Exercise Training Normalizes Sympathetic Outflow by Central Antioxidant Mechanisms in Rabbits With Pacing-Induced Chronic Heart Failure

Lie Gao, MD, PhD; Wei Wang, MD, PhD; Dongmei Liu, MD, PhD; Irving H. Zucker, PhD

**Background**—In a recent study, we demonstrated that an increase in oxidative stress in the rostral ventrolateral medulla plays a critical role in the sympathoexcitation observed in chronic heart failure (CHF). Growing evidence indicates that exercise training evokes an antioxidative effect in CHF. In the present study, we therefore hypothesized that long-term exercise exerts its beneficial effect on autonomic activity in CHF via central antioxidative mechanisms.

**Methods and Results**—Experiments were performed on New Zealand White rabbits. All rabbits were instrumented to measure mean arterial pressure, heart rate, and renal sympathetic nerve activity and to test baroreflex sensitivity. Exercise training significantly decreased baseline renal sympathetic nerve activity (65.8±5.2% to 41.3±3.9% of Max [where “Max” is the maximum renal sympathetic nerve activity induced by a 50-mL puff of smoke directed to the external nares of the rabbit], P<0.05) and increased the maximal gain of the baroreflex curves for heart rate (2.2±0.2 to 4.6±0.7 bpm per mm Hg, P<0.01) and renal sympathetic nerve activity (1.9±0.2% to 4.5±0.4% of Max per mm Hg, P<0.01) in CHF rabbits. Exercise training increased expression of CuZn superoxide dismutase (0.3±0.1 to 1.5±0.3 [ratio of CuZn superoxide dismutase to tubulin], P<0.01) and decreased NAD(P)H oxidase subunit gp91phox protein expression (1.9±0.2 to 1.2±0.1 [ratio of gp91phox to tubulin], P<0.05) in the rostral ventrolateral medulla of CHF rabbits. Central overexpression of CuZn superoxide dismutase dose-dependently decreased baseline renal sympathetic nerve activity (control, 68.5±7.1% of Max; 10^10 particles of adenovirus, 35.2±4.4% of Max; and 10^11 particles of adenovirus, 33.7±3.5% of Max; P<0.05) in CHF rabbits.

**Conclusions**—These results suggest that an upregulation in central antioxidative mechanisms and suppressed central prooxidant mechanisms may contribute to the exercise training–induced beneficial effects on autonomic activity in CHF.

(Circulation. 2007;115:3095-3102.)

**Key Words:** exercise ■ heart failure ■ nervous system, sympathetic ■ free radicals ■ oxidative stress

---

It is well accepted, on the basis of human and animal neurochemical and neurophysiological studies, that chronic heart failure (CHF) is characterized by heightened sympathetic tone.1-3 This sympathetic abnormality is a compensatory adjustment to a reduction in cardiac function and may be beneficial to provide adequate peripheral tissue perfusion in the early phase of heart failure. However, this compensatory mechanism gradually becomes more intense and sustained, which contributes to the progression of the heart failure syndrome. Indeed, a strong consensus exists as to the adverse influence of sympathetic hyperactivity on the progression and outcome of CHF.4-6

Sympathoexcitation in the CHF state, therefore, is an important therapeutic target for this syndrome, and reduction of adrenergic hyperactivity has been shown to improve patient survival.

---

**Editorial p 3042**

**Clinical Perspective p 3102**

Long-term exercise training (EX) has recently been used as a therapeutic regimen in the CHF state.7,8 Patients engaged in an EX protocol have been shown to exhibit improved exercise tolerance and an enhanced quality of life, as well as increased survival.9 In addition, our previous studies in rabbits with pacing-induced CHF demonstrated that EX reduced the sympathoexcitatory state, in part because of an enhanced arterial baroreflex sensitivity.10,11 These data help to explain the beneficial effects of EX in CHF patients. However, the exact central neural mechanisms by which EX normalizes sympathetic outflow in CHF remain to be elucidated.

