Selective contractile dysfunction of left, not right, ventricular myocardium in the SHHF rat

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Janssen, Paul M. L., Linda B. Stull, Michelle K. Leppo, Ruth A. Altschuld, and Eduardo Marbán. Selective contractile dysfunction of left, not right, ventricular myocardium in the SHHF rat. Am J Physiol Heart Circ Physiol 284: H772–H778, 2003. First published November 7, 2002; 10.1152/ajpheart.01061.2001.—The progression of hypertensive to cardiac failure involves systemic changes that may ultimately affect contractility throughout the heart. Spontaneous hypertensive heart failure (SHHF) rats have depressed left ventricular (LV) function, but right ventricular (RV) dysfunction is less well characterized. Ulbrathin (87 ± 5 μm) trabeculae were isolated from end-stage failing SHHF rats and from age-matched controls. Under near-physiological conditions (1 mM Ca2+, 37°C, 4 Hz), developed force (in mN/mm2) was not significantly different in SHHF LV and RV trabeculae and those of controls. SHHF LV preparations displayed a negative force-frequency behavior (40 ± 7 vs. 23 ± 4 mN/mm2, 2 vs. 7 Hz); this relationship was positive in SHHF RV preparations (27 ± 5 vs. 40 ± 6 mN/mm2) and controls (32 ± 6 vs. 44 ± 9 mN/mm2). The response to isoproterenol (10−6 M, 4 Hz) was depressed in SHHF LV preparations. The inotropic response to hypothermia was lost in SHHF LV trabeculae but preserved in SHHF RV trabeculae. Intracellular calcium measurements revealed impaired calcium handling at higher frequencies in LV preparations. We conclude that in end-stage failing SHHF rats, RV function is only marginally affected, whereas a severe contractile dysfunction of LV myocardium is present.

METHODS

Muscle preparation and experimental setup. Male SHHF rats (23) were housed until they developed severe heart failure at 18–19 mo of age. This was evident by clinical signs such as severe lethargic behavior, labored and high-frequency breathing (>200 breaths/min), and edema. One rat died before tissue could be harvested. Aged-matched control rats (n = 10, 18–22 mo, Wistar) were treated and housed under identical conditions. The investigation conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). Genotyping identified the vast majority (11 of 14 genotyped) of SHHF animals as “true leans,” i.e., having a complete absence of the cp gene. No obvious differences were observed between the three heterozygous animals and the true lean animals with respect to the severity of heart failure.
Rats were anesthetized by intraperitoneal injection of 1.0 ml/kg pentobarbital sodium (360 mg/ml). After intracardiac heparinization, hearts were rapidly excised and placed in Krebs-Henseleit buffer containing (in mM) 120 NaCl, 5 KCl, 2 MgSO₄, 1.2 NaH₂PO₄, 20 NaHCO₃, 0.25 Ca²⁺, and 10 glucose (pH 7.4). Additionally, 20 mM 2,3-butanedione monoxime (BDM) was added to the dissection buffer to minimize cut-end trauma. The effects of short-term BDM exposure were reversible (13, 17). Heart weight (wet, in mg) and total body weight (g) were recorded for each rat. Hearts were cannulated via the ascending aorta and retrogradely perfused with the same buffer equilibrated with 95% O₂-5% CO₂. Blood was thoroughly washed out, and thin, uniform, nonbranched trabeculae from the RV (SHHF: n = 14; control: n = 7) and/or the LV (SHHF: n = 15; control: n = 4) were carefully dissected, leaving a block of tissue at one end from the ventricular wall and a small part of the valve (or second block of tissue depending on the position of the trabecula) at the other attached to the preparation to facilitate mounting. Dimensions of the muscles (n = 40) were measured with a calibration reticule in the ocular of the dissection microscope (×10, 0.10 μm; width 218 ± 86 ± 5 μm, and length 2.34 ± 0.09 mm. Cross-sectional area was calculated assuming an ellipsoid shape; dimensions were comparable in the various experimental groups.

