Exercise training improves renal excretory responses to acute volume expansion in rats with heart failure

Hong Zheng, Yi-Fan Li, Irving H. Zucker, and Kaushik P. Patel

Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska; and Division of Basic Biomedical Science, College of Medicine, University of South Dakota, Vermillion, South Dakota

Submitted 14 December 2005; accepted in final form 23 June 2006

Zheng, Hong, Yi-Fan Li, Irving H. Zucker, and Kaushik P. Patel. Exercise training improves renal excretory responses to acute volume expansion in rats with heart failure. Am J Physiol Renal Physiol 291: F1148–F1156, 2006. First published July 5, 2006; doi:10.1152/ajprenal.00501.2005.—Experiments were performed to test the postulate that exercise training (ExT) improves the blunted renal excretory response to acute volume expansion (VE), in part, by normalizing the neural component of the volume reflex typically observed in chronic heart failure (HF). Diuretic and natriuretic responses to acute VE were examined in sedentary and ExT groups of rats with either HF or sham-operated controls. Experiments were performed in anesthetized (Inactin) rats 6 wk after coronary ligation surgery. Histological data indicated that there was a 34.9% outer and 42.5% inner infarct of the myocardium in the HF group. Sham rats had no observable damage to the myocardium. In sedentary rats with HF, VE produced a blunted diuresis (46% of sham) and natriuresis (35% of sham) compared with sham-operated control rats. However, acute VE-induced diuresis and natriuresis in ExT rats with HF were comparable to sham rats and significantly higher than sedentary HF rats. Renal denervation abolished the salutary effects of ExT on renal excretory response to acute VE in HF. Since glomerular filtration rates were not significantly different between the groups, renal hemodynamic changes may not account for the blunted renal responses in rats with HF. Additional experiments confirmed that renal sympathetic nerve activity responses to acute VE were blunted in sedentary HF rats; however, ExT normalized the renal sympathetic inhibition in HF rats. These results confirm an impairment of neurally mediated excretory responses to acute VE in rats with HF. ExT restored the blunted excretory responses as well as the renal sympathoinhibitory response to acute VE in HF rats. Thus the beneficial effects of ExT on cardiovascular regulation in HF may be partly due to improvement of the neural component of volume reflex.

sympathetic nerve activity; renal function; myocardial infarction

An impaired ability to excrete a sodium load is a hallmark of chronic congestive heart failure (HF). An acute volume expansion (with isotonic saline) produces a blunted diuresis and natriuresis in humans and various animal models of HF (3, 15, 28, 32, 37, 54). This blunted excretory response to acute volume expansion (VE) has also been reported by various investigators in the rat coronary artery ligation model of HF (3, 15, 37). Atrial stretch receptors have been shown to mediate several important reflexes that control fluid balance and heart rate (22). In rats with HF, the reflex decrease in renal sympathetic nerve activity, which occurs in response to acute VE with isotonic saline, is depressed (3, 36).

Recent evidence in patients with HF suggests that exercise training (ExT) improves the adverse cardiovascular consequences of HF, at least in patients with moderate HF (1). Thus ExT is recommended as a lifestyle therapy for patients with moderate HF (19, 27). Improvement in various cardiovascular reflexes may be, in part, responsible for the alleviation of symptomatic burden and overall prognosis (24, 31, 47). An improvement of baroreflex (24) as well as cardiopulmonary reflex function (42) has been reported in HF following ExT. The reason that ExT is beneficial in HF may relate to endothelial cell (14) and skeletal muscle function (43), cardiorespiratory conditioning (13), or improved cardiovascular reflexes (24).

ExT has been shown to enhance the input from cardiopulmonary mechanoreceptors (6, 7). We hypothesized that ExT would restore renal excretory function in rats with HF by improving the neural influences that underlie the suppressed excretory response to acute VE typically observed in HF. To test this hypothesis, experiments were performed to determine 1) whether ExT would normalize renal excretory responses to acute VE in rats with HF, 2) whether the influence of the renal nerves accounts for the effect of ExT on altered renal responses to acute VE in HF, and 3) whether ExT improves the reflex inhibition of renal sympathetic nerve activity in response to acute VE in HF. To control for changes in systemic hemodynamics and circulating hormonal factors, renal hemodynamic and electrolyte excretion responses to VE were compared in separate innervated and denervated kidneys of the same rat. This preparation is extremely powerful in detecting an effect of the renal nerves, since each kidney is exposed to the same arterial pressure and circulating hormones. Thus any difference in renal excretion between innervated and denervated kidneys in this model can be attributed to a direct or indirect impact of the renal nerves.