Patients with CHF are subjected to increased oxidative stress.12 Elevated levels of exhaled pentane13 or plasma malondialdehyde14 have been reported in this syndrome. Our recent studies15 showed that central oxidative stress plays an important role in sympathoexcitation in CHF. We found upregulation of NAD(P)H oxidase expression and activity in the rostral ventrolateral medulla (RVLM), the primary central site for the maintenance of sympathetic nerve activity. In the...
same experiment, we demonstrated that a decrease of central superoxide anion (O$_2^-$) by tempol, a superoxide dismutase (SOD) mimetic, reduced sympathetic outflow in CHF rabbits. In contrast, an increase of central O$_2^-$ due to administration of the SOD inhibitor dietylthiocarbamic acid significantly augmented sympathetic outflow in both normal and CHF rabbits.\textsuperscript{15}

Growing evidence indicates that a major benefit of EX is dependent on its antioxidant effects, which are mediated not only by increased expression of antioxidant enzymes but also by a reduced expression of prooxidant enzymes. For example, EX significantly reduced the expression of subunits of the reactive oxygen species (ROS)-producing enzyme NAD(P)H oxidase (gp91\textsuperscript{phox}, p22\textsuperscript{phox}, and Nox4).\textsuperscript{16} Increases in gene expression and enzyme activation induced by EX have also been found in the patients with CHF\textsuperscript{17,18} and, interestingly, in the central nervous system of rats with diabetes mellitus.\textsuperscript{19}

We therefore hypothesized that EX normalizes sympathetic outflow in rabbits with CHF via its antioxidant effects in those areas of the central nervous system that regulate sympathetic nerve activity. In the present study, we determined (1) the effects of EX on baseline renal sympathetic nerve activity (RSNA) and arterial baroreflex function, (2) the effects of EX on and the central expression of SOD and NAD(P)H oxidase in the RVLM, and (3) the effects of central overexpression of SOD on baseline RSNA.

Methods

Animals

Experiments were performed on 51 male New Zealand White rabbits weighing between 2.6 and 3.9 kg (Charles River Laboratories, Wilmington, Mass). These experiments were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and conformed to the guidelines for the care and use of experimental animals of the American Physiological Society and the National Institutes of Health. Rabbits were assigned to a sham group (n = 23), which was further divided into a nonexercise group (sham, n = 9), an exercise group (EX-sham, n = 8), and an adenoaviral transfection group (Sham-adSOD, n = 6). In addition, CHF groups (n = 28) were subdivided into a nonexercise group (CHF, n = 8), an exercise group (EX-CHF, n = 9), a low-dose adSOD group (CHF-adSOD 10\textsuperscript{10}, n = 6), and a high-dose adSOD group (CHF-adSOD 10\textsuperscript{14}, n = 5).

Long-Term Surgical Instrumentation

Pacing Electrodes

Pacing electrodes were implanted as described by us previously.\textsuperscript{20} The animals were treated with antibiotics (enrofloxacin 5 mg/kg SC for 5 postoperative days) and allowed to recover for 2 weeks before being used in any experiment.

RSNA Recording Electrodes

Three platinum-wire recording electrodes were wrapped around 1 or 2 renal sympathetic nerves that ran along the renal artery. A ground electrode was secured to the nearby muscle. The entire electrode assembly was then covered with a silicone gel. The electrode wires were then tunneled beneath the skin and exited in the midscapular area. During the experiment, the electrical signal from the electrode was amplified with a Grass P55 preamplifier (Grass Instruments; West Warwick, RI) with high- and low-frequency cutoffs of 1000 and 100 Hz, respectively. The output from the Grass amplifier was directed to the PowerLab system with sampling at 1000 samples per second. The signal was also rectified (full wave) and integrated. The average rectified signal (AC filtered, time constant 0.5 second) was then recorded and stored for later analysis. The frequency of nerve discharge was counted with a window discriminator and rate meter. The cursor of the window discriminator was set just above the electrical noise. Both frequency and integrated nerve activity were recorded continuously along with the raw nerve activity. The unit of RSNA we used in this experiment was “% Max,” the maximum RSNA induced by a puff of smoke (50 mL) directed to the external nares of the rabbit, which has been shown to be a suitable method of comparing RSNA baroreflex curves under a variety of different conditions.\textsuperscript{21}

Arterial Pressure and Heart Rate

A catheter connected to a radiotelemetry unit (Data Sciences International, St Paul, Minn) was inserted into the descending aorta via a brachial of the right femoral artery under general anesthesia for direct measurement of arterial pressure, from which the heart rate (HR) was derived.

