Exercise training improves endogenous nitric oxide mechanisms within the paraventricular nucleus in rats with heart failure

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Zheng, Hong, Yi-Fan Li, Kurt G. Cornish, Irving H. Zucker, and Kaushik P. Patel. Exercise training improves endogenous nitric oxide mechanisms within the paraventricular nucleus in rats with heart failure. Am J Physiol Heart Circ Physiol 288: H2332–H2341, 2005. First published January 14, 2005; doi:10.1152/ajpheart.00473.2004.—Previously, we have demonstrated that an altered endogenous nitric oxide (NO) mechanism within the paraventricular nucleus (PVN) contributes to increased renal sympathetic nerve activity (RSNA) in heart failure (HF) rats. The goal of this study was to examine the effect of exercise training (ExT) in improving the endogenous NO mechanism within the PVN involved in the regulation of RSNA in rats with HF. ExT significantly restored the decreased number of neuronal NO synthase (nNOS)-positive neurons in the PVN (129 ± 17 vs. 99 ± 6), nNOS mRNA expression and protein levels in the PVN were also significantly increased in HF-ExT rats compared with HF-sedentary rats. To examine the functional role of NO within the PVN, an inhibitor of NOS, Nω-monomethyl-arginine, was microinjected into the PVN. Dose-dependent increases in RSNA, arterial blood pressure (BP), and heart rate (HR) were produced in all rats. There was a blunted increase in these parameters in HF rats compared with the sham-operated rats. ExT significantly augmented RSNA responses in rats with HF (33% vs. 20% at the highest dose), thus normalizing the responses. The NO donor sodium nitroprusside, microinjected into the PVN, produced dose-dependent decreases in RSNA, BP, and HR in both sham and HF rats. ExT significantly improved the blunted decrease in RSNA in HF rats (36% vs. 17% at the highest dose). In conclusion, our data indicate that ExT improves the altered NO mechanism within the PVN and restores NO-mediated changes in RSNA in rats with HF.

nervous system (51). Functional studies have also revealed that the PVN is involved in cardiovascular regulation (25). In recent years, we have begun to examine the involvement of central cardiovascular processing mechanisms in the elevated neurohumoral drive in HF (57, 59). The PVN, known to dictate sympathetic outflow, demonstrates an increased neuronal activity in HF (47). The alterations in the neuronal activity of the PVN may be responsible in part for alterations in overall sympathetic nerve activity in HF.

The PVN is a nucleus on which a number of different neurotransmitters converge to influence its neuronal activity (20, 54, 55). Some studies have shown that administration of a nitric oxide (NO) donor into the PVN elicits a decrease in renal sympathetic nerve activity (RSNA) and administration of an inhibitor of NO synthase (NOS) within the PVN increases RSNA (20). This indicates that endogenous NO within the PVN is inhibitory to sympathetic outflow.

The HF condition is known to produce attenuated vasodilation in response to agonists known to operate via a NO mechanism (15). The levels of endogenous endothelial NOS (eNOS) protein and mRNA in peripheral tissue are reduced in the HF state (52). We have recently shown that the message for neuronal NOS (nNOS) in the hypothalamus and NOS-positive neurons (NADPH-positive neurons) are decreased in the PVN of rats with HF compared with sham-operated rats (59). We also found that NO-mediated inhibition of RSNA within the PVN was blunted in rats with HF (56). This set of observations suggests that an altered NO mechanism may contribute to the increased sympathetic nerve activity in the HF state.

Patients with HF benefit from a consistent exercise regimen (1, 6). It has been shown that long-term regular exercise training (ExT) of patients with HF increases the quality of life as well as survival (5). Both chronic and acute exercise has been shown to have an effect on resting arterial pressure and cardiovascular regulation (3). The mechanisms by which ExT improves the status of HF patients and animals are not known. Although ExT has been shown to enhance endothelial function in the patients and animals with HF (21), there are little data on the role of ExT in the modulation of neurohumoral drive. ExT has been shown to attenuate muscle sympathetic nerve activity in patients with dilated cardiomyopathy (50). In patients with ischemic heart disease, ExT produces a decrease in plasma concentrations of norepinephrine, an index of neurohumoral drive (50). Recently, the data have demonstrated that daily exercise increases the number of NOS-positive neurons within the PVN of hypertensive rats (12). We hypothesize that ExT may alter sympathetic outflow in HF via a NO-dependent pathway.
To determine the effects of ExT on altered NO mechanisms within the PVN in rats with HF, this study was designed to examine whether ExT would normalize 1) nNOS-positive cells and nNOS mRNA and nNOS protein levels within the PVN of HF rats compared with sham-operated control rats and 2) both endogenous and exogenous NO-mediated changes in RSNA in rats with HF compared with sham-operated control rats.