METHODS

Induction of Chronic HF

All procedures utilized in this study were approved by University of Nebraska Medical Center Institutional Animal Care and Use Committee, and the experiments were conducted according to the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings. Male Sprague-Dawley rats (250–280 g) were obtained from Sasco Breeding laboratories (Omaha, NE). The rats were assigned randomly to one of two groups, HF and sham. The method of producing a myocardial infarct and

Address for reprint requests and other correspondence: K. P. Patel, Dept. of Cellular and Integrative Physiology, Univ. of Nebraska Medical Center, 985850 Nebraska Medical Center, Omaha, NE 68198-5850 (e-mail: kpatel@unmc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
subsequent chronic HF was similar to that previously described (9, 15, 40). Briefly, each rat was anesthetized with pentobarbital sodium (50 mg/kg ip), and the trachea was cannulated to facilitate ventilation. A left thoracotomy was performed, and the heart was gently lifted out of the thorax. In the HF group, the left coronary artery was ligated between the pulmonary artery outflow tract and left atrium as it exits the aorta. The sham rats underwent thoracotomy and manipulation of the heart, but the coronary artery was not ligated. After these maneuvers, the heart was returned to its original position and the thorax was closed. The air within the thorax was removed, and the rats were observed to resume breathing. The trachea was sutured, the neck was closed, and the rats were allowed to recover from the anesthesia. The rats were then placed in individual cages until the day of the experiment. The rats were treated with analgesics (injection of Nubain-Stadol, 1 ml/kg sc) for 2 days. The animal quarters had a 12:12-h light-dark cycle with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow (Purina) and tap water were available ad libitum during these studies. Six weeks after surgery, experiments were performed on each of the rats.

**ExT Regimen**

The rats were ExT according to the protocols used by Musch and Terrell (30). Over a 3-wk period, the rats ran on a motor-driven treadmill. During this training period, rats were exercised 10 min/day at an initial treadmill speed of 10 m/min up a 0% grade. To ensure that a significant endurance ExT effect was produced, the treadmill grade and speed were gradually increased to 5–10% and 20–25 m/min, respectively, and the exercise duration was increased to 60 min/day (30). Sham and HF rats were subjected to the same ExT regimen (5 days/wk for a total of 3 wk). Animals demonstrating that they would run steadily on the treadmill with very little or no prompting (electrical stimulation) were included in the analysis. The sedentary control rats were handled daily and treated similarly to the ExT rats except they did not run. The acute experiments were carried out within 24 h of the last exercise session. To test the efficacy of ExT, citrate synthase activity was measured spectrophotometrically from muscle homogenate as previously described (44). At the time of death, muscle samples were taken from the soleus muscle, frozen at −70°C, and stored until processed.

**Renal Function Studies**

On the day of the experiment, rats were anesthetized with Inactin (100 mg/kg ip). Body temperature was maintained between 36 and 38°C by a heated stage. After tracheal intubation, the animals were allowed to breathe independently. The left femoral artery was cannulated with PE-50 polyethylene tubing and connected to a pressure transducer (Gould P23 ID) for the continuous recording of arterial pressure. The left femoral vein was cannulated with PE-50 tubing, and a constant infusion (20 µL/min) of a mixture of 2% inulin in isotonic saline was started after a 0.7-ml bolus of a priming dose of 4% inulin. Clearance of inulin was used as a measure of glomerular filtration rate (GFR).

**Renal denervation and ureteral cannulation.** The kidneys were exposed through an abdominal incision, and left renal denervation was performed by stripping the sheath and adventitia from the exposed left renal artery and vein. To destroy any remaining nerve fibers, the renal vessels were painted with 95% ethanol. Previously, this technique has been shown to decrease renal norepinephrine concentration to <5% of endogenous levels (33). In addition, the urine output from the denervated kidney was consistently greater than that of the contralateral intact kidney. Subsequently, both ureters were cannulated with PE-10 tubing. Surgery was completed within 75 min, and an additional 30-min stabilization period was allowed before the start of the first urine collection.