Induction of Heart Failure

After recovery from surgery, the heart failure groups were paced as we have described previously.\textsuperscript{22} Heart failure was characterized by a reduction in ejection fraction of \approx 50\%, a 2-mm dilation of the left ventricle in both systole and diastole, and clinical signs of CHF, such as pleural fluid, ascites, pulmonary congestion, and cachexia. In this model, left ventricular pacing was performed for 3 to 4 weeks, whereas the ejection fraction was determined weekly by echocardiography and decreased from \approx 75\% to 40\% (Tables 1 and 2).

Cardiac function was measured by echocardiography (Acuson Sequoia 512 C, Siemens Medical Solutions, Malvern, Pa) with the rabbits handheld in the conscious state. A 2-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles. M-mode tracings were recorded through the anterior and posterior left ventricular walls, and anterior and posterior wall thicknesses (end-diastolic and end-systolic) and left ventricular internal dimensions were measured.

EX Training Protocol

Rabbits were trained to run on a motor-driven treadmill. EX was performed for a total of 40 minutes per day for 6 days per week. A warm-up period of 5 minutes at 5 m/min was followed by a peak EX of 18 m/min for 30 minutes. This was followed by a cool-down period of an additional 5 minutes at 5 m/min. The training program was initiated from the first day of rapid pacing until the end of the experiment.

Evaluation of Arterial Baroreflex Function

An intravenous infusion of sodium nitroprusside was used to induce alterations of arterial pressure, by which the baroreflex function was analyzed by logistic regression over the entire pressure range. The data were acquired every 2 seconds from the threshold to the saturation points. A sigmoid logistic regression curve was fit to the data points with the following equation: $HR = A(1 + \exp[B(MAP - C)]) + D$, where $A$ is the HR or RSNA range, $B$ is the slope coefficient, MAP is the mean arterial pressure, $C$ is the pressure at the midpoint of the range (BP$_{50}$), and $D$ is the minimum HR or RSNA. The peak slope (or maximum gain) was determined by taking the first derivative of the baroreflex curve and was calculated with the equation: $\text{Gain}_{\text{max}} = A(1) \times A(2) \times (1/4)$, where $A(1)$ is the range and $A(2)$ is the average slope. The mean values for each curve parameter were used to derive composite curves for each group of rabbits.

Preparation of RVLM Tissue and Western Blot Analysis of SOD and NAD(P)H Oxidase Subunits

At the end of the experiment, the rabbits were euthanized with pentobarbital sodium. The brain was removed and immediately frozen on dry ice, blocked in the coronal plane, and sectioned at 100-μm thickness in a cryostat. The RVLM was punched according to the method of Palkovits and Brownstein\textsuperscript{23} and then was homog-
enzymatized in RIPA buffer. Protein extraction from homogenates was used to analyze CuZn SOD, Mn SOD, and gp91phox expression by Western blot, as described previously.

**Gene Transfer of CuZn SOD to the Central Nervous System**

Under sterile conditions, the skull was exposed through an incision on the midline of the scalp. After the bregma was identified, a 19-gauge stainless steel cerebroventricular cannula was implanted into the right lateral cerebral ventricle, 4 mm lateral to the bregma and 6 mm below the cerebral surface, and fixed tightly to the skull with super-glue adhesive and dental cement. After 1-week recovery from surgery, the rabbits received adenoviral vectors (1×10^{10} or 1×10^{11} particles; 50 μL) containing a human CuZn SOD (kindly supplied by Dr Robin L. Davisson of Cornell University, Ithaca, NY).

**Statistical Analysis**

Data are expressed as mean±SE. Analyses of baseline hemodynamic data, baseline RSNA, arterial baroreflex function, and Western blot density in the EX group were subjected to 2-way ANOVA followed by the Student-Newman-Keuls test. Baseline hemodynamic data and baseline RSNA in the SOD-transfected group were subjected to a 1-way ANOVA followed by the Student-Newman-Keuls test. A probability value of <0.05 was considered statistically significant. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

**Body Weight, Organ Weight, Hemodynamics, and Echocardiographic Data**

Tables 1 and 2 show the values for body weight, organ weight, hemodynamics, and echocardiographic data for the rabbits used in the present experiment. The CHF group exhibited a significantly higher wet lung weight than the sham group, but the dry lung weights were almost the same in

