Development of contractile dysfunction in rat heart failure: hierarchy of cellular events

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Daniels MCG, Naya T, Rundell VLM, de Tombe PP. Development of contractile dysfunction in rat heart failure: cellular events. Am J Physiol Regul Integr Comp Physiol 293: R284–R292, 2007. First published March 15, 2007; doi:10.1152/ajpregu.00880.2006.—The cellular mechanisms underlying the development of heart failure (HF) are not well understood. Accordingly, we studied myocardial function in isolated right ventricular trabeculae from rats in which HF was induced by left ventricular myocardial infarction (MI). Both early-stage (12 wk post-MI; E-pMI) and late, end-stage HF (28 wk post-MI; L-pMI) were studied. HF was associated with decreased sarcoplasmic reticulum Ca$^{2+}$ ATPase protein levels (28% E-pMI; 52% L-pMI). HF affected neither sodium/calcium exchange, ryanodine receptor, nor phospholamban protein levels. Twitch force at saturating extracellular [Ca$^{2+}$] was depressed in HF (30% E-pMI; 38% L-pMI), concomitant with a marked increase in sensitivity of twitch force toward extracellular [Ca$^{2+}$] (26% E-pMI; 68% L-pMI). Ca$^{2+}$-saturated myofilament force development in skinned trabeculae was unchanged in E-pMI but significantly depressed in L-pMI (45%). Tension-dependent ATP hydrolysis rate was depressed in L-pMI (49%), but not in E-pMI. Our results suggest a hierarchy of cellular events during the development of HF, starting with altered calcium homeostasis during the early phase followed by myofilament dysfunction at end-stage HF.

myofilament calcium-handling protein and mRNA levels, and these changes persisted into end-stage HF. Our results, therefore, suggest a hierarchy of cellular events during the development of HF, starting with altered calcium homeostasis during the early phase followed by myofilament dysfunction at end-stage HF. We speculate the latter may be coincident with the transition to overt end-stage decompensated HF in this model.

METHODS

Experimental animals. All procedures were performed in accordance with institutional guidelines regarding the care and use of laboratory animals. Myocardial infarction (MI) was surgically induced under appropriate anesthesia in 4-wk-old female Sprague-Dawley rats by the supplier, as previously described (7, 10). The animals received food and water ad libitum during the development of HF following the surgical procedure (12 or 28 wk). Hypothyroidism was induced in a separate group by ingestion of 0.8 g/l propylthiouracil for 6 wk via the drinking water (10, 36, 42) starting from the age of 4 wk.

Isolation and mounting of cardiac trabeculae. Right ventricular (RV) trabeculae were dissected as previously described (7, 10). Intact trabeculae were mounted in a glass-covered experimental chamber positioned on the stage of an inverted microscope (7); trabeculae were stimulated at 0.5 Hz. Skinned trabeculae were prepared by exposure to relaxing solution with 1% Triton X-100 at 4°C for at least 2 h (7, 9). Skinned trabeculae were then attached to aluminum T-clips and mounted in an experimental setup that allows for simultaneous measurement of calcium-activated myofilament force and ATP hydrolysis rate (9, 35, 36). Finally, following dissection of suitable trabeculae, the left ventricle (LV) was cut transversely at the base of the papillary muscles to measure its unstressed diameter. Residual LV and RV tissues were briefly blotted, weighed, flash-frozen, and stored at −80°C for later analyses by Northern and Western blot assays.

Solutions. The standard solution used for intact twitching trabeculae was composed of (in mM): 142.5 Na$^+$, 5.0 K$^+$, 127.5 Cl$^-$, 1.2 Mg$^{2+}$, 2.0 H$_2$PO$_4$ - 1.2 SO$_4^{2-}$, 21 HCO$_3$-, 10 p-glucose; [Ca$^{2+}$] as indicated and equilibrated with 95% O$_2$-5% CO$_2$ (25.0 ± 0.1°C). For skinned fiber studies, three bathing solutions were used (20°C): a relaxing solution with 1% Triton X-100 at 4°C for at least 2 h (7, 9). Skinned trabeculae were then attached to aluminum T-clips and mounted in an experimental setup that allows for simultaneous measurement of calcium-activated myofilament force and ATP hydrolysis rate (9, 35, 36). Finally, following dissection of suitable trabeculae, the left ventricle (LV) was cut transversely at the base of the papillary muscles to measure its unstressed diameter. Residual LV and RV tissues were briefly blotted, weighed, flash-frozen, and stored at −80°C for later analyses by Northern and Western blot assays.