**Acute VE and urine collections.** Urine was collected in preweighed tubes from both left and right kidney (via ureteral catheters), and urine volume was measured gravimetrically. Two urine collections (10 min each) were obtained before the acute VE (10% of body wt in 40 min or 0.25% of body wt/min), performed by the administration of an intravenous infusion of isotonic saline (e.g., for a 250-g rat, infusion of 0.625 ml/min for 40 min). After two 10-min control collection periods, urine was collected at 5, 10, 15, 20, and 30 min during the period of acute VE. These collections represent 1.25, 2.5, 3.75, 5.0, and 7.5% of body weight, respectively. Sodium concentration (ion-sensitive electrode; Beckman ion analyzer, Irvine, CA) of each of the urine samples was also analyzed.

**Renal nerve recording and acute VE.** In addition to the general surgery described above, the left renal artery and abdominal aorta were exposed in the peritoneal cavity. A branch of the renal nerve, generally found between the aorta and the renal artery, was isolated and placed on bipolar platinum electrodes. The distal end of the renal nerve was cut. The electrical signal from the electrode was amplified 10,000× with a Grass amplifier (P55) with high- and low-frequency cutoffs of 1,000 and 100 Hz, respectively. The output from the Grass amplifier was directed to a computer-based data-acquisition system (MacLab) by which the raw discharge and integrated voltage of the renal nerve discharge were recorded and stored for later analysis. The nerve-electrode junction was isolated electrically from the surrounding tissue with silicone rubber (Wacker Silica Gel 604). To test the validity of the recording, a bolus dose of norepinephrine (10 µg/100 µl) was administered to briefly increase arterial pressure, which produced a reflex decrease in mean rectified renal nerve activity. Since renal nerve activity represents a multifiber activity, it was expressed and compared as a relative change within a given rat before and during VE. Surgery was completed within 75 min, and an additional 30-min stabilization period was allowed before the start of data collection.

Renal nerve activity at the start of the experiment was termed 100%. The nerve activity recorded at the end of the experiment after the rat was dead was deemed as background noise and therefore denoted as 0% nerve activity. The changes in nerve activity during the experiment were subsequently expressed as percent basal activity. Renal nerve activity was averaged over a period of 2 min in the middle of each of the corresponding urine collection periods (5–10 min each). These averaged values represent the mean values during the entire 5- or 10-min periods.

**Cardiac Function**

At the end of each acute experiment, a 2-Fr micromanometer-tipped catheter (Millar Instruments) was advanced through the right carotid artery into the left ventricle to determine left ventricular (LV) pressures. LV end-diastolic pressure (LVEDP) was determined from the LV pressure recording on a computer-based data-acquisition system (Power Lab).

**Cardiac Histology**

At the end of the experiment, the hearts were removed, weighed, and fixed in 10% buffered Formalin for histological study. The hearts were coded so that determinations of infarct size were made without knowledge of the physiological data (renal function and nerve discharge data). The hearts were sectioned and analyzed for the portion of the left ventricle that was infarcted as described previously (38, 50). The infarcted portion of the myocardium was distinct in that there was a noticeable loss of cardiac cells and a thinning of the ventricular wall (9). The average fraction of the inner and outer left ventricle that was infarcted was calculated from these measurements. Previous studies have observed that, during histological evolution of myocardial infarction, there is a marked reduction in the volume of infarcted myocardium due to thinning of the infarcted wall, whereas the surface of the infarct is minimally altered (9). Thus the point of maximum thinning of the infarcted LV wall, which provides yet another measure of the myocardial damage due to coronary artery ligation (9), was also
measured. On the basis of the histological data, rats with no measurable infarct in the chronic HF group were deleted from the study.

**Statistical Analysis**

Data were subjected to a two-way ANOVA (to examine effect of HF and ExT and the interaction of these factors) followed by a multiple range (for multiple comparisons) or Student-Newman-Keuls test. Data are presented as means ± SE. \( P < 0.05 \) was considered to indicate statistical significance (48).

**RESULTS**

**Baseline Characteristics**

There was a 34% inner infarct (%inner circumference) in the HF group of rats (Table 1). The sham group had no observable damage to the myocardium. The infarction of the left ventricle was transmural and indicated there was considerable loss of cardiac muscle mass in the HF rats. The minimum ventricular thickness (maximum thinning) was significantly smaller in HF compared with the sham group. Heart weight was significantly greater in HF rats than in shams, suggesting compensatory hypertrophy of noninfarcted regions of the myocardium. This was also true when “heart weight-to-body weight” ratios were compared. Nevertheless, LVEDP was elevated significantly in HF rats compared with sham rats. These results in both groups are consistent with reports in the literature (10, 40) as well as with our previous studies (35, 37). Thus both histological and functional data disclose the presence of myocardial damage and decreased contractile function in the HF rats. The mean LVEDP values were not significantly different between sedentary and exercised HF rats, indicating that ExT per se did not improve cardiac function in HF rats. There were no statistically significant differences in basal mean arterial blood pressure and heart rate among the four groups.