Muscles were mounted between a platinum-iridium basket-shaped extension of a force transducer and a hook connected to a micromanipulator. This attachment method minimizes end-damage compliance of the muscle (2, 13, 15, 17, 24) and prevents excessive loss of force throughout the experimental protocols. Muscles were perfused with the same buffer as above (except that BDM was omitted) at 22.5°C and stimulated at 0.5 Hz, extracellular calcium concentration ([Ca²⁺]o) was raised to 1.0 mM, and muscles were allowed to stabilize for at least 30 min before the experimental protocol was initiated. Muscles were stretched to a length at which a small increase in length resulted in about equal increases in resting tension and active developed tension. This length, which is slightly below the length at which active force development is maximal, was selected to be comparable to the maximally attained length in vivo (~2.2 μm sarcomere length) (22).

**Measurement of intracellular calcium concentration in RV trabeculae.** In a subset of experiments (n = 8 muscles, 4 LV and 4 RV), the intracellular calcium concentration ([Ca²⁺]i) was measured as previously described (1, 2, 6, 12, 16). Briefly, after the muscle had stabilized (at 1.0 mM Ca²⁺, 22.5°C, and 0.5 Hz) for 30 min, [Ca²⁺]i was reduced to 0.25 mM and stimulation was stopped. Background fluorescence (340, 358, and 380 nm) was collected at both 22.5°C and 37.5°C. Fura 2 pentapotassium salt (Molecular Probes, Eugene, OR) was microinjected iontophoretically into one cell and allowed to spread throughout the muscle via the gap junctions. After fura 2 loading, stimulation was restarted and [Ca²⁺], was determined by alternating illumination at 340 and 380 nm. We used a heat-exchange system to achieve rapid (<2 s) switching to 37.5°C from 22.5°C. Limiting exposure to high temperatures to short intervals (4–6 min) enabled us to measure [Ca²⁺]i at body temperature by virtually eliminating the loss of dye that has thus far undermined the assessment of calibrated [Ca²⁺]i transients at 37.5°C. [Ca²⁺]i was calculated as described previously (1, 2, 6, 12). Hemorrhage was used to eliminate any possible loss of dye, and it was shown that the Kd for Ca²⁺ binding to fura 2 displays very similar in length resulted in about equal increases in resting tension and active developed tension. This length, which is slightly below the length at which active force development is maximal, was selected to be comparable to the maximally attained length in vivo (~2.2 μm sarcomere length) (22).

**Data analysis and statistics.** In all experiments, developed force (Fdev) and diastolic force (Fdia) were determined and normalized to the cross-sectional area of the muscle. Additionally, to assay the timing of force decay, the time from peak force to 50% relaxation (RT₅₀%o) was determined. Multiple analysis of variance was used to determine significant differences between the interventions, with post hoc t-test when appropriate. A two-sided P value of <0.05 was considered significant. Data are presented as means ± SE unless otherwise stated.

**RESULTS**

**Characteristics of SHHF animals in end-stage failure.** At the time of death, all 22 SHHF rats were in an advanced stage of heart failure with severe lethargic behavior, rapid shallow respirations, and edema. In addition, several of these rats had left atrial thrombi, which were not observed in any of the age-matched animals. As a more quantifiable parameter of hypertrophy and/or heart failure, we measured the heart weight-to-body weight ratio (HW/BW). SHHF rats had significantly larger hearts and lower body weights, resulting in a much higher HW/BW (5.4 ± 0.2 mg/g) compared with control animals (3.1 ± 0.1 mg/g; P < 0.001).