METHODS

Induction of HF

All the procedures on animals in this study were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee. The experiments were conducted according to the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings” and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Male Sprague-Dawley rats weighing 180–200 g were obtained from Sasco Breeding Laboratories (Omaha, NE) and were randomly assigned to a sham-operated group and a HF group. HF was produced by coronary artery ligation, as previously described (22, 49). Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip). The trachea was intubated, and the rat was placed on a ventilator. A left thoracotomy was performed, and the left coronary artery was ligated. Sham-operated rats underwent thoracotomy and manipulation of the heart, but the coronary artery was not ligated. Analgesics (Nubain-Stadol, 1 ml/kg sc) were administered on each of the first 2 days after the surgery. Each rat was caged individually in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow (Harlan; Madison, WI) and tap water were available ad libitum.

ExT Protocol

ExT was conducted 4 wk after ligation of the coronary artery or sham surgery in the rats. The rats were exercised according to the protocols used by Musch and Terrell (41). For a 3-wk period, the rats run 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 to a 0% grade. To ensure that a significant endurance ExT effect was produced, the treadmill grade and speed were gradually increased to 10% and 20–25 m/min, respectively, and the exercise duration was increased to 60 min/day. Sham and HF rats had the same total work load (5 days/wk for a total of 3 wk). In the study, about 10% of rats did not run adequately on the treadmill without electric shock and were thus eliminated from the analysis. The number of rats eliminated was similar between sham and HF groups. Electric shock was seldom used to aid running performance (just in the last minutes of the running, less than twice every 5 min). The sham-operated control rats were handled daily and treated similarly to the ExT rats except for the running. The time after the last exercise session to conduct the acute experiments was 20–24 h.

Skeletal Muscle Oxidative Enzyme Activity

To test the efficacy of ExT, at the time of death, muscle samples were taken from the soleus muscle, frozen at −70°C, and stored until processed. Citrate synthase activity was measured spectrophotometrically from whole muscle homogenate as previously described (53).

Experimental Series 1: NADPH-Diaphorase Activity as a Marker of nNOS Activity

After ExT, the rats from all four groups (sham-sedentary, n = 9; sham-ExT, n = 7; HF-sedentary, n = 8; and HF-ExT, n = 8) were anesthetized with pentobarbital (65 mg/kg) and perfused through the left ventricle of the heart with 100–150 ml of heparinized saline followed by 200–250 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer. The brain was removed and postfixed at 4°C for 4 h in 4% paraformaldehyde solution and then placed in 20% sucrose for 24 h. The brain was blocked in the coronal plane, and sections of 30 μm in thickness were cut with a cryostat. Every third section was kept from the anterior commissure (0.4 mm posterior to bregma) posterior to where the optic tracts were observed to be in their most lateral position on the ventral surface of the brain (2.6 mm posterior to bregma). The sections were collected in 0.1 M phosphate containing 0.3% Triton X-100, 0.1 mg/ml nitroblue tetrazolium, and 1.0 mg/ml β-NADPH. The sections in nitroblue tetrazolium solution were then placed in an oven at 37°C for 1 h. After the reaction, the sections were rinsed in phosphate buffer and mounted onto slides.

The presence of NADPH-diaphorase in the PVN, supraoptic nucleus (SON), lateral hypothalamus (LH), and median preoptic area (MnPO) were examined under a microscope. The density of the staining was evaluated by counting the number of cells that were positively stained for NADPH-diaphorase. The number of NADPH-diaphorase-stained cells was counted for each individual nucleus at the same coronal level. Three adjacent sections were considered to represent one coronal level because the numbers of cells counted were within 5% of each other. The number of cells in the middle section was taken to represent the number of cells within a given nucleus.