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Measurement of twitch force and myofilament function. Sarcomere length (SL) was measured by laser diffraction techniques (7). Muscle length was controlled by servomotor (Cambridge Technology); force was measured by semiconductor strain gauge (Sensonor). Stress, expressed as mN/mm$^2$, was calculated as developed force normalized to cross-sectional area of the trabecula estimated from the width and thickness of the muscle; these were measured at ~10-μm resolution either in the dissection dish (intact twitching trabeculae) or in the experimental apparatus (skinned trabeculae). On average, intact
twitching trabeculae (n = 28) were 2.8 ± 0.1 mm in length, 275 ± 26 μm in width, and 127 ± 8 μm in thickness; skinned trabeculae (n = 28) were 1.7 ± 0.1 mm in length, 218 ± 11 μm in width, and 163 ± 9 μm in thickness. The rate of ATP hydrolysis was measured in skinned trabeculae over a range of contractile activation levels induced by varying concentrations of free calcium, as described previously (9).

Measurement protocols. Intact electrically stimulated trabeculae were stretched to maintain resting SL at 2.10 μm, while the extracellular calcium concentration ([Ca<sup>2+</sup>]o) was gradually increased to 1.5 mM followed by 1 h of stabilization. The active force-SL relationship was then measured, as previously described (10). Next, twitch force was measured over a range of [Ca<sup>2+</sup>]o at SL = 2.0 μm to evaluate the twitch force-[Ca<sup>2+</sup>]o relationship (7, 10). Skinned trabeculae were bathed in a series of solutions with varied activating calcium levels to compile the steady state contractile force-[Ca<sup>2+</sup>]o and force-ATPase relationships (9, 35, 36).

Northern blot mRNA analysis. Total RNA was isolated from RV tissues by acid guanidinium thiocyanate-phenol-chloroform extraction, separated on 1% formaldehyde-agarose gels (10 μg), and transferred to nylon membranes, according to standard protocols. The nylon membranes were successively hybridized with randomly primed 32P-labeled rat cDNA probes; human GAPDH (Y02278, Gibco), and rat 18S rRNA (Ambion) were used to control gel loading. All cDNA fragments were subcloned into cloning vectors, and their integrity was confirmed by direct sequencing. Digitized photographic films were analyzed using image processing software (NIH image). To allow for quantitative comparison between samples analyzed on separate gels, each gel also contained lanes loaded with RNA extract obtained from standard sample (a 7-wk-old rat ventricle; thus, an identical reference sample was included in each analysis). Linearity for each cDNA probe was confirmed in preliminary experiments.

Western blot protein analysis. Cardiac RV whole homogenates (20 μg total protein) were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes using standard protocols. Total protein content was determined by the bicinchoninic acid method (in separate runs): monoclonal sarcoplasmic reticulum calcium pump (SERCA2; 2A7-A1, ABR), polyclonal sodium-calcium exchanger (NCX; pai11-13, Swant), monoclonal ryanodine receptor 2 (RyR2; C3-33, ABR), or monoclonal phospholamban (PLBm; 2D-12, ABR); incubation with the appropriate horseradish peroxidase-conjugated antibody was done according to the supplier’s instructions (cardiac sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase, α- and β-myosin heavy chain distribution analysis. The isofrom distribution of myosin heavy chain (MHC) was estimated using SDS-PAGE as described previously (36). Briefly, whole RV homogenates (5–10 μg total protein) were separated using a 6% resolving and 5% stacking gel at 45 mA for 6.5 h at 4°C. Gels were stained with Cooamase brilliant blue R-250 and digitally scanned. Images were analyzed by commercially available software (Kodak 1D) to measure relative densities of α-MHC and β-MHC.

