food Science and Technology and 4Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio 43210

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Dumitrescu, Cristian, Prakash Narayan, Igor R. Efimov, Yuanna Cheng, M. Judith Radin, Sylvia A. McCune, and Ruth A. Altschuld. Mechanical alternans and restitution in failing SHHF rat left ventricles. J Physiol Heart Circ Physiol 282:H1320–H1326, 2002. First published December 6, 2001; 10.1152/ajpheart.00466.2001.—We examined mechanical alternans and electromechanical restitution in normal and failing rat hearts. Alternans occurred at 5 Hz in failing versus 9 Hz in control hearts and was reversed by 300 nM isoproterenol, 6 mM extracellular Ca2+, 300 nM BAY K 8644, or 50 nM ryanodine. Restitution curves comprised phase I, which was completed before relaxation of the steady-state beat, and phase II, which occurred later. Phase I action potential area and developed pressure ratios were significantly reduced in the failing versus control hearts. Phase II was a monoexponential increase in relative developed pressure as the extrasystolic interval was increased. The plateau of phase II was significantly elevated in failing hearts. Thapsigargin (3 μM) plus ryanodine (200 nM) potentiated phase I to a significantly greater extent in control versus failing hearts and abolished phase II in both groups. The results suggest that both regulation of Ca2+ influx across the sarcolemma and Ca2+ release by the sarcoplasmic reticulum may contribute to altered excitation-contraction coupling in the failing spontaneously hypertensive heart failure prone rat heart.

Heart failure; calmodulin; sarcoplasmic reticulum; calcium current facilitation

THE SPONTANEOUSLY HYPERTENSIVE heart failure prone rat (SHHF/Mccfa+); i.e., SHHF is a reliable genetic model of hypertensive cardiomyopathy that progresses to four-chamber enlargement, dilatation, and end-stage congestive heart failure (20). Using failing SHHF rat hearts to investigate possible failure-related abnormalities in excitation-contraction coupling, we have previously reported sustained mechanical alternans at relatively low pacing frequencies (22), a flattened force-frequency relation (22), and declines in the gain of excitation-contraction coupling (6) and maximal Ca2+-activated force per cross-sectional area (23). In the present study, we have extended our studies of mechanical alternans to include 1) a systematic comparison of failing SHHF hearts and hearts from age-matched controls and 2) a survey of interventions designed to reverse alternans. We have also examined another important aspect of excitation-contraction coupling: electromechanical restitution, the recovery of electrical and mechanical responses as a function of time following a twitch, in failing SHHF hearts and in hearts from age-matched controls.

As shown in the companion paper (4), mechanical restitution in perfused rat left ventricles consists of two components. The early component, phase I, appears to be independent of Ca2+ release from the sarcoplasmic reticulum (SR) and may reflect changes in Ca2+ influx across the sarcolemma. The more widely studied phase II occurs later and may reflect recovery of the ability of the SR to release Ca2+. The present study demonstrates that both phases of electromechanical restitution in failing SHHF rat hearts differ to some extent from those of hearts from age-matched controls. Phase I is markedly blunted, whereas the plateau of phase II is elevated, suggesting the possibility that the process(es) leading to the recovery of SR Ca2+ release may begin slightly later and occur somewhat more slowly in heart failure. Thus regulation of Ca2+ influx across the sarcolemma and of Ca2+ release from the SR may both contribute to the altered excitation-contraction coupling observed in the failing SHHF rat heart.

MATERIALS AND METHODS

SHHF rats were bred and housed at The Ohio State University. Male Wistar-Furth (WF) and Wistar rats were purchased from Harlan (Indianapolis, IN) at 8–9 mo of age and housed at The Ohio State University until use at 15–19 mo of age. Male Brown Norway (BN) rats, 19 mo of age, were purchased through the National Institute on Aging. The animal housing facilities are fully accredited and are under the supervision of licensed veterinarians. All protocols were

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Phenotypically lean male SHHF rats were selected for the study when they exhibited signs of lethargy and dyspnea. This occurred between 15 and 19 mo of age. Some of the SHHF rats used in these studies were examined by M-mode echocardiography several days before being euthanized. As has been reported previously for failing SHHF rats (6, 23), all had fractional shortenings \( \frac{1}{40\%} \) compared with \( \frac{1}{40\%} \) for all age-matched controls. At the time of euthanasia, all of the failing SHHF rats exhibited pleural effusion and most had well-resolved atrial thrombi and ascites. Heart weight-to-body weight ratios (mg/g) for failing SHHF rats were similar to those described in earlier studies (6, 22, 23): \( \frac{6.1}{0.2} \) compared with \( \frac{3.8}{0.2} \) for controls.