Experimental Series 2: Semiquantitative RT-PCR Measurement for nNOS mRNA and Western Blot Analysis for nNOS Protein

RT-PCR for measurement of mRNA expression of nNOS. Four groups of rats (sham-sedentary, n = 6; sham-ExT, n = 6; HF-sedentary, n = 6; and HF-ExT, n = 6) were over anesthetized with pentobarbital and killed. The brain was removed, frozen on dry ice, and then stored at −70°C. A thick coronal section (600 μm) was cut through the hypothalamus at the level of the PVN, and the PVN area was punched out with a 15-gauge needle stub using the Palkovits technique (43). The punched tissue was put in 0.5 ml of Tri-Reagent and homogenized. Total RNA and proteins in the homogenate were extracted according to the Tri-Reagent manufacturer’s instructions.

Semiquantitative RT-PCR assays were performed to assess relative mRNA levels. RNA was isolated followed by RT for 40 min at 37°C in the presence of 1.5 μM random hexamers and 400 units of Moloney murine leukemia virus reverse transcriptase. Each 1 μl aliquot of the RT product was used for nNOS cDNA amplification. The following PCR primers were used: nNOS, 5'-GGGAGGCAGAGGCGGCCT-TAT-3' (sense); 5'-TTTGGTGAGGAAGGAGGG-3' (antisense); and β-actin, 5'-GGAATCTGTGGGTACACT-3' (sense); 5'-CG-GATGTCAACGTCACATT-3' (antisense). The PCR mixture contained 0.7 μM primers, dNTP, and BSA and 1 unit of Taq DNA polymerase. β-Actin was coamplified with each receptor cDNA as an internal control. After 10 min of denaturing at 94°C, the amplification was performed at 94°C for 1 min, at 56°C for 1 min, and at 72°C for 1 min or 34 cycles with the final extension at 72°C for 10 min. PCR products (7 μl) were fractionated in a 1% agarose gel and visualized by ethidium bromide staining and ultraviolet transillumination. The visualized DNA bands were captured by a Kodak digital camera and loaded into the computer. The intensities of the bands were analyzed with Kodak 1D image analysis software.

Western blot analysis of nNOS protein. The protein extraction was used for Western blot analysis of nNOS in samples obtained above. The protein concentration was measured using a protein assay kit (BCA kit, Pierce). The protein sample was mixed with an equal volume of 2× 4% SDS sample buffer. The sample was boiled for 5 min and then loaded onto the 7.5% SDS-PAGE gel for electrophoresis at 40 mA/each gel for 50 min. The fractionated proteins on the gel were electrophoretically transferred onto the polyvinylidene difluoride membrane at 300 mA for 90 min. The membrane was incubated

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with 5% milk-Tris-buffered saline-Tween 20 solution for 30 min at room temperature. The membrane was incubated with primary antibody (rabbit anti-rat nNOS polyclonal antibody. Santa Cruz Biotechnology, 1:1,000) at 4°C overnight. After being washed three times, the membrane was incubated with secondary antibody (goat anti-rabbit IgG, peroxidase conjugated, Pierce, 1:5,000) for 30 min at room temperature. After being washed three times, the membrane was treated with enhanced chemiluminescence reagent for 5 min and detected by exposing a film. The visualized bands on the developed film were captured with a Kodak digital camera and loaded into the computer. The intensities of the bands were analyzed with Kodak 1D image analysis software.

Experimental Series 3: Recording of RSNA, Blood Pressure, Heart Rate, Left Ventricular End-Diastolic Pressure, and dP/dt

Four groups of rats were used (sham-sedentary, sham-ExT, HF-sedentary, and HF-ExT). On the day of the experiment, the rat was anesthetized with urethane (0.75 g/kg ip) and α-chloralose (70 mg/kg ip). Additional anesthesia was administered as needed (0.25 g/kg urethane and 20 mg/kg ip α-chloralose). This supplemental anesthesia was not different between the groups. The animal was ventilated to breath room air. The body temperature was monitored during the experiment period and kept 37°C using a heating pad. The left femoral vein was cannulated for drug administration if necessary, and the left femoral artery was cannulated and connected to MacLab via a pressure transducer for recording of arterial blood pressure (BP) and heart rate (HR). During the experiments, we infused isotonic saline to maintain hydration (2-3 ml/h) and stabilize arterial BP.