Data analysis. Statistical significance was tested by one- or two-way ANOVA; P < 0.05 was considered significant. Data are expressed as means ± SE. Sigmoidal force-[Ca<sup>2+</sup>]o relations were fit using a nonlinear fit procedure to a modified Hill equation:

\[ F = F_{\text{max}} \cdot \frac{[\text{Ca}^{2+}]^n}{[\text{Ca}^{2+}]^n + EC_{50}} \]  

where F is peak twitch force (intact) or steady-state force (skinned), F<sub>max</sub> is the maximum saturated value F can attain, EC<sub>50</sub> is the concentration of [Ca<sup>2+</sup>]o at which F is 50% of F<sub>max</sub>, and H represents the slope of the force-[Ca<sup>2+</sup>]o relation (the Hill coefficient). In skinned trabeculae, the ATP hydrolysis rate as a function of steady-state force over a range of free [Ca<sup>2+</sup>]o was fit by linear regression, the slope of which represents tension-dependent myofilament ATP consumption rate (tension cost parameter; a direct measure of average cross-bridge cycling rate).

RESULTS

Signs of heart failure. At the time of the study, the animals in the end-stage HF group (L-pMI; 24 wk following LV myocardial infarction) showed evidence of congestive heart failure (CHF), as we observed previously in this experimental HF model (see Table 1) (5, 10). Signs of CHF included a more than twofold dilatation of the left ventricle (123%), increased maximum LV wall thickness (11%), and RV hypertrophy (165% increase in RV-to-body-weight ratio). Similar findings were apparent in the early stage heart failure group (E-pMI; 12 wk after LV myocardial infarction), albeit to a significantly lesser degree (LV dilation 59%; LV wall thickness 7%; RV-to-body-weight ratio 109%). In addition, variable amounts of pleural fluid were found in the chest upon removal of the heart in about half of the animals in the L-pMI group, but none in the E-pMI group. Thus, development of HF in this model was associated with progressive LV dilation and RV hypertrophy. These anatomical findings are consistent with the findings of the Northern blot analyses of RV tissues as summarized in Figs. 1 and 2. Fig. 1 shows that both early and late HF was associated with a decrease in “adult genes” mRNA levels (cardiac sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase, α-myosin heavy chain), as well as an increase in “fetal genes” mRNA levels (skeletal α-actin, β-myosin heavy chain). Atrial natriuretic factor and brain natriuretic factor expression were also enhanced in both groups. Aging by itself, on the other hand, did not affect mRNA expression of these genes. In contrast, in either group, neither cardiac α-actin mRNA nor NCX mRNA levels were affected (results not shown). These qualitative Northern blot analysis results are consistent with the more quantitative results generally found during the development of HF. It was well established that cardiac dysfunction of
diverse etiologies in the small rodent is associated with a shift in MHC expression to the beta isoform (8, 42), and this was also observed in the present study (cf. Fig. 2). Early HF resulted in a decrease in \(\alpha\)-MHC mRNA, concomitant with an increase in \(\beta\)-MHC mRNA, and furthermore, these alterations were more pronounced in late HF. As expected, signals from a probe that hybridized with either \(\alpha\)- or \(\beta\)-MHC mRNA showed no significant alteration during the development of HF. In addition, aging by itself was associated with a small, but significant increase in \(\beta\)-MHC mRNA, as has been observed previously (42).

Intact electrically stimulated trabeculae. Fig. 3, top, illustrate the impact of HF on twitch force measured in intact electrically stimulated trabeculae over a range of \([Ca^{2+}]_o\). The data from each individual trabecula were fit to a modified Hill equation; the average fit parameters are summarized in Table 2. Twitch force at saturating levels of \([Ca^{2+}]_o\) (the \(F_{\text{max}}\) parameter) was significantly depressed in both early and late HF groups, while aging per se did not affect \(F_{\text{max}}\). The sensitivity of twitch force to \([Ca^{2+}]_o\) was slightly increased in the early stage of HF and significantly increased at the late stage of HF (compare E-pMI and L-pMi EC50 parameters in Table 2). Aging by itself was also associated with a small, but significant, reduction in the EC50 parameter, independent of the development of HF. Finally, the slope of the twitch force-\([Ca^{2+}]_o\) relationship, as indexed by the Hill coefficient, was significantly reduced in older animals, regardless of HF status. Note that due to the increased sensitivity to extracellular \([Ca^{2+}]_o\), twitch force at an intermediate level of \([Ca^{2+}]_o\) (~1 mM) was not appreciably affected by the development of HF. Finally, at \([Ca^{2+}]_o = 1.5\) mM, the entire twitch force-sarcomere length relationship was markedly depressed both in the early phase (33%) and late phase (47%) of HF (results not shown). Thus, depressed myocardial function following myocardial infarction in both phases was associated not only with depressed maximum twitch force at saturating \([Ca^{2+}]_o\), but also with a depression of the Frank-Starling relationship.

