Origin of Contractile Dysfunction in Heart Failure
Calcium Cycling Versus Myofilaments

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Background—Chronic congestive heart failure is a common, often lethal disorder of cardiac contractility. The fundamental pathophysiology of the contractile failure remains unclear, the focus being on abnormal Ca\(^{2+}\) cycling despite emerging evidence for depressed myofilament function.

Methods and Results—We measured intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and contractile force in intact ventricular muscle from SHHF rats with spontaneous heart failure and from age-matched controls. At physiological concentrations of extracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_e\)), [Ca\(^{2+}\)]\(_i\) transients were equal in amplitude in the 2 groups, but [Ca\(^{2+}\)]\(_i\) peaked later in SHHF muscles. Twitch force peaked slowly and was equivalent or modestly decreased in amplitude relative to controls. Steady-state analysis revealed a much greater (53%) depression of maximal Ca\(^{2+}\)-activated force in SHHF muscles, which, had other factors been equal, would have produced an equivalent suppression of twitch force. Phase-plane analysis reveals that the slowing of Ca\(^{2+}\) cycling prolongs the time available for Ca\(^{2+}\) to activate the myofilaments in failing muscle, partially compensating for the marked dysfunction of the contractile machinery.

Conclusions—Our results indicate that myofilament activation is severely blunted in heart failure, but concomitant changes in [Ca\(^{2+}\)]\(_i\), kinetics minimize the contractile depression. These results challenge prevailing concepts regarding the pathophysiology of heart failure: the myofilaments emerge as central players, whereas changes in Ca\(^{2+}\) cycling are reinterpreted as compensatory rather than causative. (Circulation. 1999;99:1077-1083.)

Key Words: calcium • contractility • heart failure • myocardium

Heart failure is a systemic illness in which cardiac output is diminished. Excitation-contraction coupling is abnormal in human heart failure: diastolic intracellular [Ca\(^{2+}\)]\(_i\) ([Ca\(^{2+}\)]\(_i\)) is elevated, whereas [Ca\(^{2+}\)]\(_i\) transients are blunted and slow to decay.\(^1\) The function of the myofilaments has been argued not to be much altered,\(^2\) although carefully controlled steady-state analysis has revealed marked depression of the maximal force-generating capacity in a rat ischemic injury model of heart failure.\(^3\) Multiple biochemical alterations of the contractile proteins have been described, including switching of the isoforms of troponin T,\(^4\) suppression of α-myosin heavy chain expression,\(^5\) and decreased basal cAMP-dependent phosphorylation.\(^6\) The realization that heart failure increases the likelihood of myocardial apoptosis\(^7\) gives us further reason to wonder whether depression of the contractile apparatus (by loss of functional units) might play a major unanticipated role.

To assess the relative importance of abnormal Ca\(^{2+}\) cycling versus depressed myofilaments in heart failure, we measured [Ca\(^{2+}\)]\(_i\), and force simultaneously in intact ventricular muscle from rats with spontaneous heart failure. These SHHF/Mccfa\(^{ab}\) (spontaneous hypertension and heart failure) rats recapitulate many of the features of the human disease, including systemic vascular congestion, ventricular dilatation, and decreased ejection fraction.\(^8\)–\(^10\) We find that myofilament force-generating capacity is strongly depressed in heart failure, far out of proportion to the observed depression of force during physiological twitch contractions. The salient abnormality of Ca\(^{2+}\) cycling turns out to be slowing of the [Ca\(^{2+}\)]\(_i\) transients, including a 3-fold increase in the time to peak [Ca\(^{2+}\)]\(_i\). Phase-plane analysis reveals that [Ca\(^{2+}\)]\(_i\), comes closer to equilibrium with the myofilaments in the SHHF rats than in age-matched controls. The kinetic changes in [Ca\(^{2+}\)]\(_i\), can thus be construed as adaptive, insofar as they minimize the contractile failure, which would otherwise track the marked depression of maximal force production. This integrative analysis of excitation-contraction coupling in failing heart muscle highlights the importance of the myofilaments, necessitating a fundamental revision of...
prevailing concepts regarding the pathophysiology of heart failure.