**Effects of temperature.** In age-matched controls, none of the contractile parameters in any of the protocols was significantly different between RV and LV. Thus, in normal animals of this age, contractile function is very similar in the RV and LV. Under baseline conditions of 1.0 mM [Ca²⁺]o and a stimulation frequency of 0.5 Hz, at room temperature the active Fdev of both SHHF RV and LV trabeculae was depressed compared with that of the age-matched controls (P < 0.05; Fig. 1). The observation of depressed RV muscle function in this study confirms that observed previously in this animal model (20). Although we do not see major differences in the time course of twitch contractions, the present data further reveal that the function of LV trabeculae is also depressed compared with age-matched controls. Next, we investigated whether this depression of contractile function is present at physiological temperature. Therefore, we slowly raised the temperature of the perfusate to 37.5°C and measured contractile function at 2.5°C intervals. SHHF RV and all control trabeculae behaved as expected (23); with increasing temperature, Fdev remained relatively un-
changed up to 30°C and thereafter declined to ~30–40% of the values obtained at 22.5°C (reversed hypothermic inotropy; Fig. 2). Surprisingly, despite the similar behavior of SHHF LV and RV trabeculae at room temperature, SHHF LV trabeculae did not decline in F_{dev} with increasing temperature, as would be expected. Active F_{dev} actually increased up to 35°C (P < 0.05 vs. 22.5°C at 30, 32.5, and 35°C) and then slightly declined. At 37.5°C, F_{dev} was not significantly different from that observed at 22.5°C, in sharp contrast to SHHF RV and control trabeculae.

**Force-frequency relationship.** At the low stimulation frequency of 0.5 Hz and body temperature, SHHF LV trabeculae displayed higher F_{dev} (43.7 ± 7.7 mN/mm²) than SHHF RV trabeculae (24.3 ± 4.6, mN/mm²; P < 0.05). However, when we increased the frequency to 4 Hz, approaching the physiological frequency of the rat, all four groups had similar forces (Fig. 3). SHHF LV trabeculae displayed a negative contractile response to increasing frequency, whereas both the SHHF RV and control trabeculae exhibited an increase in F_{dev} when paced more rapidly up to ~6–7 Hz (ranging from 5 to 10 Hz). Thus the optimum frequency was near 6–7 Hz.
in SHHF RV and control trabeculae but only 1 Hz (range 0.5–3 Hz) in SHHF LV trabeculae. At a frequency of 9–10 Hz, which is maximal for a rat in vivo (3), the difference between the SHHF LV and all other groups was most prominent; SHHF LV trabeculae decreased to 40 ± 21% of their initial force at 0.5 Hz, whereas SHHF RV trabeculae significantly increased (actually almost doubled their initial individual values). Twitch timing parameters (Fig. 4) were quite similar; the only significant difference observed between SHHF LV and SHHF RV preparations was in regard to time to peak tension, which was significantly higher in SHHF LV preparations at all frequencies, and a faster rate of relaxation in RV preparations at 0.5 and 10 Hz (vs. LV or control).

Also, mechanical alternans developed at frequencies exceeding 6 Hz in 8 of 11 SHHF LV preparations but only in 2 of 10 SHHF RV preparations and in 3 of 10 control preparations. When mechanical alternans occurred, the trabeculae were not paced at higher frequencies, to avoid irreversible damage; in pilot experiments, we observed that pacing these small preparations at frequencies 2 or 4 Hz above the frequency at which alternans developed caused a loss of \( F_{\text{dev}} \), even after the frequency was reduced back to lower levels. In cases of alternans, the frequency of stimulation was maximally increased by 2 Hz and then returned to the 4-Hz baseline.

**β-Adrenergic response.** Next, we investigated the response to isoproterenol. A reduced β-adrenergic response is a classic feature of end-stage heart failure (4). Starting from baseline conditions (37.5°C, 4 Hz, 1 mM \([\text{Ca}^{2+}]_o\)), a cumulative concentration-response curve was obtained by raising the isoproterenol concentration stepwise from \(10^{-9}\) to \(10^{-6}\) M. For this protocol, we pooled the control trabeculae (\(n = 2\) LV and \(n = 7\) RV; no difference between LV and RV). No differences in EC\(_{50}\) [SHHF LV (\(n = 6\)): 18 ± 11 nM; SHHF RV (\(n = 7\)): 24 ± 12 nM; control (\(n = 9\)): 27 ± 9 nM] were observed between the groups (calculated as the average of the individual curve fits). However, the maximal response to isoproterenol was significantly reduced in the SHHF LV specimens (Fig. 5); in SHHF LV trabeculae, \( F_{\text{dev}} \) increased only 23.8 mN/mm\(^2\) on average compared with 43.1 mN/mm\(^2\) in SHHF RV and 47.3 mN/mm\(^2\) in control trabeculae.