**Citrate Synthase Activity**

In this experiment, ExT results in a significant increase (63%) in citrate synthase activity in the soleus muscle, consistent with previous reports (20, 30). The level of citrate synthase activity in the soleus muscle was comparable in the two ExT groups, indicative of similar levels of ExT, which are consistent with reports in the literature as well as with our previous studies (53).

| Table 1. Characteristics of sham-operated and heart failure rats |
|-------------------|-----------------|-----------------|-----------------|
|                   | Sham-Sedentary  | HF-Sedentary    | Sham-ExT        | HF-ExT          |
|                   | \( n = 10 \)    | \( n = 10 \)    | \( n = 8 \)     | \( n = 10 \)    |
| Body weight, g    | 380 ± 8         | 417 ± 16        | 360 ± 6         | 356 ± 8†        |
| Heart weight, g    | 1.17 ± 0.01     | 1.47 ± 0.09*    | 1.20 ± 0.06     | 1.60 ± 0.05*    |
| Lang wet weight, g | 2.16 ± 0.39     | 2.84 ± 0.27*    | 2.09 ± 0.11     | 2.95 ± 0.44*    |
| Basal mean blood pressure, mmHg | 106 ± 5 | 101 ± 2 | 98 ± 4 | 103 ± 6 |
| Basal heart rate, beats/min | 378 ± 21 | 388 ± 15 | 345 ± 40 | 351 ± 20 |
| Infracit size, % of epicardial LV | 0 | 34.1 ± 3.4* | 0 | 34.0 ± 4.9* |
| Minimum ventricular thickness, mm | 2.8 ± 0.2 | 0.7 ± 0.1* | 2.0 ± 0.2 | 0.5 ± 0.1* |
| LVEDP, mmHg       | 1.2 ± 1.0       | 16.8 ± 6.4*     | 1.0 ± 0.40      | 14.5 ± 4.9*     |
| Plasma protein, g/dl | 3.64 ± 0.12    | 2.59 ± 0.11*    | 3.93 ± 0.19     | 3.84 ± 0.09†    |
| Citrate synthase, \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \) | 12.4 ± 1.3     | 9.8 ± 1.5       | 19.3 ± 1.5†     | 16.7 ± 1.9†     |

Values are means ± SE. HF, heart failure; ExT, exercise training; LV, left ventricle; LVEDP, left ventricular end-diastolic pressure. \(* P < 0.05 \) vs. sham-operated rats. \( \dagger P < 0.05 \) vs. sedentary rats.

**Responses from Intact Kidneys**

**Basal hemodynamic and renal characteristics.** During VE, there was no significant difference in mean arterial pressure in both sedentary and ExT groups (Fig. 1). There was no significant difference between the groups either. There were no significant differences in kidney weights (right intact kidneys) between the groups of rats (Table 2). Urine flow from intact kidneys (right kidneys) before acute VE from sedentary rats and ExT rats was not significantly different between the two groups. Similarly, sodium excretion from the sedentary rats and ExT rats was not significantly different between these two groups, nor was fractional excretion of sodium, before acute VE.

**Diuretic and natriuretic responses to acute VE.** Acute VE produced diuresis and natriuresis from the intact kidneys in both groups of rats (Figs. 2 and 3). Both the diuresis and the natriuresis were significantly blunted in the sedentary HF group compared with the corresponding sedentary sham rats during acute VE. During VE, there was a significantly greater urine flow and sodium excretion in the HF-ExT compared with the HF-sedentary group \( (P < 0.05; \text{Figs. 2B and 3B}) \). Although the diuresis and natriuresis were greater from kidneys of sham-ExT and HF-ExT groups, ExT abolished the difference in diuresis and natriuresis between the sham and HF rats. In other words, ExT improved the renal excretory responses to acute VE in rats with HF. Although urine flow and sodium excretion in ExT rats were greater than in sedentary rats (ANOVA showed an effect of ExT), there were no significant differences between sham-sedentary and sham-ExT groups \( (P < 0.08; \text{Figs. 2A and 3A}) \).