Methods

In Vivo Characterization of Rats

Lean male SHHF rats were bred and housed at The Ohio State University. Animals were chosen for study when they exhibited clinical signs of heart failure (tachypnea, dyspnea, cyanosis, peripheral edema). Age-matched (17 to 18 months) male Wistar-Furth rats (Harlan, Indianapolis, Ind) were purchased at ~9 months of age and housed under conditions identical to those for the SHHF rats until use. M-mode echocardiography was performed under light ketamine/xylazine anesthesia, as described previously. Afterward, rats were transported to Baltimore, Md, and used within 2 weeks for studies of isolated trabeculae.

Rat Trabeculae

Rats were anesthetized by intra-abdominal injection of sodium pentobarbital 0.1 to 0.2 mL, and the hearts were quickly removed and perfused retrogradely with a high-potassium (20 mmol/L) Krebs-Henseleit (K-H) solution equilibrated with a mixture of 95% O2/5% CO2 at room temperature (21°C to 22°C). The K-H solution was composed of (in mmol/L) NaCl 120, NaHCO3 20, KCl 20, MgSO4 1.2, glucose 10, and CaCl2 0.5, pH 7.35 to 7.40. Trabeculae from the right ventricles of these hearts were dissected and mounted between a force transducer and a micromanipulator in a perfusion chamber placed on the stage of an inverted microscope according to methods already described. Not every heart yields geometrically suitable (long, thin, nonbranching) trabeculae: 6 of 11 control hearts and 5 of 6 SHHF hearts did so. The dimensions of the trabeculae were not significantly different in the 2 groups (in mm: control, 3.25±0.10 long, 0.20±0.02 wide, and 0.10±0.01 thick [n=6]; SHHF, 3.14±0.25 long, 0.17±0.04 wide, and 0.11±0.02 thick [n=5]). The cross-sectional area was calculated by multiplying thickness and width and was corrected by a factor of 0.75, assuming an ellipsoidal shape, and a reduction in thickness of ~5% because of the stretch to a sarcomere length of ~2.2 μm. All muscles were superfused with a K-H solution of the same composition as the one described above, but in this case with normal [KCl] (5 mmol/L), at a flow rate of ~10 mL/min, and stimulated at 0.5 Hz. All experiments were performed at room temperature. Force was measured as described previously by a silicon strain gauge (model AEM 801, SensoNor) and expressed as mN/mm2 of cross-sectional area. All experiments were carried out at the length at which the muscles developed maximal twitch force (end-diastolic sarcomere length of 2.2 to 2.3 μm).

Measurement of Intracellular [Ca2+] in Trabeculae

[Ca2+]i was measured with fura 2 pentapotassium salt, according to the method described by Backx and ter Keurs. Briefly, after 40 to 60 minutes of stabilization at 0.5-Hz stimulation frequency, pacing was stopped, and fura 2 pentapotassium salt was microinjected iontophoretically into 1 cell and allowed to spread throughout the muscle via gap junctions. After fura 2 loading, stimulation was stopped, and fura 2 pentapotassium salt was microinjected into the trabeculae placed on the stage of an inverted microscope according to the method described by Backx and ter Keurs. Briefly, after 40 to 60 minutes of stabilization at 0.5-Hz stimulation frequency, pacing was stopped, and fura 2 pentapotassium salt was microinjected into the trabeculae. After fura 2 loading, stimulation was stopped, and fura 2 pentapotassium salt was microinjected into the trabeculae. After fura 2 loading, stimulation was stopped, and fura 2 pentapotassium salt was microinjected into the trabeculae.

Figure 1. In vivo indices of heart failure (HF). Ejection fraction is from echocardiography. *Two-factor ANOVA indicates that all differences between 2 groups are statistically significant: EF, P=0.001; heart wt–to–body wt ratio, P=0.012; lung wt–to–body wt ratio, P=0.028.