---

**TABLE 1. Baseline Hemodynamic Data From Exercise-Trained Rabbits**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=9)</th>
<th>EX-Sham (n=8)</th>
<th>CHF (n=6)</th>
<th>EX-CHF (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>3.2±0.4</td>
<td>3.4±0.5</td>
<td>2.9±0.3</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>2.3±0.4</td>
<td>2.5±0.3</td>
<td>3.1±0.4</td>
<td>3.6±0.5</td>
</tr>
<tr>
<td>WLW/BW, g/kg</td>
<td>4.2±0.3</td>
<td>4.0±0.3</td>
<td>6.9±0.4*</td>
<td>6.2±0.4‡</td>
</tr>
<tr>
<td>DLW/BW, g/kg</td>
<td>1.0±0.3</td>
<td>1.1±0.1</td>
<td>1.3±0.1</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>73.7±6.8</td>
<td>69.3±6.1</td>
<td>73.1±7.4</td>
<td>72.6±8.9</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>206.2±9.3</td>
<td>201.1±11.6</td>
<td>258.6±12.5*</td>
<td>247.9±13.5‡</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>83.7±6.7</td>
<td>79.7±6.9</td>
<td>82.8±6.5</td>
<td>82.6±9.2</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.6±0.8</td>
<td>3.8±0.5</td>
<td>12.5±1.4†</td>
<td>13.7±1.6‡</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>7.9±0.6</td>
<td>8.1±0.5</td>
<td>15.9±0.9*</td>
<td>14.6±0.8‡</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>13.8±1.1</td>
<td>14.5±0.8</td>
<td>18.7±0.9*</td>
<td>17.6±0.6‡</td>
</tr>
<tr>
<td>EF, %</td>
<td>75.8±4.4</td>
<td>77.2±5.1</td>
<td>38.5±5.2*</td>
<td>42.2±4.6‡</td>
</tr>
</tbody>
</table>

Values are mean±SE. BW indicates body weight; HW, heart weight; WLW, wet lung weight; DLW, dry lung weight; MAP, mean arterial pressure; HR, heart rate; LVSD, left ventricular systolic diameter; LVEDD, left ventricular end-diastolic diameter; LVSP, left ventricular peak systolic pressure; LVEDP, left ventricular end-diastolic pressure; and EF, ejection fraction.

*P<0.05 compared with sham; ‡P<0.05 and §P<0.01 compared with sham. 0.05 compared with EX-sham.

**TABLE 2. Baseline Hemodynamic Data From SOD-Transfected Rabbits**

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=9)</th>
<th>Sham SOD-V-10^{10} (n=6)</th>
<th>CHF SOD-V-10^{10} (n=6)</th>
<th>CHF SOD-V-10^{11} (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, kg</td>
<td>3.2±0.4</td>
<td>3.2±0.5</td>
<td>2.9±0.3</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>2.3±0.4</td>
<td>2.4±0.5</td>
<td>3.1±0.4</td>
<td>3.4±0.6</td>
</tr>
<tr>
<td>WLW/BW, g/kg</td>
<td>4.2±0.3</td>
<td>4.0±0.3</td>
<td>6.9±0.4*</td>
<td>6.5±0.5§</td>
</tr>
<tr>
<td>DLW/BW, g/kg</td>
<td>1.0±0.3</td>
<td>1.0±0.2</td>
<td>1.3±0.1</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>73.7±6.8</td>
<td>70.4±6.5</td>
<td>73.1±7.4</td>
<td>71.3±5.5</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>206.2±9.3</td>
<td>201.1±8.5</td>
<td>258.6±12.5*</td>
<td>261.1±5.1†</td>
</tr>
<tr>
<td>LVSP, mm Hg</td>
<td>83.7±6.7</td>
<td>81.6±7.5</td>
<td>82.8±6.5</td>
<td>78.4±5.2</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.6±0.8</td>
<td>3.9±0.4</td>
<td>12.5±1.4†</td>
<td>13.2±1.5†</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>7.9±0.6</td>
<td>8.4±0.6</td>
<td>15.9±0.9*</td>
<td>14.4±1.6‡</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>13.8±1.1</td>
<td>13.4±0.5</td>
<td>18.7±0.9*</td>
<td>17.7±1.5§</td>
</tr>
<tr>
<td>EF, %</td>
<td>75.8±4.4</td>
<td>73.4±4.5</td>
<td>38.5±5.2*</td>
<td>40.4±3.5‡</td>
</tr>
</tbody>
</table>

Values are mean±SE. Sham SOD-V-10^{10} indicates sham rabbits treated with SOD adenovirus 10^{10} particles; CHF SOD-V-10^{10}, CHF rabbits treated with SOD adenovirus 10^{10} particles; and CHF SOD-V-10^{11}, CHF rabbits treated with SOD adenovirus 10^{11} particles. Other abbreviations as in Table 1.