Calcium homeostasis protein levels. To determine whether depressed twitch function during the development of HF in this model is correlated with altered calcium homeostasis, Western blot analysis of key calcium handling proteins was performed. The results are summarized in Fig. 4. Early HF was associated with a significant decrease (28%) in SERCA2 level, decreasing further to 52% at late HF; aging per se did not affect SERCA2 levels. There was a trend toward increased NCX levels in early HF (14%), followed by a decrease at late HF (13%); neither changes were significant, however. Again, aging per se did not affect NCX levels. Neither PLBm nor RyR2 protein levels were affected by either aging or HF (results not shown).

Chemically permeabilized (skinned) trabeculae. To assess whether the depression of twitch force was due to altered myofilament function, a separate group of trabeculae was chemically permeabilized (skinned) to allow direct study of contractile protein function. Fig. 3, middle, shows myofilament force-free \([Ca^{2+}]_o\) relationships in the early HF (left) and the late HF (right) groups. The data from each individual trabecula were fit to a modified Hill equation; the average fit parameters are summarized in Table 2. Late HF was associated with a 45% decrease in maximum \(Ca^{2+}\) saturated force (\(F_{\text{max}}\) parameter), \(F_{\text{max}}\) in early HF, on the other hand, was not affected. There were no differences in the Hill coefficient parameter. Finally, the sensitivity of myofilament force to free \(Ca^{2+}\), the EC50 parameter, was not affected by HF status in either early or late HF group. Aging by itself, however, was associated with a significant (14%) increase in \(Ca^{2+}\) sensitivity. To assess the impact of HF on cross-bridge cycling rate, we also measured the rate of ATP hydrolysis as a function of myofilament force development over a range of free \(Ca^{2+}\) in the bathing medium. These results are illustrated in Fig. 3, bottom. Late HF was associated with a significant decrease (49%) in tension-dependent ATP hydrolysis rate (tension cost). Early HF, on the other hand, did not affect tension cost. Aging, by itself, was associated with a 28% decrease in tension cost.

Relation of tension cost to \(\beta\)-myosin content. HF and aging were associated with a shift in isomyosin expression from \(\alpha\)- to
By MHC (cf. Fig. 2). We have recently demonstrated that increased MHC content in rat myocardium correlates with reduced tension cost (35, 36). Therefore, the depression of and tension cost seen in the late HF group could be due merely to altered isomyosin composition. To investigate this possibility, we measured force and tension cost in a separate group of skinned trabeculae that were obtained from animals that were rendered hypothyroid (see METHODS), which resulted in the virtually exclusive synthesis of RV/MHC (Fig. 2). Early HF was associated with a significant increase (16%) in RV/MHC protein, which increased further to 39% MHC in end-stage HF (39%). Aging by itself was associated with a small (4%), but significant, increase in RV MHC content. Neither maximum force nor calcium sensitivity was affected by hypothyroidism, as reported by us previously (10, 36). Tension cost was significantly depressed in the late HF group to levels below that of the hypothyroidism group, despite the fact that the hearts still contained ~60% MHC (Fig. 5, middle). This finding is further illustrated in Fig. 5, bottom, where tension cost is plotted as a function of MHC content for all the study groups. Relative to non-HF muscle groups (solid line), tension cost in relation to MHC content was ~55% depressed in the late HF group.

Discussion

Myocardial infarction frequently results in heart failure. However, the cellular mechanisms underlying the transition from a compensatory state to end-stage failure following myocardial infarction is largely unknown. We studied contractile function in both intact and skinned cardiac RV trabeculae from rat hearts at 12 and 28 wk post LV infarction. These time points during the development of HF in this small rodent model represent early, compensated, and end-stage HF. We found depressed twitch function at both stages of HF in intact, electrically stimulated trabeculae. Myofilament function, however, was depressed only at end-stage HF.