Experimental Protocols

To characterize excitation-contraction coupling, muscles were subjected to the following conventional experimental protocols. We first studied the response to extracellular [Ca2+] ([Ca2+]o) (0.5, 1.0, 1.5, and 2.0 mmol/L) during twitch contractions elicited by field stimulation (pulse duration, 5 ms) at a rate of 0.5 Hz. Thereafter, the muscles were exposed to 5 μmol/L ryanodine for 30 minutes and stimulated periodically (~1 minute−1) at 10 Hz to elicit tetani of 4- to 5-second duration. By varying extracellular [Ca2+], different levels of steady-state activation were achieved during tetani until maximal force was reached.

Steady-state force-[Ca2+]o relations were fit with a function of the following form (Hill equation): F=Fmax([Ca2+]i)/(Ca50+[Ca2+]i), where Fmax is the maximal Ca2+-activated force, Ca50 is the [Ca2+] required for 50% of maximal activation, and n is the Hill coefficient.

Statistics

Student’s t test was used for simple comparisons. Two-way ANOVA was used to analyze differences between the SHHF and Wistar-Furth rats. ANOVA with repeated measures was used to analyze [Ca2+]i, and force data for trabeculae superfused at varying [Ca2+]o. Appropriate post hoc tests were then applied where indicated. Data are expressed as mean±SEM.

Results

General Characteristics

We studied 17- to 18-month-old SHHF rats, just after the animals had developed overt heart failure. This was confirmed by echocardiography in the living animals. Figure 1 shows pooled data for ejection fraction from echocardiography; this was significantly lower in the heart failure group than in age-matched controls. To further characterize the clinical phenotype of these failing rats, we determined the ratios of heart weight to body weight and lung weight to body...
weight; both were increased in the heart failure group (Figure 1), consistent with previous findings (eg, see Reference 10).

Force-\([\text{Ca}^{2+}]_i\) Relationships During Twitch Contractions in Control Versus Failing Trabeculae

When the trabeculae were homogeneously loaded with fura 2, we measured \([\text{Ca}^{2+}]_i\) transients and the corresponding force development at various different \([\text{Ca}^{2+}]_o\) s. Figure 2 shows \([\text{Ca}^{2+}]_i\) transients and force in typical experiments from the control (left) and failing (right) groups in 1 mmol/L (A and B; 1.5 mmol/L in C and D).

Figure 2. Calcium transients and force in representative control (left [A and C]) and failing (HF) (right [B and D]) muscles. Each panel shows \([\text{Ca}^{2+}]_i\) (top) and force (bottom) during field-stimulated twitch contractions; recordings were made at 2 extracellular \([\text{Ca}^{2+}]_o\) concentrations in each muscle (1 mmol/L in A and B; 1.5 mmol/L in C and D).

The \([\text{Ca}^{2+}]_i\) transient amplitudes in the 2 groups are not superimposed on major differences in diastolic \([\text{Ca}^{2+}]_i\). Figure 4 shows pooled data for end-diastolic \([\text{Ca}^{2+}]_i\), (top) and end-diastolic force (bottom). Although there was a tendency for end-diastolic \([\text{Ca}^{2+}]_i\), to be higher in the heart failure group at any given \([\text{Ca}^{2+}]_o\), the differences were not significant.

Time Courses of \([\text{Ca}^{2+}]_i\), Transients and Twitch Force

Despite the similarity in the values of \([\text{Ca}^{2+}]_i\), achieved during twitch contractions in the 2 groups, we do find marked differences in the time courses of both \([\text{Ca}^{2+}]_i\), transients and force. Such differences are apparent even from a casual inspection of the results in Figure 2: the times to peak \([\text{Ca}^{2+}]_i\), and peak force are prolonged in the failing muscle, as are the decay phases. These differences were consistent and significant. Figure 5 shows pooled data for time to peak \([\text{Ca}^{2+}]_i\), (top) and time to 50% decay of \([\text{Ca}^{2+}]_i\), transient amplitude (bottom) and force (bottom panel). At any given \([\text{Ca}^{2+}]_o\), the times to peak \([\text{Ca}^{2+}]_i\), and peak force were prolonged in the failing group. The time to 50% decay of the \([\text{Ca}^{2+}]_i\), transient was also prolonged, but the increase in the time to 50% decay of twitch force did not reach statistical significance. The physiological importance of the striking
Weighs the modest (25%) increase in Ca\textsuperscript{2+}maximal force is lower in the failing muscles (K\textsubscript{d}).