*P<0.05 and †P<0.01 compared with sham; ‡P<0.05 compared with sham SOD-V-10^{10}. 


the 2 groups, which suggests that pulmonary edema that resulted from the failing heart did not further result in pulmonary fibrosis in the CHF rabbits. Moreover, additional clinical signs of heart failure were evident. These included pleural effusion, ascites, and subcutaneous edema. The CHF group also exhibited a significantly higher HR, left ventricular end-diastolic pressure, left ventricular systolic diameter, and left ventricular end-diastolic diameter and a significantly lower ejection fraction than sham rabbits; however, only the change in CuZn SOD expression reached significance in sham rabbits. EX restored these proteins to near the level seen in EX-sham animals. Conversely, the gp91phox protein was significantly increased in CHF rabbits, which was normalized by EX. EX also downregulated this protein expression in sham rabbits.

Effect of EX on Baseline RSNA

A representative recording and the mean data for baseline RSNA are shown in Figure 1A. As can be seen in Figure 1B, there was a significantly higher baseline RSNA than sham rabbits, which was normalized by EX. **P<0.01. AP indicates arterial pressure; Inte, integrity.

Composite arterial baroreflex curves and gain curves for control of HR and RSNA in the 4 groups of rabbits are shown in the lower panels of Figure 2. The group data indicate a significant attenuation of maximum gain in CHF rabbits that was restored by EX.

Protein Expression of SOD and NAD(P)H Oxidase Subunit in the RVLM

In this experiment, we measured 2 of the 3 major SOD isoforms (CuZn SOD and Mn SOD) and the catalytic subunit of NAD(P)H oxidase, gp91phox. As shown in Figures 3 and 4, protein expression of both CuZn SOD and Mn SOD was significantly downregulated in the CHF state. EX upregulated both isoforms in sham and CHF rabbits; however, only the change in CuZn SOD expression reached significance in sham rabbits. EX restored these proteins to near the level seen in EX-sham animals. Conversely, the gp91phox protein was significantly increased in CHF rabbits, which was normalized by EX. EX also downregulated this protein expression in sham rabbits (Figure 5).

Effects of Central Nervous System Overexpression of SOD on Baseline RSNA

To determine whether augmentation of SOD altered RSNA in CHF rabbits, we transfected the brain with an SOD adenovirus. As shown in Figure 6, after AdCuZnSOD transfection into the central nervous system in CHF rabbits, baseline RSNA was decreased in a dose-dependent manner. On the other hand, overexpression of SOD had no effect on RSNA in sham rabbits. The control adenoviral vector encoding the bacterial β-galactosidase gene (AdLacZ) had no effect on baseline RSNA in either the CHF or the sham rabbits (data not shown).

Discussion

EX has been shown to provide a beneficial effect in patients with CHF. As secondary prevention or as adjunctive therapy, EX was associated with a significant reduction of morbidity and mortality.24–26 The benefits of EX to these patients include an improvement in exercise tolerance,27,28 a significant drop in epinephrine levels,29,30 and restoration of endothelium-dependent vasodilation.31,32 Moreover, most recent studies show that an antioxidant effect is induced by EX via reduction of expression of NAD(P)H oxidase33 and that augmentation of the activity of radical scavenger enzymes also benefits CHF patients.

CHF is characterized by an impaired arterial baroreflex and augmented sympathetic nerve activity, which contributes to the progression of this disease and is one of the most important therapeutic targets in this syndrome. Indeed, reduction of sympathetic hyperactivity has been shown to improve patient survival. Since the seminal study by Cohn and colleagues identified the presence of high plasma levels of norepinephrine in CHF as being associated with an adverse outcome more than 20 years ago, β-blockers have been widely used as a first-line therapy in patients with CHF. Evidence from our laboratory has shown beneficial effects of EX in rabbits with pacing-induced CHF by reduction of sympathetic outflow and enhancement of arterial baroreflex sensitivity, augmentation of vagal tone,10

Figure 1. Effects of EX on baseline RSNA in conscious rabbits. A, Original recordings of RSNA, HR, and mean arterial pressure (MAP) in sham and CHF rabbits; B, group data of baseline RSNA. Baseline RSNA is expressed as a percent of maximum nerve activity induced by cigarette smoke in each group of rabbits. Rabbits with CHF had significantly higher baseline RSNA than sham rabbits, which was normalized by EX. **P<0.01. AP indicates arterial pressure; Inte, integrity.