Heart perfusion protocols were identical to those described in the companion paper (4) except that the temperature was raised to 37°C for studies of mechanical alternans. For the latter, hearts were paced at a basic cycle length of 333 ms (3 Hz) until left ventricular developed pressure (LVDP) stabilized. A quick jump in pacing frequency was then imposed in steps of 1 Hz until the appearance of sustained mechanical alternans. Sustained mechanical alternans was arbitrarily defined as alternating large and small beats that differed by at least 10% in LVDP amplitude and that lasted for at least 20 s. A variety of interventions were tested for their ability to reverse alternans. When an agent was effective, the experiment was repeated with at least two additional hearts to verify the observation. We describe no situations where an intervention was effective in only some of the trials.

Restitution curves were analyzed using Origin 5.0 software and a resident statistical package (one-way ANOVA with repeated measures) with Newman-Keuls post hoc analysis. A P value <0.05 was considered to be statistically significant.

Reagents.–BAY K 8644 and ryanodine were purchased from Calbiochem (LaJolla, CA). Verapamil HCl was purchased from Knoll Pharmaceutical (Whippany, NJ). KB-R7943 was the generous gift of Kanebo Pharmaceutical Laboratories (Osaka, Japan). All other reagents were purchased from Sigma Chemical (St. Louis, MO).

RESULTS

Both normal and failing hearts were able to develop sustained mechanical alternans with an abrupt increase in pacing frequency, but the threshold frequency for sustained mechanical alternans was significantly lower in the failing SHHF hearts compared with those from the age-matched WF controls (Fig. 1). Figure 2 illustrates the reversal of alternans when failing hearts were switched to perfusate containing either elevated extracellular Ca\(^{2+}\) (Fig. 2A) or the nonselective \(\beta\)-adrenoceptor agonist isoproterenol (Fig. 2B). Figure 3 shows the typical response of an age-matched control heart in alternans to the addition of isoproterenol. Isoproterenol reversed alternans, but isoproterenol plus verapamil was ineffective. Similar experiments indicated that alternans could be reversed
with 50 nM ryanodine, 0.1 mM isobutyl methyl xanthine (phosphodiesterase inhibitor), 300 nM -BAY K 8644 (Ca\(^{2+}\) channel agonist), 500 nM norepinephrine (\(\beta_1\)- and \(\alpha\)-adrenoceptor agonist), or 10 \(\mu\)M zinterol (selective \(\beta_2\)-adrenoceptor agonist) plus 300 nM CGP-20712A (selective \(\beta_1\)-adrenoceptor antagonist).

Because mechanical alternans has been attributed to reactions of the SR (13, 17), we investigated another facet of SR function, mechanical restitution (14, 32). Baseline functional data for hearts from failing SHHF rats and age-matched WF controls are given in Table 1. Mechanical restitution curves and the curve fit data for phase II are given in Fig. 4. Note the flattening of phase I and the significantly elevated plateau for phase II in the failing hearts. Figure 5 summarizes electrical restitution data for the two types of hearts. Note the early increase in action potential areas at 50% repolarization (APD\(_{50}\) areas) corresponding to phase I mechanical restitution in the control hearts and the lack of increase in APD\(_{50}\) areas for the failing hearts. The lack of change in the SHHF heart action potentials with very early extrasystoles may be related to the prolonged action potentials at steady state (APD\(_{50}\) areas) corresponding to phase I mechanical restitution in the control hearts and the lack of change in APD\(_{50}\) areas for the failing hearts. The APD\(_{50}\) values at an extrasystolic interval of 150 ms were 42 ± 4 and 51 ± 5 ms for the control WF and failing SHHF hearts, respectively.

Isoproterenol accelerated phase II restitution in both the failing and age-matched control hearts to the extent that the phase II plateau was decreased almost to 100% (i.e., complete restitution was nearly achieved by the basic cycle length, Fig. 6). However, isoproterenol did not restore phase I increases in \(P_t\) in the failing hearts.