GFR data from this experiment are shown in Table 2. GFR did not differ significantly between sham and HF rats regardless of whether they were sedentary or ExT. In both groups of rats, VE significantly elevated GFR. However, the effect of VE on this parameter did not differ between sham and HF groups, either in sedentary or ExT groups.

**Responses from Denervated Kidneys**

**Basal renal characteristics.** There were no significant differences in kidney weights (left denervated kidneys) among the four groups of rats (Table 2). Urine flow before acute VE from sedentary rats and ExT rats was not significantly different between the two groups. Similarly, sodium excretion from the
sedentary rats and ExT was not significantly different between the two groups before acute VE.

Diuretic and natriuretic responses to acute VE. Acute VE produced an increase in diuresis and natriuresis from the denervated kidneys in both groups of rats (Figs. 4 and 5). Both diuresis and natriuresis were not significantly different in the sedentary sham rats during acute VE. Again, although the diuresis and natriuresis were greater from kidneys of sham-ExT and HF-ExT groups, ExT did not alter the lack of difference in diuresis and natriuresis between the sham and HF rats (Figs. 4 and 5). In other words, ExT did not alter the renal excretory responses to acute VE from denervated kidneys of rats with HF.

Similar to intact kidneys, GFR did not differ significantly from denervated kidneys between sham and HF rats regardless of whether they were sedentary or ExT. Furthermore, the effect of VE on GFR from denervated kidneys did not differ between sham and HF groups, either in sedentary or ExT groups.

Renal Sympathoinhibition in Response to Acute VE

Renal sympathoinhibition in response to acute VE in sham-operated and HF rats is shown in Fig. 6. The renal sympathoinhibition was significantly blunted in the HF group compared with the sedentary sham group. After ExT, there was a significant difference in the renal sympathoinhibition to acute VE between the HF-ExT and HF sedentary group (Fig. 6B). However, there was no significant difference in the renal sympathoinhibition to acute VE between the HF-ExT and sham groups (Fig. 6). This indicates that ExT improved renal sympathoinhibition in the HF group. There was no significant effect of time per se on renal nerve activity during 30 min, the time required for acute VE. ExT had a significant effect on renal sympathoinhibition in the sham group as well (ANOVA showed an effect of ExT; Fig. 6).

DISCUSSION

This study demonstrated that acute VE (with isotonic saline) produced a blunted natriuresis and diuresis in a rat model of HF.

Table 2. Basal renal characteristics of sham-operated and heart failure rats

<table>
<thead>
<tr>
<th></th>
<th>Sham-Sedentary (n = 10)</th>
<th>HF-Sedentary (n = 10)</th>
<th>Sham-ExT (n = 8)</th>
<th>HF-ExT (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact kidneys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.44±0.07</td>
<td>1.55±0.11</td>
<td>1.32±0.05</td>
<td>1.36±0.02</td>
</tr>
<tr>
<td>Urine flow, µl·min⁻¹·gkw⁻¹</td>
<td>4.13±2.38</td>
<td>2.61±1.45</td>
<td>8.60±2.56</td>
<td>13.6±8.7†</td>
</tr>
<tr>
<td>Sodium excretion, µeq·min⁻¹·gkw⁻¹</td>
<td>0.51±0.34</td>
<td>0.20±0.05</td>
<td>1.10±0.37</td>
<td>1.29±0.80†</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>0.73±0.27</td>
<td>0.63±0.15</td>
<td>0.71±0.10</td>
<td>0.64±0.25</td>
</tr>
<tr>
<td><strong>Denervated kidneys</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.43±0.05</td>
<td>1.51±0.13</td>
<td>1.34±0.05</td>
<td>1.37±0.02</td>
</tr>
<tr>
<td>Urine flow, µl·min⁻¹·gkw⁻¹</td>
<td>12.00±5.10</td>
<td>8.50±4.45</td>
<td>18.20±5.56</td>
<td>19.25±7.13</td>
</tr>
<tr>
<td>Sodium excretion, µeq·min⁻¹·gkw⁻¹</td>
<td>1.05±0.79</td>
<td>1.20±0.86</td>
<td>4.10±1.29</td>
<td>5.29±1.89</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g⁻¹</td>
<td>1.09±0.23</td>
<td>0.75±0.16</td>
<td>1.29±0.28</td>
<td>0.99±0.24</td>
</tr>
</tbody>
</table>

Values are means ± SE. GFR, glomerular filtration rate; gkw, gram kidney weight. †P < 0.05 vs. sedentary rats.