The left kidney was exposed through a left retroperitoneal flank incision. A branch of the renal nerve was isolated. The nerve was placed on a bipolar steel electrode. The nerve-electrode junction was fixed and insulated electrically from the surrounding tissues with a mixture of Wacker SilGel 604 and 601. The electrical signal from the electrode was amplified 10,000 times with a Grass amplifier with high- and low-frequency cutoffs of 1,000 and 100 Hz. The output from the Grass amplifier was directed to the MacLab, and the raw discharge and integrated voltage of the renal nerve discharge were recorded and stored for later analysis. Effferent RSNA at the beginning of the experiment was defined as basal nerve discharge. The nerve discharge recorded at the end of the experiment after the rat was injected with hexamethonium (intravenous) or dead was deemed background noise. The value of nerve discharge was calculated by subtracting the background noise from the actual recording value. The response of renal nerve discharge to the administration of drugs into the PVN during the experiment was subsequently expressed as the percent change from the basal value.

At the end of each acute experiment, the left ventricle was cannulated via the carotid artery and connected to MacLab via a pressure transducer for recording of left ventricular end-diastolic pressure (LVEDP) and dP/dt.

Microinjection Into the PVN

The rat was placed in a stereotaxic apparatus. A burr hole was placed in the skull. The coordinates for the right PVN were determined from the Paxinos and Watson rat atlas (48), which is 1.5 mm posterior, 0.5 mm lateral to the bregma, and 8.0 mm ventral to the dura. A cannula connected to a microsyringe (0.5 μl) was advanced into the right side PVN with a manipulator. The volumes of injection were 50, 100, and 200 nl. At the end of the experiments, monoaqueous blue dye was injected into the brain for histological verification of the injection. After the rat was killed, the brain was removed and fixed in 4% formaldehyde for at least 24 h. The brain was then frozen, and serial transverse sections (30 μm) were cut using a cryostat at −18°C. The sections were thaw mounted on microscope slides and stained with 1% aqueous neutral red staining procedures. The presence of the blue dye within the PVN was verified under a microscope with ×40 magnification.

Experiment protocol 1. RSNA, BP, and HR were measured in sham-sedentary (n = 10), HF-sedentary (n = 10), sham-ExT (n = 8), and HF-ExT (n = 10) rats. An inhibitor of NOS, Nω-monomethyl-L-arginine (L-NMMA), was injected into the PVN in three doses (50, 100, and 200 pmol in 50, 100, and 200 nl, respectively). The doses were given consecutively at intervals of 20 min. Maximum changes in RSNA, BP, and HR were determined after each dose.

Experiment protocol 2. RSNA, BP, and HR were measured in sham-sedentary (n = 10), HF-sedentary (n = 10), sham-ExT (n = 8), and HF-ExT (n = 10) rats. A NO donor, sodium nitroprusside (SNP), was injected into the PVN in three doses (5, 10, and 20 nmol in 50, 100, and 200 nl, respectively). The doses were given consecutively at intervals of 20 min. Maximum changes in RSNA, BP, and HR were determined after each dose.

Cardiac Histology

At the end of each experiment, the heart was removed, weighed, and fixed in 4% formaldehyde. The infarct size was determined as previously described (46, 47). Briefly, the hearts were sectioned into four major segments located from the atrium to the apex: segments A-D. Segment A (the atria and the connecting inflow and outflow tracts) and segment D (mainly the apex) were not analyzed for histological damage. Segments B and C (representing the bulk of the left ventricle) were subjected to graded dehydration with ethanol, embedded in paraffin, cut into sequential 10-μm sections, and stained with hematoxylin-eosin. Every 10th section was projected onto a screen, and the outline of the tissue was diagramed after microscopic examination of the infarcted areas of the ventricle to identify the edges of the infarct area. The infarct size was expressed as a fraction of the total cross-sectional epicardial circumferences of the left ventricle.

Data Analysis

Responses of RSNA to the drugs were reported as the percent change from the basal value. Responses of mean BP and HR were expressed as the difference between the basal value and the value after each dose of a drug. Data were subjected to two-way ANOVA followed by Newman Keuls test (for multiple comparisons) or Student’s t-test. P < 0.05 was considered to indicate statistical significance.

RESULTS

Baseline Data (Group Characteristics)

Table 1 summarizes the salient characteristics of sham and HF rats utilized in the present study. Heart weight, body weight, and lung wet weight were significantly higher in HF-sedentary rats compared with sham-sedentary rats (P < 0.05). This indicates that retention of water occurs in HF rats. ExT significantly lowered the body weight and lung wet weight in the HF group.