Figure 6 shows pooled steady-state activation by tetanizing muscles in the 2 experimental groups. There were no significant differences between groups.

Discussion

This study is the first to measure [Ca\textsuperscript{2+}], and force simultaneously in intact muscles from a well-controlled model of spontaneous congestive heart failure. The results indicate that although Ca\textsuperscript{2+} cycling is not normal, the dysfunction of the myofilaments turns out to predominate quantitatively. Moreover, the Ca\textsuperscript{2+} cycling changes can be construed as adaptive, insofar as they largely negate the profound myofilament depression at low stimulation frequencies and physiological [Ca\textsuperscript{2+}]o.

Phase-Plane Analysis

The fact that [Ca\textsuperscript{2+}]s, transients are slowed in SHHF muscles (Figure 5) might offset the marked depression of F\textsubscript{max} by providing more time for Ca\textsuperscript{2+} to interact with the contractile proteins during each cardiac cycle. We tested this idea by plotting [Ca\textsuperscript{2+}c], versus force on a point-by-point basis during representative twitch contractions, then superimposing the resultant phase-plane loop on the steady-state [Ca\textsuperscript{2+}], force relationship obtained from the same muscle (Figure 7). Normally, the rise of [Ca\textsuperscript{2+}], transients is far too rapid to allow [Ca\textsuperscript{2+}], to reach equilibrium with the contractile proteins\textsuperscript{11}; this is evident in Figure 7 as the discrepancy between the peak [Ca\textsuperscript{2+}], in each loop and the [Ca\textsuperscript{2+}], that produces the same force at steady state in the same muscle. This discrepancy of [Ca\textsuperscript{2+}], (Δ[Ca\textsuperscript{2+}]), highlighted graphically in both panels of Figure 7, is much greater in the control example (left panel) than in the SHHF (right panel). On average, Δ[Ca\textsuperscript{2+}], was 5 times greater in controls than in SHHF (0.20±0.07 versus 0.04±0.02 μmol/L, P<0.05). This analysis confirms that the phase lag between [Ca\textsuperscript{2+}], and force is much smaller in SHHF, giving Ca\textsuperscript{2+} more time for interaction with the myofilaments during each cardiac cycle.
studies of human trabeculae from transplant recipients failed to detect a decline in maximal force production. With human studies, an additional difficulty can be the severely limited availability of truly normal control subjects.

The conclusions of the present study differ from those of Gómez et al, who used the same SHHF heart failure model to investigate Ca$^{2+}$ sparks in voltage-clamped ventricular myocytes. These investigators argued that coupling between sarcolemmal L-type Ca$^{2+}$ channels and the Ca$^{2+}$ release channels of the sarcoplasmic reticulum (SR) is a primary cause of altered contractile performance in hypertrophy and failure. In their experiments, [Ca$^{2+}$]$_i$ transients and isotonic shortening were markedly blunted in failing SHHF myocytes, but the relation between [Ca$^{2+}$]$_i$ and fractional shortening was not altered relative to that in cells from age-matched controls. There are several reasons for the apparent discrepancies. In the voltage-clamp experiments, the duration of depolarization was controlled (200 ms) and was identical for the normal and failing myocytes. The present study, however, used field stimulation. Previous work has shown that the action potential is markedly prolonged in cells from failing SHHF hearts, and action potential duration has been shown to regulate the Ca$^{2+}$ content of the SR. In addition, changes in Ca$^{2+}$ release from the SR tend to be offset by compensatory changes in Ca$^{2+}$ filling of the SR in physiologically contracting myocytes. Thus, the more normal [Ca$^{2+}$]$_i$ transient amplitudes in SHHF trabeculae in the present study probably reflect the longer action potentials in the SHHF versus control trabeculae as well as other homeostatic mechanisms that regulate the Ca$^{2+}$ content of the SR.