Fig. 1. Mean arterial pressure (mmHg) during acute volume expansion (VE; % of body wt) with isotonic saline. A: sham-sedentary rats and sham-ExT rats. B: HF-sedentary and HF-ExT rats. BW, body wt; HF, heart failure; ExT, exercise training. Values represent means for each parameter ± SE (n = 6–7). There were no differences between groups.
HF, confirming previous reports by us and other investigators (3, 15, 37, 45). ExT restored the blunted renal responses to an acute volume load in rats with HF. This abrogated response was abolished in renal denervated kidneys. Additionally, this study demonstrated that ExT restored the blunted renal sympathoinhibition to acute VE in rats with HF. These results indicate that ExT improves blunted renal excretory responses to acute VE by improving renal sympathoinhibition in rats with HF.

The HF condition was characterized by an increased cardiac filling pressure, reduced LV contractility, and a large myocardial infarct over the left ventricle. These rats also demonstrated cardiac hypertrophy and an increased wet lung weight, indicative of pulmonary edema. ExT did not improve any of these parameters of cardiac dysfunction. However, there was significant improvement in the body weights of ExT HF rats vs. sedentary HF rats, indicative of possible improvement in peripheral edema and ascites. This was consistent with the observation of improvement in plasma protein concentrations in ExT HF rats compared with sedentary HF rats. These data suggest that ExT improved peripheral circulatory function in the chronic congestive phase of HF but not the cardiac dysfunction per se. However, it is recognized that LVEDP may not be the most precise and accurate measure of cardiac function.

HF, confirming previous reports by us and other investigators (3, 15, 37, 45). ExT restored the blunted renal responses to an acute volume load in rats with HF. This abrogated response was abolished in renal denervated kidneys. Additionally, this study demonstrated that ExT restored the blunted renal sympathoinhibition to acute VE in rats with HF. These results indicate that ExT improves blunted renal excretory responses to acute VE by improving renal sympathoinhibition in rats with HF.

The HF condition was characterized by an increased cardiac filling pressure, reduced LV contractility, and a large myocardial infarct over the left ventricle. These rats also demonstrated cardiac hypertrophy and an increased wet lung weight, indicative of pulmonary edema. ExT did not improve any of these parameters of cardiac dysfunction. However, there was significant improvement in the body weights of ExT HF rats vs. sedentary HF rats, indicative of possible improvement in peripheral edema and ascites. This was consistent with the observation of improvement in plasma protein concentrations in ExT HF rats compared with sedentary HF rats. These data suggest that ExT improved peripheral circulatory function in the chronic congestive phase of HF but not the cardiac dysfunction per se. However, it is recognized that LVEDP may not be the most precise and accurate measure of cardiac function.

HF, confirming previous reports by us and other investigators (3, 15, 37, 45). ExT restored the blunted renal responses to an acute volume load in rats with HF. This abrogated response was abolished in renal denervated kidneys. Additionally, this study demonstrated that ExT restored the blunted renal sympathoinhibition to acute VE in rats with HF. These results indicate that ExT improves blunted renal excretory responses to acute VE by improving renal sympathoinhibition in rats with HF.

The HF condition was characterized by an increased cardiac filling pressure, reduced LV contractility, and a large myocardial infarct over the left ventricle. These rats also demonstrated cardiac hypertrophy and an increased wet lung weight, indicative of pulmonary edema. ExT did not improve any of these parameters of cardiac dysfunction. However, there was significant improvement in the body weights of ExT HF rats vs. sedentary HF rats, indicative of possible improvement in peripheral edema and ascites. This was consistent with the observation of improvement in plasma protein concentrations in ExT HF rats compared with sedentary HF rats. These data suggest that ExT improved peripheral circulatory function in the chronic congestive phase of HF but not the cardiac dysfunction per se. However, it is recognized that LVEDP may not be the most precise and accurate measure of cardiac function.

HF, confirming previous reports by us and other investigators (3, 15, 37, 45). ExT restored the blunted renal responses to an acute volume load in rats with HF. This abrogated response was abolished in renal denervated kidneys. Additionally, this study demonstrated that ExT restored the blunted renal sympathoinhibition to acute VE in rats with HF. These results indicate that ExT improves blunted renal excretory responses to acute VE by improving renal sympathoinhibition in rats with HF.