The HF group displayed an average myocardial infarct size over 30% of the left ventricle. Sham rats had no observable damage to the myocardium. LVEDP was significantly elevated in HF rats compared with sham rats (P < 0.05). +dP/dt_max was significantly decreased in HF rats, indicating a decreased contractility that was manifest as an increase in LVEDP. −dP/dt_max had a similar trend in rats with HF. These data suggest that the rats in the HF group had decreased cardiac contractile and diastolic function. ExT did not significantly change LVEDP in either sham or HF groups. In the HF group, the LVEDP level tended to be lower in the ExT group versus the sedentary group. Furthermore, ExT had no effect on ±dP/
Table 1. Characteristics of sham-operated and HF rats in the microinjection experiments

<table>
<thead>
<tr>
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<th>n</th>
<th>Sham-Sedentary 10</th>
<th>HF-Sedentary 10</th>
<th>Sham-ExT 8</th>
<th>HF-ExT 10</th>
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<tr>
<td>Body weight, g</td>
<td></td>
<td>390±14</td>
<td>423±7*</td>
<td>366±9</td>
<td>364±6†</td>
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<td>Heart weight, g</td>
<td></td>
<td>1.30±0.11</td>
<td>1.60±0.23*</td>
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<td>1.46±0.13*</td>
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<td>Wet lung weight, g</td>
<td></td>
<td>1.83±0.03</td>
<td>2.06±0.09*</td>
<td>1.61±0.11</td>
<td>1.64±0.11†</td>
</tr>
<tr>
<td>Basal mean blood pressure, mmHg</td>
<td>73±7</td>
<td>78±5</td>
<td>75±5</td>
<td>75±5</td>
<td></td>
</tr>
<tr>
<td>Basal heart rate, beats/min</td>
<td>361±12</td>
<td>366±21</td>
<td>349±7</td>
<td>348±9</td>
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<td>Basal integrated RSNA, µV·s</td>
<td>4.43±0.52</td>
<td>5.65±0.80</td>
<td>4.19±0.57</td>
<td>5.06±0.59</td>
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<td>Infarct size, % epicardial LV</td>
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<td>0</td>
<td>30.8±3.6*</td>
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<td>LVEDP, mmHg</td>
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<td>0.04±0.93</td>
<td>9.33±0.65*</td>
<td>0.54±0.40</td>
<td>7.75±1.19*</td>
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<td>+dP/dt max, mmHg/s</td>
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<td>6.014±514*</td>
<td>7.782±673</td>
<td>6.318±792*</td>
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<tr>
<td>−dP/dt max, mmHg/s</td>
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<td>−5.001±641</td>
<td>−4.190±339*</td>
<td>−5.765±424</td>
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<td>Citrate synthase, µmol·g⁻¹·min⁻¹</td>
<td>17.0±2.3</td>
<td>15.8±1.5</td>
<td>25.4±2.4†</td>
<td>24.2±2.3†</td>
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</table>

Values are means ± SE; n, no. of animals. HF, heart failure; ExT, exercise training; RSNA, renal sympathetic nerve activity; LV, left ventricle; LVEDP, LV end-diastolic pressure. *P < 0.05 vs. respective sham-operated rats; †P < 0.05 vs. respective sedentary rats.

was significantly less than that found in the other groups (30% less, P < 0.05). In rats with HF, ExT restored the decreased number of nNOS-positive neurons in the PVN. The number of cells stained for diaphorase in the PVN in the sham-sedentary, sham-ExT, and HF-ExT groups was significantly greater than that in HF-sedentary group (Fig. 2). There were no significant changes in the number of NADPH-diaphorase-positive cells in the SON, LH, or MnPO.

Experimental Series 2: Semiquantitative RT-PCR Measurement and Western Blot Analysis for nNOS Protein

The mRNA levels of nNOS were 0.93, 0.58, 0.99, and 1.02 (ratio of intensity) in sham-sedentary, HF-sedentary, sham-ExT, and HF-ExT rats, respectively. There was a significantly lower level of nNOS mRNA in HF-sedentary rats (P < 0.05). ExT enhanced nNOS mRNA significantly in the HF-ExT rats (Fig. 3). Western blot analysis showed 155-kDa bands in the PVN of sham-sedentary and HF-sedentary rats. HF-sedentary rats had significantly lower protein levels of nNOS compared with the other three groups (P < 0.05). Protein expression of nNOS was significantly improved in the HF-ExT rats compared with the HF-sedentary group (Fig. 4).
Experimental Series 3: Microinjection of l-NMMA and SNP into the PVN