### Table 1. Contractility parameters for failing SHHF and age-matched control rat hearts

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>LVDP, mmHg</th>
<th>TTP, ms</th>
<th>(+dP/dt_{\text{max}})</th>
<th>(-dP/dt_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age-matched WF 3 Hz</td>
<td>11</td>
<td>130 ± 8</td>
<td>118 ± 3</td>
<td>2,150 ± 140</td>
<td>1,330 ± 120</td>
</tr>
<tr>
<td>Failing SHHF 3 Hz</td>
<td>18</td>
<td>146 ± 9</td>
<td>121 ± 2</td>
<td>2,260 ± 160</td>
<td>1,370 ± 100</td>
</tr>
<tr>
<td>Age-matched BN 2 Hz</td>
<td>6</td>
<td>135 ± 9</td>
<td>169 ± 3</td>
<td>1,560 ± 110</td>
<td>1,060 ± 50</td>
</tr>
<tr>
<td>Failing SHHF 2 Hz</td>
<td>6</td>
<td>140 ± 11</td>
<td>156 ± 11</td>
<td>1,690 ± 160</td>
<td>976 ± 100</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n\), number of rat hearts. LVDP, left ventricular developed pressure; TTP, time to peak pressure. Rates of contraction (+dP/dt\(_{\text{max}}\)) and relaxation (-dP/dt\(_{\text{max}}\)) are measured in mmHg/s. Hearts were paced at 2 or 3 Hz, 32°C. WF, Wistar-Furth rats; SHHF, SHHF/M\(\text{k}_{\text{e}}\) rats; BN, Brown Norway rats. There were no statistically significant differences in contractility between the failing SHHF and control left ventricles.
SHHF hearts. Figure 7 compares the effects of thapsigargin plus ryanodine on mechanical restitution in failing SHHF hearts and age-matched controls. The exaggeration of phase I in the presence of a disabled SR was extremely pronounced in hearts from the 19-month-old BN rats but was substantially lower in the failing SHHF hearts.

**DISCUSSION**

Mechanical alternans is the hallmark of heart failure (18, 31). During mechanical alternans, oscillations in contractile force are associated with oscillations in SR Ca$^{2+}$/H$^{1+}$ release, and this alternans can be abolished by compounds such as caffeine (22) and ryanodine (15) that render the SR leaky to Ca$^{2+}$. Ischemia and acidosis are known to facilitate the development of alternans (7, 16) and to reduce the rate of SR Ca$^{2+}$/H$^{1+}$ uptake (8), but $^{31}$P NMR studies of failing SHHF and age-matched WF rat hearts demonstrated no fall in intracellular pH with the induction of alternans (21). Moreover, as shown in a previous study, reducing the rate of SR Ca$^{2+}$/H$^{1+}$ accumulation with thapsigargin does not decrease the threshold pacing frequency at which sustained mechanical alternans develops in healthy hearts (22). Thus acidic inhibition of SR Ca$^{2+}$ accumulation or reduced activity of the sarco(endo)plasmic reticulum Ca$^{2+}$ pump does not fully explain the propensity for mechanical alternans in the failing SHHF heart.

The results of the present study show that alternans can be abolished by interventions that increase Ca$^{2+}$ influx via the L-type Ca$^{2+}$ channels – BAY K 8644, isoproterenol, norepinephrine, zinterol) and that may therefore increase SR Ca$^{2+}$ load. It is noteworthy that the effects of isoproterenol were not observed in the presence of partial L-type Ca$^{2+}$ channel blockade with verapamil, indicating that the increase in L-type Ca$^{2+}$ current secondary to an increase in the activity of cAMP-dependent protein kinase A most likely accounted for the reversal of alternans by the β-adrenoceptor agonist.
In isolated cardiomyocytes, activation of glycolysis has been invoked to explain cessation of alternans in response to β-adrenergic receptor stimulation (10). The mechanisms responsible for the development of alternans in isolated cells and intact tissues may differ, however. Unexplained alternans often complicates the study of single ventricular cardiomyocytes during low (0.2–0.5 Hz) rates of field stimulation (unpublished observations). In the present study, an abrupt jump in pacing frequency from 8 to 9 Hz was required for the development of sustained mechanical alternans in the majority of the healthy intact rat left ventricles examined (Fig. 1). This had no effect on global ATP concentration as measured by 31P NMR (21). Nevertheless, the hearts used in these studies were perfused with 5 mM pyruvate, and we cannot wholly discount the possibility that there were localized areas of decreased [ATP] secondary to inhibition of glycolytic ATP production.