Figure 2 shows original recordings and mean data for arterial baroreflex function in sham and CHF rabbits. The recordings clearly show that the changes in RSNA were smaller and the bradycardia was less in CHF rabbits than in sham rabbits.
and correction of the reduced cardiopulmonary reflex response to volume expansion. All of these findings provide a novel explanation for the therapeutic effects of EX on CHF by its actions on the central nervous system to restore autonomic function. However, the cellular and molecular mechanisms remain to be elucidated.

We recently demonstrated that upregulation of NAD(P)H oxidase expression and the subsequent increased superoxide production in the RVLM play a critical role in sympathoexcitation in rabbits with pacing-induced CHF. Given the above-mentioned antioxidative effects of EX in CHF, we hypothesized that EX normalizes sympathetic nerve activity...
via its central antioxidative effects. The data obtained from the present experiment confirm our previous findings\(^1\) that EX is associated with less sympathoexcitation and greater arterial baroreflex sensitivity in rabbits with pacing-induced CHF. We further demonstrated that EX not only upregulated SOD but also downregulated protein expression of the NAD(P)H oxidase subunit gp91\textsuperscript{phox} in the RVLM of CHF rabbits, which implicates this as a potential molecular mechanism underlying the normalized sympathetic outflow after EX in this syndrome. This speculation was further strengthened by another finding of the present study that demonstrated a decrease in baseline RSNA by central overexpression of SOD via gene transfection with AdCuZnSOD in CHF rabbits.

The close association of oxidative stress with sympathoexcitation in the RVLM in some pathological states is becoming increasingly evident. In a previous study, we found increased RSNA along with upregulation of NAD(P)H oxidase expression and superoxide generation in RVLM in CHF rabbits\(^1\) and in rabbits receiving long-term intracerebroventricular infusion of angiotensin II.\(^3\) Another demonstration that oxidative stress is important in the sympathoexcitatory process can be found in the effects of statins on this process. For instance, the increased RSNA and oxidative stress in the RVLM of CHF rabbits was normalized after treatment with simvastatin.\(^20\) Several studies have characterized simvastatin as an antioxidant.\(^35\text{-}37\) Oxidative stress in the RVLM also plays an important role in spontaneous hypertension, another cardiovascular disease characterized by sympathoexcitation. Increasing protein expression and enzyme activity of SOD in the RVLM of spontaneous hypertensive rats by gene transfer decreases oxidative stress and lowers arterial pressure by suppression of the augmented sympathetic vasomotor activity.\(^38\) Chan et al.\(^39\) further pointed out the crucial interplay between elevated O\(_2\)\textsuperscript{=} levels and the reduced mRNA protein and activity of SOD in the RVLM, which leads to oxidative stress and the pathogenesis of hypertension. These findings have led to the hypothesis that the elevated O\(_2\)\textsuperscript{=} levels in RVLM might increase the excitability of the presympathetic neurons. Consistent with this, Sun et al.\(^33\) have provided evidence to demonstrate the ROS-induced increase in neuronal excitability and therefore firing rate via inhibition of the delayed rectifier potassium current in primary neuronal cells from rat brain stem.

It has been reasonably well established that EX has an influence on the prooxidant and antioxidant system in peripheral tissues of CHF patients. As early as 2001, in an experiment to determine the molecular basis for the improvement of vascular endothelial function in patients with CHF subjected to physical training, Ennezat et al.\(^18\) found that EX increased the expression of CuZn SOD in skeletal muscle. Also using skeletal muscle of CHF patients, Linke et al.\(^17\) demonstrated an augmented activity of glutathione peroxidase and catalase by EX. In patients with symptomatic coronary artery disease, EX reduced the expression of subunits of the NAD(P)H oxidase enzyme complex and the enzymatic activity of the internal mammary artery, resulting in a diminished overall production of ROS.\(^16\) The data presented in the current study demonstrate a central antioxidative effect of EX via both upregulation of SOD and downregulation of the NAD(P)H oxidase subunit gp91\textsuperscript{phox} in the RVLM of an animal model of CHF. Interestingly, several investigators have determined the effects of EX on antioxidant enzymes in brain regions in normal animals and in animals with other diseases. Somani et al.\(^40\) reported an increase in SOD activity in the brain stem and cerebral cortex in male Sprague-Dawley rats after treadmill exercise. However, in male Kunming albino mice, Qiao et al.\(^41\) found that intermittent anaerobic swimming significantly increased total antioxidant capacity but had no effect on SOD activity in the
brain. In streptozotocin-induced diabetic rats, EX markedly increased CuZn SOD activity but did not alter catalase activity in the brain.49 Other studies have provided evidence for an EX-induced increase in SOD activity in the striatum52 and an increase in glutathione peroxidase in the hippocampus and cerebral cortex.43 We realize that the results of the present study cannot necessarily be extrapolated to the clinical situation; however, the data from the present study do provide useful information relevant to therapy in CHF patients. In the present study, the beneficial effects of exercise training in the CHF state were, at least in part, dependent on the enhanced central antioxidative activity, which induced normalization of sympathetic nerve activity. On the basis of the present work, it may be possible to design an exercise training regimen to maximize the upregulation of antioxidative mechanisms.