Experimental protocol 1. An example of the peak responses in RSNA, BP, and HR to l-NMMA in the four groups is illustrated in Fig. 5. The administration of l-NMMA (50, 100, and 200 pmol) into the PVN elicited a dose-dependent increase in RSNA, BP, and HR in the sham and HF groups (Fig. 6). The HF-sedentary group showed an increase in RSNA that was significantly blunted with all three doses compared with sham rats (*P < 0.05). It should be noted that, although HF rats tended to have an increased basal RSNA, the absolute increases in RSNA to l-NMMA were still significantly less in rats with HF. Therefore, the smaller percent changes in RSNA in response to l-NMMA are not due the possible difference in baseline values between groups. ExT significantly normalized the attenuated RSNA responses in rats with HF (33% compared with 20% at the highest dose). There was no significant difference between sham-sedentary rats and HF-ExT rats. A similar trend was observed in BP and HR responses to SNP.

DISCUSSION

The results of the present study indicate that there are a decreased number of NOS-positive cells in the PVN of rats with HF. The decreased number of NOS-positive cells in the PVN of rats with HF may contribute to the increased sympathoexcitation in chronic HF. Daily ExT restored the number of NOS-positive neurons in the PVN of rats with HF. Consistent with these results, nNOS mRNA expression and protein levels in the PVN were also normalized after ExT in rats with HF. Furthermore, blockade of endogenous NO production within the PVN with l-NMMA produced a blunted increase in RSNA,
BP, and HR in rats with HF compared with sham-operated rats. Again, ExT normalized the attenuated RSNA responses in rats with HF. Conversely, SNP, microinjected into the PVN, produced a dose-dependent decrease in RSNA in sham and HF rats. ExT significantly improved the blunted decrease in RSNA in HF rats. These data suggest that the sympathoinhibition associated with daily ExT in rats with HF (34) may be due, in part, to the improvement of nNOS levels and NO-mediated mechanisms within the PVN of rats with HF.

The HF condition was documented by the increased cardiac filling pressure, reduced left ventricular contractility, and the size of the myocardial infarct. These rats also demonstrated cardiac hypertrophy and an increased lung wet weight, indicative of pulmonary edema. ExT tended to improve the cardiac hypertrophy, but there was no significant difference between the sedentary and ExT HF rats. Similarly, the contractile function tended to improve in ExT HF rats but was not significantly different between sedentary and ExT HF rats as well as between HF-ExT rats and sham-sedentary rats. However, there was a significant improvement in lung wet weight in ExT HF rats, an index of water accumulation in the pulmonary circulation. Consistent with these observations, there was also a significant difference in the body weight in the sedentary and ExT HF rats, indicative of improvement in peripheral edema and ascites. This would suggest that ExT improved the peripheral circulation and the chronic congestive phase of HF but not the cardiac dysfunction per se.

It is recognized that basal hemodynamic parameters can be affected by acute surgery and anesthesia. We tried to minimize the effects of acute surgery by doing the acute experiments at least an hour after the surgery, when we had stabilized BP and HR. In terms of anesthesia, because these experiments required extensive manipulations, we had to do the experiments in anesthetized rats. In our experience, the level of anesthesia is maintained with urethane-chloralose with a slight depression in BP, but the cardiovascular reflexes remain intact (17). The lower BP may cause a consequent increase in basal RSNA, but because we were able to increase RSNA we believe that this does not confound the data. We are assuming that the effects of surgery and anesthesia are similar between sham and HF groups, because ExT was able to reverse these effects in HF rats but not sham rats (again under these manipulations).

It has been well established that increased sympathetic nerve activity is a characteristic symptom of chronic HF. This sympathoexcitatory state increases the progression and risk of mortality during HF. In patients with HF, elevated sympathetic activity and altered baroreflexes have been reported (39). Norepinephrine release from the failing heart at rest in untreated patients is increased as much as 50-fold (16). Similarly, with the use of an animal model of HF produced by coronary artery ligation, some studies have revealed that RSNA is elevated in both conscious and anesthetized rats with HF (8). It was also found that the volume reflex (7) and the baroreflex (24) were blunted in rats with HF. Recently, several studies have suggested that altered central mechanism(s) may be responsible for the elevated neurohumoral drive in HF (44). In addition to the classical negative feedback of arterial and cardiopulmonary baroreflexes, there are positive feedback, sympathoexcitatory reflexes and humoral regulatory mechanisms that modulate sympathetic outflow centrally.