The results of the present study support the suggestion by Bers (2) that mechanical alternans in the intact heart may be related to an incomplete, time-dependent recovery of the ability of the SR to release Ca2+. As shown in Figure 4, at a pacing frequency of 3 Hz, $Y_{\text{max}}$, the mean maximum value for $F/F_0$ is 1.51 for control hearts and 1.89 for the failing hearts. This suggests that at steady-state normal hearts have achieved a 66% restitution (1/1.51 × 100%) of the ability to release SR Ca2+ compared with 53% for the failing hearts. The introduction of a premature beat or an abrupt increase in pacing frequency would be predicted to place the next beat lower on the restitution curve for both groups of hearts (cf., Fig. 4) and a smaller amount of Ca2+ should be released from the SR. With the following beat, the SR may no longer be refractory, so a larger than normal Ca2+ release may occur. But the SR may again be refractory with the next stimulus, and SR Ca2+ load may be smaller due to faster inactivation of the Ca2+ current ($I_{\text{Ca,L}}$) by the larger intracellular Ca2+ concentration ([Ca2+]i) transient and enhanced Ca2+ extrusion via Na+/Ca2+ exchange during the previous large beat (2). Absent compensatory changes in other Ca2+ cycling reactions, this oscillatory behavior could persist indefinitely in normal and failing hearts. The reversal of alternans by interventions shown to accelerate phase II of mechanical restitution (isoproterenol, −BAY K 8644) would tend to support this hypothesis. Acceleration of phase II of mechanical restitution most likely represents a faster recovery of the ability of the SR to release Ca2+, which could reverse the oscillatory behavior described above. As would be predicted by assuming incomplete time-dependent restitution as the basis for alternans, the differences in amplitude between large and small beats increase linearly with increases in pacing frequency (21).

The slightly but not significantly delayed initiation of phase II restitution found for failing SHHF hearts (Fig. 4), which may be related to the prolonged action potentials and less synchronized SR Ca2+ release events observed in failing hearts (19), is unlikely to account for the large difference in the alternans threshold between healthy and failing hearts. Instead, the cross talk between the L-type Ca2+ channels and ryanodine receptors that normally might tend to dampen alternans appears to be deficient in heart failure (6). This cross talk includes the local control of SR Ca2+ release by Ca2+ influx through adjacent L-type Ca2+ channels (28, 30) and the Ca2+ calmodulin-dependent inactivation and facilitation of the L-type Ca2+ current (34). The observed flattening of the early phase I of mechanical restitution in failing SHHF hearts supports this hypothesis. That this difference persists and is in fact magnified by agents that disable the SR (Fig. 7) suggests that it may result from differences in Ca2+ influx across the sarcolemma. We have shown that the putative Na+/Ca2+ exchange inhibitor KB-R7943 has little effect on mechanical restitution (4), suggesting that the differences in phase I between normal and failing hearts might be attributable to differences in the L-type Ca2+ current.

The transient action potential prolongation that coincides with increases in LVDP during phase I restitution in normal hearts further suggests that phase I may be due, at least in part, to Ca2+ or voltage-dependent Ca2+ current facilitation (27). On the other hand, phase I of mechanical restitution was nearly flat in the failing SHHF hearts, and there was no further prolongation of the already prolonged action potentials with the introduction of early extrasystoles. Action potential prolongation is common in heart failure (1, 11, 33) and is important in cross talk between healthy and failing hearts. Instead, the trabecular force–time alternans that normally might tend to dampen mechanical restitution in the intact heart persists and is in fact magnified in heart failure (6). This cross talk includes the local control of SR Ca2+ release by Ca2+ influx through adjacent L-type Ca2+ channels (28, 30) and the Ca2+ calmodulin-dependent inactivation and facilitation of the L-type Ca2+ current (34). The observed flattening of the early phase I of mechanical restitution in failing SHHF hearts supports this hypothesis. That this difference persists and is in fact magnified by agents that disable the SR (Fig. 7) suggests that it may result from differences in Ca2+ influx across the sarcolemma. We have shown that the putative Na+/Ca2+ exchange inhibitor KB-R7943 has little effect on mechanical restitution (4), suggesting that the differences in phase I between normal and failing hearts might be attributable to differences in the L-type Ca2+ current.