Depressed twitch function. The twitch function of isolated trabeculae was depressed at both stages of HF, a result that is consistent with our previous observations in this model (7, 10). Depressed function manifested as a reduction in twitch force at saturating levels of Ca2+ in the bathing medium (Fig. 3, top). It is interesting to note that at an intermediate and, thus, more physiological concentration of Ca2+, twitch force was virtually identical between the control and HF groups. This was due to a significant increase in the sensitivity to extracellular Ca2+ at both stages of HF, but particularly at end-stage HF. Despite the clear defect in myocardial E-C coupling, physiological twitch force, and, as a consequence, we presume ventricular pump function, may well have been preserved in our HF rats. A similar observation has also been reported for another small rodent model of HF, the spontaneous hypertensive HF rat (32). The mechanisms that underlie this phenomenon cannot directly be determined from our study. HF was associated with a decrease in SERCA both at the mRNA (Fig. 1) and protein level (Fig. 4), as has been described previously in both human and animal HF (3, 26, 38), albeit not universal (8). In addition, reduced phosphorylation of phospholamban has been reported in HF (1, 31, 47). It is reasonable to conclude that the pool of calcium available for release by the SR is depressed in the HF myocyte. It has been established that action potential duration is prolonged in HF in general (6, 33), and this has indeed also been observed in the rat myocardial infarct model (46). Hence, in HF the source of calcium that activates the cardiac myofilament is shifted from the intracellular store to sarcolemmal calcium influx during the action potential, a process that apparently saturates at a relatively low extracellular calcium concentration. The mechanisms underlying this are unknown but may involve increased Ca2+ channel function. In addition, the duration of the calcium transient has also been shown to be prolonged in HF in general (6, 33), as well as in the rat MI HF model (46), suggesting that myofilament activation may approach a condition that is closer to steady state and thus higher levels of activation (4, 32, 37, 46). Although beneficial for maintaining pump function in HF, the sustained force development comes at a “cost” in the form of a high sensitivity toward extracellular calcium Ca2+ and a prolonged calcium.
stimulated RV trabeculae in early HF (left) and late, end-stage HF (RV trabeculae. Symbols and fit as in top panels. Ca\(^{2+}\) for all groups.

panels are summarized in Table 2. Data are presented as means ly sis rate was depressed in late, end-stage HF. Average fit parameters of all (dashed lines indicate HF groups). Myofilament force-dependent ATP hydro-

development in skinned RV trabeculae. Data were fit by linear regression: myofilament force as function of free ionized \([\text{Ca}^{2+}]_\text{i}\).

Transient, which may increase the incidence of ventricular arrhythmias (6, 33). Another mechanism that may underlie the increase in sensitivity toward extracellular Ca\(^{2+}\) is an increase in the sensitivity of the SR calcium release process triggered by calcium influx, as has been suggested by the Marks group (45).

It is important to note that we found no indication of altered RyR mRNA or protein levels, which is consistent with previous reports (26). A change in NCX function has been reported in some (15, 24) but not all (33, 39) studies. In our study, we found no significant changes in NCX protein content in early and late HF. Regardless of the underlying mechanisms, the enhanced sensitivity of twitch force to extracellular Ca\(^{2+}\) may function as a cellular compensation aimed to maintain twitch function in the face of depressed myofilament function in the late HF myocyte.

Depressed myofilament function. Myofilament function was depressed at end-stage HF but not in the early stage of HF (Figs. 3 and 5). This manifested itself as a depression of Ca\(^{2+}\)-saturated myofilament steady-state force development and a reduction in cross-bridge cycling rate. Myofilament Ca\(^{2+}\) sensitivity on the other hand, was not affected by HF in the present study on isolated skinned cardiac trabeculae (cf. Table 2), consistent with previous studies (13, 17).

Previous studies in single skinned myocytes in contrast, both by others and us have reported either increases or decreases in myofilament Ca\(^{2+}\) sensitivity both in human and experimental HF (5, 22, 44). The reasons for these different findings are not entirely clear; one explanation for these different findings may relate to the different experimental preparations that were studied (multicellular vs. single myocyte skinned myocardium). It is unlikely that depressed myofilament function was due to a reduction in the cross-sectional myofilibrillar protein content, resulting from a relative increase in extracellular matrix protein (fibrosis). First, our measurement of cross-bridge cycling rate is not affected by this confounding factor, since ATP hydrolysis rate is measured as a function of myofilament force development. Second, we found no evidence of increased passive force development in our skinned muscle preparations, as may be expected from a significant increase in fibrosis. Finally, we also observed decreased myofilament force development in single skinned myocytes in this HF model (5), an approach that altogether eliminates the extracellular matrix.