Fig. 5. Segment of an original recording from individual rats demonstrating the baseline parameters and peak changes in renal sympathetic nerve activity (RSNA), blood pressure (BP), and heart rate [HR, in beats/min (bpm)] by administration of \( N^\text{G} \)-monomethyl-l-arginine (l-NMMA) into the PVN in the four groups of rats: sham-sedentary, HF-sedentary, sham-Ext, and HF-Ext. Shown are the baseline parameters with 0 and 200 pmol l-NMMA into the PVN.
The PVN is a major site of integration of autonomic and endocrine cardiovascular responses. The parvocellular neurons of the PVN (pPVN) are involved in the mediation of the neural component of cardiovascular reflexes by influencing RSNA (19). Specifically, these studies demonstrated that the PVN is involved in the baroreflex regulation of lumbar sympathetic nerve activity (45). Stimulation of the PVN has been shown to elicit an increased discharge from several sympathetic nerves, including renal (25), adrenal (26), and splanchnic nerves (25). The observations suggest that the PVN plays an essential role in the mediation of RSNA under resting and reflex conditions. Studies from our laboratory have shown increased neuronal activity in the PVN during HF (47), suggesting the involvement of the PVN in the altered sympathetic activity in HF. In contrast, adjacent areas within the hypothalamus such as the SON, LH, and MnPO did not show a significant change in number of NOS-positive cells in rats with HF (59). This further suggested that the change in NOS within the PVN was unique within the hypothalamic areas examined in rats with HF. Whether similar changes in NOS activity within brain stem areas involved in regulating sympathetic outflow, such as the nucleus tractus solitarii, rostral ventrolateral medulla, etc., are altered in HF remains to be elucidated. It is conceivable that such changes within the brain stem may also contribute to the altered sympathetic outflow noted in the HF condition.

Increasing evidence indicates that NO acts as a neuromodulator to affect synaptic function in the central nervous system (2). In the hypothalamus, NOS-positive neurons are found primarily in the PVN and SON (2). NO through a GABA mechanism within the PVN has been shown to decrease RSNA and HR in rats (58). Work from our laboratory (57) and others (20) have shown that administration of the NO donor SNP into
the PVN produces a decrease in RSNA and depressor responses in rats. Conversely, it has been observed that administration of the NOS blockers L-NMMA or N-nitro-L-arginine methyl ester into the PVN increases RSNA in rats (57). These responses are significantly blunted, and the sympathoinhibitory effects of NO are less effective in rats with HF (56). These results suggest that the lack of endogenous as well as exogenous NO within the PVN is less effective in decreasing sympathetic nerve activity in rats with HF. The results of the present study are consistent with these previous observations and specifically identify a decreased message for nNOS and protein levels of nNOS in the PVN of rats with HF. Adjacent areas within the hypothalamus such as the LH and SON did not demonstrate similar changes in rats with HF.

Daily exercise is associated with a reduction in arterial pressure (29) and sympathetic nerve activity (4). ExT in rabbits with HF has also been shown to decrease RSNA (34). ExT induced an increase in the density of nNOS-positive neurons, nNOS message, and nNOS protein in rats with HF and may lead to an increase in synthesis of NO in the PVN, which subsequently causes an increased inhibitory effect on RSNA. This suggestion is consistent with improved RSNA responses to microinjection of L-NMMA and SNP into the PVN concomitant with improved nNOS in the PVN of rats with HF.

It has been shown that long-term regular exercise of patients with HF increases the quality of life as well as survival (5). The quality of life was assessed by a questionnaire (Minnesota Living With Heart Failure Questionnaire) consisting of 21 items focused on the patients perceptions concerning the effects of congestive heart failure on their physical, psychological, and socioeconomic lives. This questionnaire is shown to be responsive to changes in quality of life in patients with HF (5). The mechanisms by which ExT improves survival and enhances the quality of life in HF are not completely understood. Experimental evidence indicates that ExT reduces renal sympathetic nerve activity and improves arterial baroreflex in animals with HF (34). It has been found that ExT lowers BP in hypertensive animals, most likely through a NO-dependent mechanism (23). ExT has been shown to upregulate eNOS and endothelium-dependent responses in the coronary circulation of dogs with HF (52). In our present study, the results suggest that ExT enhanced the function and gene expression of endogenous nNOS to regulate sympathetic outflow during HF in the PVN. The time after the last exercise session to conduct the
experiments was 20–24 h. A single session of exercise may have effects on hemodynamic lasting 24 h, but we believe that the functional and molecular biological changes that we observe in our experiments are most likely due to long-term ExT rather than an acute effect.