The present study also differs from that of Gómez et al in that they measured unloaded cell shortening, whereas this study measured genuine contractile force. Force per cross-sectional area is exquisitely sensitive to the number of contractile units, whereas isotonic shortening is relatively insensitive to this parameter. In this regard, it should be noted...
that histological and ultrastructural studies of failing SHHF ventricles have noted marked interstitial and perivascular fibrosis, a reduction in myofibrils, myofibrillar disorganization, and streaking of Z bands. These ultrastructural changes could well lead to a decrease in functional force-producing units and account for at least a part of the decline in maximal Ca\(^{2+}\)-dependent force observed in the present study. Nevertheless, the fact that single skinned myocytes from a rat pressure-overload model of heart failure also exhibit a marked reduction of maximal Ca\(^{2+}\)-activated force hints that the predominant change resides within the myofilaments themselves, not in extracellular factors or myocyte loss.

A decline in maximal Ca\(^{2+}\)-activated force production similar to that described in the present study has been observed in skinned trabeculae from a postinfarct rat model of heart failure and from failing SHHF hearts (P.J. Reiser, MD et al, oral personal communication). Although 85% to 100% of the myosin in the failing SHHF heart is the slow, \(\nu_1\) (\(\beta\)-myosin heavy chain) subtype, this isoform switch should be adaptive changes of myofilaments. Nevertheless, these “adaptive” changes of myofilaments themselves are depressed; in any case, the changes in myofilaments per cross-sectional area (eg, due to loss of functional units and replacement fibrosis). The single-cell results showing unequivocal depression of maximal force in other heart failure models provides some reassurance that the myofilaments are depressed; in any case, the changes in \(K_1\) and Hill coefficient that we have observed cannot be rationalized by nonmyofibrillar alterations. Second, we examined only the terminal stage of heart failure; thus, our results may shed light on the mechanism of the dysfunction, but they do not tell us how the heart failure developed in the first place. For such insights, further studies in the premorbid and in the hypertrophic, prefailure stages of this model may prove to be useful. Third, all our experiments have been performed at a stimulation frequency of 0.5 Hz. It would be of interest in the future to examine the force-frequency relation, given the evidence that this is altered in heart failure.

**Relationship to Reversible, Postischemic Contractile Failure**

The changes of myofilament Ca\(^{2+}\) responsiveness revealed in this study are reminiscent of those previously described in postischemic “stunned” myocardium. This reversible form of contractile failure occurs immediately, without compensatory changes in Ca\(^{2+}\) cycling. Thus, the profound depression of maximal force (typically 50% to 60%, comparable to that seen here) is matched by an equally profound depression of twitch contractions. In chronic heart failure, Ca\(^{2+}\) transients become slower and somewhat blunted in amplitude (particularly at higher [Ca\(^{2+}\)])..

The slowing of calcium transients may reflect broadening of the subspace surrounding the SR Ca\(^{2+}\) release sites, resulting in reduced coupling between L-type Ca\(^{2+}\) channels and ryanodine receptors; however, whatever the underlying mechanism, the changes of Ca\(^{2+}\) cycling will tend to mitigate the functional depression during physiological excitation-contraction coupling. The kinetic changes enable [Ca\(^{2+}\)] to approach equilibrium with the contractile proteins during each cardiac cycle; therefore, a lower Ca\(^{2+}\) transient amplitude suffices to achieve equivalent (or greater) fractional activation of the myofilaments. Nevertheless, these “adaptive” changes of Ca\(^{2+}\) homeostasis exact their own toll, impairing relaxation and predisposing to diastolic dysfunction.

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