Heart failure and cardiac hypertrophy in small rodents are associated with a shift in isomyosin synthesis from predominantly α-myosin toward β-myosin (42). Our study confirms this observation (Figs. 2 and 5). Such a change in isomyosin composition does not occur in larger mammals such as the human, at least not to the extent as observed in the small rodent models (27). However, it is unlikely that the depressed myofilament force development that we observed at end-stage HF was the result of altered isomyosin composition. That is, we have consistently found that an alteration in isomyosin composition in the rat does not affect myofilament force development and calcium responsiveness (10, 36), which is in line with studies by others (14, 21). A new finding in the present study is the reduction in cross-bridge cycling rate that accompanied the depression of myofilament force development at end-stage HF. It is well established that β-myosin is associated with a reduction in cross-bridge cycling (35, 36). Therefore, reduced cross-bridge cycling may merely have been the result of altered isomyosin composition in HF. This was clearly not the case; tension-cost, an index of cross-bridge cycling, was higher in muscles composed exclusively of β-myosin than that observed in end-stage HF myocardium, despite the fact that end-stage HF RV tissue was composed of a mixture of α- and β-myosin.
In fact, cross-bridge cycling rate in end-stage HF was only about half the rate that would be expected based solely on MHC content. We have previously shown a linear relationship between tension-cost and the \( \frac{H_9}{H_9+H_2} \)-myosin ratio in both rat and mouse myocardium (25, 36); a similar result was also reported in guinea-pig myocardium, albeit over a smaller range of \( \frac{H_9}{H_9+H_2} \)-myosin ratio (43). Likewise, human ventricular myocardium displays a lower tension cost than human atrial myocardium that is largely explained by the difference in myosin composition (29). It should be noted that the tension-cost parameter is measured during an isometric contraction, that is, under conditions in which the cross-bridges are under high strain and developing force. A linear relationship between tension-cost and \( \frac{H_9}{H_9+H_2} \)-myosin ratio implies independent cycling of cross-bridges, generating force and consuming ATP independently. Although measurement of thin filament sliding speed (an unloaded, zero-strain situation) has demonstrated a curvilinear relationship between sliding velocity and \( \frac{H_9}{H_9+H_2} \)-myosin ratio (19), there is still a general decline in cross-bridge cycling rate in proportion to an increase in \( H_2 \)-myosin content, even under those conditions. However, since here, we measured isometric contractions, it is appropriate to compare

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Values are expressed as mean ± SE; \( n = 7 \) for all groups. Average Hill fit parameters of individual trabecule in intact and skinned right ventricular fibers from the E-Ctrl, E-pMI, L-Ctrl, and L-pMI groups. \( F_{\text{max}} \) is maximum developed tension (force per fiber cross-sectional area) at saturating extracellular [Ca\(^{2+}\)] (Intact trabeculae) or free ionized [Ca\(^{2+}\)] (Skinned trabeculae). The Hill coefficient is an index of the level of cooperative activation. \( E_{\text{50}} \) is the [Ca\(^{2+}\)] at which developed tension is half-maximal. Statistical analysis was performed by two-way ANOVA. \( P \) values are listed in the final three columns: \( P \) MI indicates the impact of HF, regardless of age; \( P \) Time indicates the impact of aging, regardless of HF status; \( P \) Interaction indicates the influence of aging on HF or vice versa.

(Fig. 5). In fact, cross-bridge cycling rate in end-stage HF was only about half the rate that would be expected based solely on MHC content. We have previously shown a linear relationship between tension-cost and the \( \alpha/\beta \)-myosin ratio in both rat and mouse myocardium (25, 36); a similar result was also reported in guinea-pig myocardium, albeit over a smaller range of \( \alpha/\beta \)-myosin ratio (43). Likewise, human ventricular myocardium displays a lower tension cost than human atrial myocardium that is largely explained by the difference in myosin composition (29). It should be noted that the tension-cost parameter is measured during an isometric contraction, that is, under conditions in which the cross-bridges are under high strain and developing force. A linear relationship between tension-cost and \( \alpha/\beta \)-myosin ratio implies independent cycling of cross-bridges, generating force and consuming ATP independently. Although measurement of thin filament sliding speed (an unloaded, zero-strain situation) has demonstrated a curvilinear relationship between sliding velocity and \( \alpha/\beta \)-myosin ratio (19), there is still a general decline in cross-bridge cycling rate in proportion to an increase in \( \beta \)-myosin content, even under those conditions. However, since here, we measured isometric contractions, it is appropriate to compare