The HF condition was characterized by an increased cardiac filling pressure, reduced LV contractility, and a large myocardial infarct over the left ventricle. These rats also demonstrated cardiac hypertrophy and an increased wet lung weight, indicative of pulmonary edema. ExT did not improve any of these parameters of cardiac dysfunction. However, there was significant improvement in the body weights of ExT HF rats vs. sedentary HF rats, indicative of possible improvement in peripheral edema and ascites. This was consistent with the observation of improvement in plasma protein concentrations in ExT HF rats compared with sedentary HF rats. These data suggest that ExT improved peripheral circulatory function in the chronic congestive phase of HF but not the cardiac dysfunction per se. However, it is recognized that LVEDP may not be the most precise and accurate measure of cardiac function.

HF, confirming previous reports by us and other investigators (3, 15, 37, 45). ExT restored the blunted renal responses to an acute volume load in rats with HF. This abrogated response was abolished in renal denervated kidneys. Additionally, this study demonstrated that ExT restored the blunted renal sympathoinhibition to acute VE in rats with HF. These results indicate that ExT improves blunted renal excretory responses to acute VE by improving renal sympathoinhibition in rats with HF.

The HF condition was characterized by an increased cardiac filling pressure, reduced LV contractility, and a large myocardial infarct over the left ventricle. These rats also demonstrated cardiac hypertrophy and an increased wet lung weight, indicative of pulmonary edema. ExT did not improve any of these parameters of cardiac dysfunction. However, there was significant improvement in the body weights of ExT HF rats vs. sedentary HF rats, indicative of possible improvement in peripheral edema and ascites. This was consistent with the observation of improvement in plasma protein concentrations in ExT HF rats compared with sedentary HF rats. These data suggest that ExT improved peripheral circulatory function in the chronic congestive phase of HF but not the cardiac dysfunction per se. However, it is recognized that LVEDP may not be the most precise and accurate measure of cardiac function.
Other parameters of cardiac function such as change in pressure over time (dP/dt) or echo-based measures may be more discriminating. Therefore, it is possible that ExT enhances peripheral vascular function (29) with concomitant subtle improvement of cardiac function leading to overall improvement of volume regulation in HF.

It is of interest to note that renal sympathetic nerve activity has been reported to be elevated in rats with HF produced by coronary occlusion in the conscious state (3, 5, 40) and that renal denervation prevents the blunted natriuresis and diuresis in response to acute VE (3, 40). This was confirmed in the present study. Furthermore, this study shows that ExT normalizes the blunted diuretic and natriuretic responses in HF rats. This effect of ExT is absent in renal denervated HF rats. These data could be interpreted to mean that intact renal nerves/renal nerve response contributes to the improvement in the blunted volume reflex observed in the rats with HF. Consistent with this hypothesis, measurement of renal sympathetic nerve activity demonstrated that acute VE produced a reduced renal sympathoinhibition in the HF group compared with sham-treated rats (3, 37). ExT improved the renal sympathoinhibition in HF rats, comparable to sham rats. This observation, combined with the observation that renal denervation normalized the renal responses (as compared with normal rats with intact renal nerves) to VE, suggests that renal nerves are involved in the blunted renal excretory responses to VE in rats with HF.

**Fig. 4.** Urine flow (μl·min⁻¹·g/kW) in response to acute VE (% of body wt) with isotonic saline from denervated kidneys. A: sham-sedentary rats and sham-ExT rats. B: HF-sedentary and HF-ExT rats. Values represent means for each parameter ± SE (n = 6–7).

**Fig. 5.** Sodium excretion (μEq·min⁻¹·g/kW) in response to acute VE (% of body wt) with isotonic saline from denervated kidneys. A: sham-sedentary rats and sham-ExT rats. B: HF-sedentary and HF-ExT rats. Values represent means for each parameter ± SE (n = 6–7).
myocardial infarction. The effect of ExT on sympathetic nerve activity may reflect a generalized normalization of all known cardiovascular reflexes. Aside from the restoration of the volume reflex as shown here, others have previously demonstrated that baroreflex (24), chemoreflex, and the Bezold-Jarisch reflexes (42, 52) are also improved in experimentally induced HF following ExT. This might imply that other mechanisms, such as increased sensitivity of the arterial chemoreceptor and/or activation of reflexes by the abnormal skeletal muscle, stimulate the sympathetic activation in HF and that ExT appears to induce its beneficial effects by reducing activation of these sensory afferents (26, 41). Conversely, ExT would be involved in enhancing the baroreflex and volume-mediated mechanoreceptors to reduce overall sympathetic outflow in HF. The role of ExT on sensory input and the integration among these various inputs during HF remain to be elucidated.