The mechanisms that underlie the EX-induced upregulation of SOD expression and downregulation of gp91phox expression in RVLM of CHF rabbits observed in the present study are unknown. Finkel and Holbrook44 elegantly stated that the best strategy to enhance endogenous antioxidant levels may actually be oxidative stress itself, which has been nicely reviewed by Ji45 to explain the mechanisms of EX that stimulate the expression of specific antioxidant enzymes. Indeed, it has been demonstrated for some time that increased amounts of ROS are generated by EX.46,47 Various transcriptional factors, including nuclear factor-κB, can be activated by oxidative stress,58–50 and Mn SOD contains nuclear factor-κB–binding sites in its gene-promoter region.50 These data imply a potential mechanism for exercise-activated upregulation of SOD expression via the nuclear factor-κB–signaling pathway. Indeed, in some of CHF rabbits in the present study, we did find an increase in plasma lipid peroxide from the blood sample drawn immediately after EX (data not shown). Unfortunately, this change in plasma lipid peroxide did not reach significance because of the remarkable variety among the individual rabbits.

In the present study, we also noted that in contrast to CHF rabbits, the increase in CuZn SOD expression in EX-sham rabbits or in sham rabbits transfected with an adenovirus encoding CuZn SOD was not associated with alterations in baseline RSNA or baroreflex function. The reason for this discrepancy is not clear. We speculate that in the normal state, ROS plays a small role in the maintenance of baseline sympathetic activity due to the balance between prooxidative and antioxidative mechanisms, so that no surplus of ROS exists in the RVLM. Therefore, the upregulation of CuZn SOD had little effect on baseline RSNA. In contrast to the normal animal, the CHF animal is characterized by augmented oxidative stress, which provides ample substrate for the upregulated CuZn SOD to scavenge, which results in a decrease in sympathetic outflow. Even though we do not know exactly how much CuZn SOD is actually involved in the regulation of the RSNA and baroreflex function in the normal state, we can say with confidence that EX-induced upregulation of CuZn SOD plays a critical role in the normalization of sympathetic activity.

In summary, we found that EX normalized sympathetic outflow and arterial baroreflex function in rabbits with CHF, accompanied by an upregulation of SOD expression and a downregulation of gp91phox expression in the RVLM. We further provided evidence to demonstrate that central overexpression of SOD decreased baseline sympathetic activity in CHF rabbits. We therefore conclude that the enhancement of antioxidative pathways and the suppression of prooxidant mechanisms in the RVLM of CHF rabbits contribute to the normalization of sympathetic nerve activity and arterial baroreflex function after EX.

Acknowledgments

The authors acknowledge the expert technical assistance of Johnnie F. Hackley, Pamela Curry, Kaye Talbitzer, and Phyllis Anding.

Sources of Funding

The present study was supported by National Institutes of Health grants PO-1-HL-62222 and RO-1-HL-38690. Dr Gao was supported by a Scientist Development Grant from the American Heart Association National Center (award No. 0635007N).

Disclosures

None.

References


Exercise Training Normalizes Sympathetic Outflow by Central Antioxidant Mechanisms in Rabbits With Pacing-Induced Chronic Heart Failure
Lie Gao, Wei Wang, Dongmei Liu and Irving H. Zucker

Circulation. 2007;115:3095-3102; originally published online June 4, 2007; doi: 10.1161/CIRCULATIONAHA.106.677989
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/115/24/3095

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/