The mechanisms responsible for the ExT-induced increase in nNOS within the PVN remain unknown. However, it is well known that the PVN receives information from various cardio-pulmonary receptors including myocardial vagal afferents (37, 38). Some studies suggest that ExT increases various hemodynamic parameters within the heart (HR, cardiac contractility, preload, and afterload) that dictate cardiac vagal activity (9, 10). Some data support the view that enhancement of baroreflex control of HR after ExT is due to an augmentation of vagal tone (35). Accordingly, the activation of cardiac vagal activity during ExT may mediate an increase of nNOS within the PVN resulting in overall sympathoinhibition. This is consistent with the enhanced inhibitory influence of cardiac vagal afferents on directly measured sympathetic activity and regional vascular resistance after ExT (11). The specific role for cardiac vagal afferents remains to be examined.

Angiotensin II has also been implicated as a contributor in the sympathoexcitatory observation in HF (33). The levels of angiotensin II are increased in HF, and angiotensin-converting enzyme inhibitors are effective in reducing sympathoinhibition during HF (18, 28). Modulation of NO synthesis and the interaction of NO with angiotensin II in the central nervous system may be an important mechanism for the regulation of sympathetic nerve activity during exercise in HF. Several studies suggest that NO and angiotensin II interact in such a way with regard to sympathetic outflow (33, 36). Angiotensin II acts as a sympathoexcitatory substance that potentiates the effects of NO blockade in normal animals. Blockade of angiotensin II receptors in combination with a NO donor reduces sympathetic nerve activity in animals with HF (36). It has also been shown that ExT of rabbits with HF resulted in a decrease in plasma angiotensin II and restoring RSNA despite little improvement in several parameters of cardiac function (34, 40). It is conceivable that one mechanism by which ExT improves the endogenous NO mechanism is related to angiotensin II. This possibility remains to be examined more thoroughly.

There is a body of literature that indicates that voluntary exercise and forced exercise may induce different behavioral and hypothalamic-pituitary-adrenocortical (HPA) responses to stress (13, 14). It has been shown that treadmill ExT has an effect on the metabolism of norepinephrine and the secretion of adrenocorticotropic hormone (ACTH) after foot-shock stress (13). Treadmill ExT has a hyperadrenergic effect in brain areas that modulate hypothalamic regulation of ACTH release during stress (14). Therefore, differential activation of the HPA in response to exercise between the two groups might have different effects on the hypothalamus (including the PVN) during stress. Measures of HPA activity and plasma catecholamines would help to address the question of whether the animals were responding comparably to the exercise challenge (13). However, we believe that this was not the major factor dictating the changes we are reporting here. In this study, we included rats that ran as well (as willingly) in both groups. Those that required more prompting (higher level of potential stress) were not used in this study. There was no difference in the prompting to run between the sham and HF groups, suggesting a similar level of unintended “stress.” It should be recognized that this is a mild to moderate level of exercise, which is relatively easy, and the rats were not resistant to exercising, even the HF rats. However, it is recognized that a similar level of stress (even if it was small) may produce different levels of a stress response in HF rats than in sham rats, and this may potentially be involved in these changes. Finally, we did the terminal experiments 1 day after the last bout of running. This would suggest any of the effects of potential stress were perhaps not responsible for the differences observed. These reasons would suggest that stress per se is not likely responsible for the changes observed in the HF-ExT rats.

In conclusion, our data suggest that a reduction of nNOS/NO within the PVN contributes to sympathoexcitation in HF. Furthermore, ExT improves the altered NO mechanisms within the PVN involved in dictating RSNA in rats with HF. The increased NOS may increase the level of NO and contribute to the ExT-induced sympathoinhibition. Although patients with HF have limited exercise capacity, which is, in part, due to a reduced cardiac output, ExT may be an important therapeutic tool for modulating central NO mechanisms dictating sympathetic drive in patients with HF.

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