The mechanisms by which ExT alters reflex renal sympathoinhibition and diuresis and natriuresis in response to acute volume are not completely understood. We have previously shown that acute VE produces an increase in nitric oxide (NO) in the microdiasylate from the paraventricular nucleus (PVN) (21). Furthermore, inhibition of nitric oxide synthase (NOS) within the PVN causes a blunting of the renal sympathoinhibitory as well as renal excretory responses to acute VE similar to that observed in HF rats (21). These data suggest that neuronal NOS (nNOS) within the PVN is crucial in the normal function of the volume reflex in normal rats. Interestingly, we have previously observed that there is decreased nNOS within the PVN of rats with HF (51). Thus it is conceivable that improving central nNOS within the PVN of rats with HF may be partially responsible for the improvement and restoration of renal sympathoinhibition and subsequent diuresis and natriuresis. Consistent with these observations, we have recently shown that ExT in rats with HF improves the decreased levels of nNOS message and protein. Additionally, the functional responses to either blocking NOS with 1-NMMA or administering sodium nitroprusside into the PVN are also restored in ExT HF rats (53). Consistent with these observations, an increase in nNOS in the PVN of rabbits with pacing-induced HF was also demonstrated (56), suggesting that ExT increases the level of nNOS within the PVN of different animal models of HF. Additionally, we have previously shown that ExT increases the number of NOS-stained cells within the PVN of hypertensive rats (8). Thus these studies could be interpreted to show that the improvement in the nNOS mechanism within the PVN of rats with HF after ExT may contribute to the improvement in renal sympathoinhibition induced by acute VE, thus improving the renal excretory responses.

An alternate explanation may relate to plasma levels of angiotensin II (16, 25). The levels of angiotensin II are increased in the HF condition (49, 55), and angiotensin II is known to exaggerate sympathetic outflow and blunt various cardiovascular reflexes (4, 12). ExT is known to reduce plasma levels of angiotensin II in HF (23). It is possible that the reduction of plasma levels of angiotensin II may contribute to the improvement of the renal nerve responses to acute VE in ExT HF rats. This possibility remains to be examined.

It is also possible that interactions between neural and humoral mechanisms evoked by VE are altered in HF. Among the humoral substances that may be involved is atrial natriuretic factor (ANF), which is released during an acute VE. Excretory responses to ANF are impaired in rats with HF (17, 37, 39). Since ANF has been shown to have different effects in the presence and absence of renal nerves (18, 34), it is conceivable that the renal responses to ANF would be different in rats with HF. It has been reported that renal denervation reversed the blunted renal excretory responses to ANF in rats.
with HF (39). Other data demonstrate that renal excretory responses to ANF are blunted in rats with HF regardless of the presence of renal nerves (2, 11, 37). These data are consistent with the reported downregulation of ANF receptors in the renal inner medulla of rats with HF (46). Regardless of the effects of ANF, it may be that ExT may reduce the levels of ANF in HF and thus improve this component of the volume reflex. The improvement in the index of blood volume (plasma protein concentration) due to ExT in HF rats may be related to improving the ANF component of the volume reflex. This improvement would also account for the improvement in the volume reflex in rats with HF. This possibility remains to be examined.

Finally, apart from the neural and hormonal changes that may account for the improvement in excretory responses to acute VE in ExT HF rats, intrarenal factors such as medullary blood flow and renal interstitial pressure may also contribute. However, this is unlikely, since there was no difference in the excretory responses between the denervated kidneys from HF and sham rats. Furthermore, there was a difference in intact kidneys that were exposed to the same hormonal milieu as the denervated kidneys. That is, in the absence of renal nerves, the beneficial effects of ExT on the excretory responses were abolished. This would suggest that intact renal innervation is essential for the beneficial effects of ExT in HF rats.

In summary, the present study demonstrated that 1) ExT normalized the blunted excretory response to acute VE in rats with HF and 2) ExT improved renal sympathoinhibition to acute VE in rats with HF, which was responsible for the improved renal excretory responses to acute VE in rats with HF. In the HF state, conditioning by ExT is proposed to be one mechanism to beneficially affect fluid retention/balance as well as the subsequent progression of cardiovascular complications.

ACKNOWLEDGMENTS

We thank Dr. Kurtis G. Cornish and Jill Skaroupa for invaluable assistance in these experiments.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-62222.

REFERENCES