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**Fig. 4.** Ca\(^{2+}\) homeostasis RV protein content following myocardial infarction. **Top:** representative Western blot analysis illustrating protein content of several calcium-handling proteins: ryanodine receptor (RyR), cardiac SERCA2, sodium-calcium exchanger (NCX), and phospholamban (PLB) in E-Ctrl, E-pMI, L-Ctrl, and L-pMI tissue. Whole RV homogenate (20 \( \mu \)g protein/lane) was loaded on SDS-gels and transferred to polyvinylidene difluoride membranes. Specific antibody binding was detected by chemiluminescence. **Bottom:** bar graphs of average SERCA2 (left) and NCX (right) protein content normalized to a stock 7-wk-old rat ventricle. SERCA2 protein content was diminished in early HF and further decreased in L-pMI. Values are expressed as means ± SE; \( n = 7 \) for all groups. *\( P < 0.05 \) control vs. HF.
Our results suggest a hierarchy of cellular events during the development of HF. We employed hypothyroidism to induce synthesis of β-myosin. Hypothyroidism potentially affects many aspects of cardiac myocyte biology. However, in the present study a hypothyroid model was used only for the purpose of comparing tension-cost between sham and HF myofilaments. Neither myofilament maximum force, calcium sensitivity, or level of cooperativity is altered between euthyroid and hypothyroid derived myocardium (10, 14, 21, 25, 36), strongly suggesting that, as far as the contractile proteins are concerned, hypothyroidism only affects myosin isoform expression and, thereby, cross-bridge cycling rate; we would have expected a change in these functional parameters should hypothyroidism have caused other changes in myofilament function apart from cross-bridge cycling rate observed in this model of HF could not be directly determined from our present study.

Limitations. Heart failure was induced by infarction of the left ventricle. We adopted this small rodent model of HF because the RV of rats can provide thin and homogeneous cardiac trabeculae. This feature is essential to ensure both the metabolic stability of the preparation and to allow for the measurement and control of sarcomere length by laser diffraction techniques (7, 10). Furthermore, by studying trabeculae from the right ventricle, we ensured that only noninfarcted tissue was studied. Nevertheless, by studying the RV, important changes in the LV during the development of HF may have gone unnoticed in this study. The finding of depressed function of isolated right ventricular myocardium may seem surprising since congestive heart failure was induced by left ventricular infarction. An explanation may be that, in this HF model, both the right and left ventricle are exposed to increased mechanical load and circulating neurohormonal stimuli during the development of HF. We employed hypothyroidism to induce synthesis of β-myosin. Hypothyroidism potentially affects many aspects of cardiac myocyte biology. However, in the present study a hypothyroid model was used only for the purpose of comparing tension-cost between sham and HF myofilaments. Neither myofilament maximum force, calcium sensitivity, or level of cooperativity is altered between euthyroid and hypothyroid derived myocardium (10, 14, 21, 25, 36), strongly suggesting that, as far as the contractile proteins are concerned, hypothyroidism only affects myosin isoform expression and, thereby, cross-bridge cycling rate; we would have expected a change in these functional parameters should hypothyroidism have caused other changes in myofilament function apart from cross-bridge cycling rate under our conditions (23, 40). Finally, our study was performed at 25°C, at a relatively slow heart rate (0.5 Hz), and in the absence of catecholamine stimulation. Therefore, the contractile function that we measured in the isolated RV trabeculae in this HF model, at least at the early stage of HF (7). Finally, PKC-induced contractile protein phosphorylation is associated with decreased cross-bridge cycling rate (8, 40, 41). It has recently been suggested that PKC-α is upregulated in HF (18, 23, 40), and this kinase may mediate the changes in myofilament function that we observed in HF, particularly via phosphorylation of troponin in heart failure (5). However, it should be noted that the underlying molecular mechanisms that may be responsible for the reduced cross-bridge cycling rate observed in this model of HF could not be directly determined from our present study.
development of HF, starting with altered calcium homeostasis during the early phase followed by myofilament dysfunction at end-stage HF. We speculate that the latter may be coincident with the transition to overt end-stage decompensated HF in this model.

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