**Calcium transients.** In a subset of experiments (\(n = 8\); 4 SHHF RV and 4 SHHF LV), we measured intracellular calcium transients. Figure 6 shows experimental records from individual RV and LV muscles at 4 and 10 Hz; Fig. 7 shows average values for calcium transient amplitude and developed force. In RV trabeculae, at 10 Hz the amplitude of the calcium transient increased compared with 4 Hz but force remained unaltered, whereas diastolic calcium was slightly elevated (from 180 ± 50 to 250 ± 60 nM; \(P < 0.05\)). In sharp contrast, in LV trabeculae, \( F_{\text{dev}} \) decreased on an increase in stimulation frequency from 4 to 10 Hz. This was accompanied by a larger elevation of diastolic calcium transients.
calcium (from 210 ± 40 to 320 ± 60 nM; \( P < 0.05 \)) without a significant change in the amplitude of the calcium transient. Thus, at higher frequencies, more calcium appears to be required to maintain a similar amount of force in SHHF RV trabeculae; failure to increase calcium with frequency in SHHF LV preparations resulted in a loss of force. However, the rather small difference in the change of the calcium transient amplitude may not fully explain the effects on twitch force amplitude. As can be seen in Fig. 6, bottom (and in Fig. 3), \( F_{\text{dev}} \) clearly declined considerably in the LV trabeculae but not in the RV trabeculae. Thus, besides the obvious alterations in calcium handling, altered myofilament properties may potentially play an additional role in the contractile dysfunction observed in failing SHHF myocardium.

**DISCUSSION**

The goal of the present study was to conduct a comparison of contractile function of right and left myocardium from SHHF rats that develop heart failure due to long-term hypertension. We found that the conclusions depended strongly on experimental conditions. Under conditions designed to mimic those occurring in vivo, we observed severe contractile dysfunction of LV myocardial preparations. In contrast, RV preparations displayed little dysfunction and behaved very similarly to age-matched preparations from healthy rats. In contrast, at low rates of stimulation and at room temperature, both RV and LV SHHF preparations developed significantly less force than respective control preparations. The magnitude of this cardiac depression at room temperature was similar to that observed previously (20). Interestingly, at body temperature, at a stimulation frequency near the lowest achievable in a rat in vivo (4 Hz), active \( F_{\text{dev}} \) was similar in SHHF and control preparations as well as between the RV and LV. At higher, more physiological frequencies (5–9 Hz), it became apparent that LV function was severely depressed; active \( F_{\text{dev}} \) decreased considerably when frequency was increased. RV function displayed a normal positive force-frequency relationship similar to that of age-matched controls and previous studies on...
isolated rat myocardium under these physiologically relevant conditions (16, 17). Compared with controls, RV preparations from SHHF rats were not significantly depressed under near-physiological conditions. Thus, except for the depressed contractile behavior at nonphysiological temperature and stimulation frequencies, a finding consistent with Perez et al. (20), RV function was similar to that of age-matched controls.

LV dysfunction was readily apparent in this study. The characteristic positive force-frequency behavior of healthy rat myocardium (16, 17) was absent, or even negative, in SHHF LV preparations. In addition, the amplitude of the intracellular calcium transients was depressed at higher frequencies, isoproterenol response was reduced in amplitude, time to peak tension was prolonged, and normal hypothermic inotropism was absent. In addition, diastolic calcium levels were elevated at physiologically relevant frequencies by 110 nM on average but only by 70 nM on average in RV preparations. These observations are in line with prior assessments of LV function of the SHHF rat in whole heart models, which showed depressed contractions and the development of alternans at frequencies >6 Hz (19). In addition, many aspects of the SHHF LV dysfunction observed here are reminiscent of failing human myocardium. Blunted or negative force-frequency behavior (18, 21), reduced β-adrenergic response (4), and impaired calcium handling (9, 10) are hallmarks of end-stage heart failure. Unfortunately, few data are presently available regarding contractile behavior of RV myocardium from human end-stage failing hearts for comparison with the present study. It was shown, however, that a large number of small RV preparations from end-stage failing human hearts displayed a positive force-frequency behavior (reminiscent of healthy myocardium) in two recent studies (11, 14). Thus, also in human end-stage heart failure, RV contractile function may be significantly less impaired than that in LV myocardium.