The lack of change in action potential duration with early extrasystoles in the failing SHHF hearts raises the possibility that voltage- or Ca2+–dependent facilitation of L-type Ca2+ channels may be reduced in heart failure. A previous study demonstrated that Ca2+–dependent Ca2+ current inactivation is slowed in failing rat cardiomyocytes (6). It is therefore noteworthy that Ca2+–dependent facilitation and inactivation of cardiac L-type Ca2+ channels are both dependent on calmodulin binding to the COOH-terminal tail of the α1-subunit (34). The flattening of phase I of mechanical restitution in the failing SHHF hearts might therefore be due to alterations in calmodulin (5) or the Ca2+ channel itself.

The significantly elevated plateau for phase II of mechanical restitution in the failing hearts was striking. It was not related to differences in $F_0$ LVDP: steady-state LVDP was 130 mmHg for control vs. 146 mmHg (not significant) for the failing hearts. Also, studies of trabeculae from failing SHHF hearts have shown no decline in twitch force under conditions similar to those used in the present study (i.e., 1 mM extracellular Ca2+) (23). However, the trabecular twitches and [Ca2+]i transients were significantly prolonged compared to controls. Part of this prolongation may have been related to the asynchronous SR Ca2+ release described by Litwin et al. (19) in failing cardiomyocytes from infarcted hearts, part to slowed Ca2+ uptake.
by the SR (24), and part to action potential prolongation. Regardless, time constant (τ) values for mechanical restitution were very slightly, but not significantly, increased in the failing SHHF hearts. The curve-fit values for mechanical restitution (T₉₀) were larger in the failing hearts, but again, the difference failed to reach statistical significance. The significantly elevated plateau of phase II shown in Fig. 4 probably resulted from the combined effects of a modest delay in the initiation of restitution combined with a slight decrease in the rate of restitution.

Despite the fact that all of the SHHF rats used in the present study exhibited clear signs of congestive heart failure and had reduced ejection fractions by M-mode echocardiography, LVDP for the isolated perfused hearts paced at 2 or 3 Hz was not depressed relative to that for age-matched controls. There are several possible explanations for the apparent differences between baseline in vivo and in vitro cardiac function. As pacing frequencies are increased, there are significant differences between the LVDP of normal and failing SHHF hearts (22). Because the SHHF rat is spontaneously hypertensive, the SHHF heart in vivo is forced to pump against an increased vascular resistance, whereas end-diastolic pressure was adjusted to 8 mmHg in both groups of isolated perfused hearts. Finally, hearts in the present study were perfused with high concentrations of glucose, insulin, and pyruvate, which may have helped to correct any bioenergetic defect(s) that may have existed in vivo.

The propensity for mechanical alternans, the negative force-frequency relationship, and the prolonged action potentials seen in the failing SHHF rat heart are also observed in failing human hearts (3, 9, 12, 25, 26, 29, 31). Moreover, cardiomyocytes isolated from nonfailing human hearts typically display increases in L-type Ca²⁺ currents as pacing frequency is increased, whereas cells from failing hearts do not (12, 26), suggesting that Ca²⁺ current facilitation might also be deficient in human heart failure.

In summary, failing SHHF hearts develop mechanical alternans at physiological pacing frequencies, whereas control hearts do so only at higher heart rates. In both groups of hearts, alternans can be reversed by agents that either render the SR leaky to Ca²⁺ (caffeine, ryanodine) or increase SR Ca²⁺ load (isoproterenol, BAY K 8644). Agents that increase SR Ca²⁺ load also accelerate phase II of mechanical restitution, the time constants of which are similar in normal and failing hearts under basal and pharmacologically accelerated conditions. In normal hearts, phase I exhibits a transient phase of enhanced LVDP and a parallel increase in action potential area at 50% repolarization, whereas phase I electromechanical restitution curves are flat for failing hearts. Altered excitation-contraction coupling in the failing SHHF rat heart cannot be explained by a single molecular abnormality.

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