**Calcium handling.** Despite the clear differences in contractile function of LV versus RV preparations, calcium handling may not necessarily be the sole culprit underlying this dysfunction. In healthy rat myocardium, an increase in frequency within the physiological range results in a parallel increase in calcium transient amplitude and maximal twitch force (16). In end-stage failing SHHF rats, despite an increase in calcium transient amplitude, RV preparations could not generate more force at 10 Hz compared with 4 Hz. The absence of an increase in calcium with increasing stimulation frequency in SHHF LV preparations resulted in a loss of contractile force. Thus changes in the calcium transient may not solely explain the depressed contractions compared with normal myocardium as described previously (16). Contractile behavior critically depends on two major factors, calcium handling and myofilament responsiveness. Thus myofilament properties are also altered in this model of heart failure. This would be in line with results obtained from tetanized SHHF trabeculae at room temperature; these experiments revealed a depressed maximal force development (20). However, a potential myofilament problem does not directly explain the difference between LV and RV preparations but could possibly affect function of preparations of both ventricles slightly differently. At a higher stimulation rate, the changing relaxation balance between intracellular calcium decline and force decline shifts the governing of relaxation to the myofilament properties (16). Because the twitch timing properties are somewhat different in RV and LV preparations, it is not impossible that an underlying myofilament dysfunction (20) may have a larger impact on LV preparations than on RV preparations, contributing to the observed differences. However, if such a myofilament component of dysfunction did contribute, this would clearly be a minor effect compared with the obvious defect in calcium handling.

The loss of hypothermia-induced inotropy in SHHF LV trabeculae may likewise be due to altered myofilament abnormalities. Compared with body temperature, at room temperature $F_{dev}$ was not higher, as it was in control and SHHF RV trabeculae. Although a more focused investigation would be needed to unambiguously determine the relative roles of calcium cycling and myofilament abnormalities, we speculate that myofilament alterations may result in an altered $Q_{10}$ for cross-bridge cycling kinetics, causing the observed loss of hypothermia-induced inotropy. Altered temperature dependence of cross-bridge cycling kinetics would also fit with the observed timing parameters; time to peak tension was slowed at body temperature but not at room temperature in SHHF LV trabeculae, whereas such changes were not observed in SHHF RV trabeculae.

**Technical limitations.** Although we have now shown the feasibility of assessing cytosolic calcium transients from iontophoresically loaded cardiac trabeculae at physiological temperature, the short period over which these can be collected unfortunately prevents an in vivo calibration that typically takes several hours. Thus, because it is impossible to obtain an in situ $K_d$, we currently have to rely on the previously assessed $K_d$ for fura-Ca$^{2+}$ obtained at room temperature to calculate free calcium from the fluorescence ratio of 340 to 380 nm. It has been reported that the $K_d$ of fura 2 displays very little temperature dependence (8, 25). However, even if a shift in the $K_d$ with temperature were to occur, this would not affect the central conclusions. Such a shift in $K_d$ would alter the exact calcium concentration calculated from the ratio equally in all groups; thus the central conclusions regarding the calcium transient observations (difference in amplitude at high stimulation rates) would not be affected.

In summary, in the SHHF rat, LV contractile dysfunction is much more prominent than in the RV. Although the $F_{dev}$ of SHHF LV preparations is comparable to that of controls and SHHF RV preparations at subphysiological frequencies, at higher, more physiological frequencies baseline contractility and β-adrenergic responsiveness are severely impaired in SHHF LV preparations. This indicates a severe reduction in
cardiac reserve of SHHF LV preparations in contrast to SHHF RV preparations.

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