Exercise training enhances baroreflex sensitivity by an angiotensin II-dependent mechanism in chronic heart failure

Tarek M. Mousa, Dongmei Liu, Kurtis G. Cornish, and Irving H. Zucker

Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska

Submitted 5 June 2007; accepted in final form 12 December 2007

Mousa TM, Liu D, Cornish KG, Zucker IH. Exercise training enhances baroreflex sensitivity by an angiotensin II-dependent mechanism in chronic heart failure. J Appl Physiol 104: 616–624, 2008. First published December 13, 2007; doi:10.1152/japplphysiol.00601.2007.—Exercise training (EX) has become an important modality capable of enhancing the quality of life and survival of patients with chronic heart failure (CHF). Although 4 wk of EX in animals with CHF evoked a reduction in renal sympathetic nerve activity and ANG II plasma levels and an enhancement in baroreflex sensitivity at rest (Liu JL, Irvine S, Reid IA, Patel KP, Zucker IH, Circulation 102: 1854–1862, 2000; Liu JL, Kulakofsky J, Zucker IH, J Appl Physiol 92: 2403–2408, 2002), it is unclear whether these phenomena are causally related. CHF was induced in rabbits by ventricular pacing (360–380 beats/min) for 3 wk. CHF rabbits were EX for 4 wk at 15–18 m/min, 6 days/wk, 30–40 min/day. Three groups of rabbits were studied: CHF (with no EX), CHF-EX, and CHF-EX + ANG II infusion [in which ANG II levels were kept at or near levels observed in CHF (non-EX) rabbits by subcutaneous osmotic minipump infusion]. EX prevented the increase in plasma ANG II levels shown in CHF rabbits. CHF and CHF-EX + ANG II infusion rabbits had significantly depressed baroreflex sensitivity slopes (P < 0.01 for sodium nitroprusside and P < 0.001 for phenylephrine) and higher baseline renal sympathetic nerve activities than CHF-EX animals. EX downregulated mRNA and protein expression of ANG II type 1 receptors in the rostral ventrolateral medulla in CHF rabbits. This was prevented by ANG II infusion. These data are consistent with the view that the reduction in sympathetic nerve activity and the improvement in baroreflex function in CHF after EX are due to the concomitant reduction in ANG II and angiotensin receptors in the central nervous system.

SYMPATHOEXCITATION is a well-documented consequence of severe chronic heart failure (CHF) (14). Initially, the sympathoexcitatory excitation that occurs in CHF state is beneficial because it acts as a protective mechanism to maintain arterial blood pressure, and hence peripheral tissue perfusion, in the face of reduced cardiac output. However, chronic stimulation of neurohumoral pathways ultimately becomes deleterious and results not only in a progressive downward spiral of cardiovascular depression but also has been shown to be prognostically significant for increased complications and sometimes death (15). Most of the modern therapeutic interventions for CHF have targeted the sympathetic nervous system and the renin-angiotensin system with beta blockers, angiotensin-converting enzyme inhibitors, angiotensin II (ANG II) receptor blockers, and aldosterone receptor antagonists. Over the past several years, exercise training (EX) has become a therapeutic modality capable of enhancing the quality of life of patients with CHF (9, 10, 34, 55). Recent data on a small number of patients with CHF have shown that a long-term EX program prolongs life and reduces adverse effects, including hospitalization (2, 38). Nevertheless, the mechanism(s) by which EX is beneficial to patients with CHF is not well understood. In the normal condition, EX increases cardiac vagal tone and reduces sympathetic outflow at rest (8, 12, 21). On the other hand, variable changes in arterial baroreflex sensitivity (BRS) have been reported to be reduced (32, 43, 49, 51) in subjects after a course of EX. Few data exist on the role of EX in the modulation of neurohumoral function in CHF. Recent data from our laboratory (26, 30) showed that, in rabbits with CHF, EX reduced renal sympathetic nerve activity (RSNA) and plasma ANG II levels. In addition, BRS was improved by EX in CHF rabbits compared with CHF rabbits without EX (26). Furthermore, EX augmented vagal efferent tone in CHF rabbits (30).

Although it seems clear that EX in the CHF state evokes a reduction in sympathetic nerve activity and plasma ANG II, it is still not known whether these phenomena have cause and effect relationships. The available data do not differentiate between a primary effect of ANG II on lowering sympathetic nerve activity in CHF after EX and a secondary effect of RSNA in lowering plasma ANG II. The present study was designed to address the question of whether the reduction in ANG II levels and/or central ANG II type 1 (AT1) receptors is responsible for the reduction in sympathoexcitation and the improvement of the BRS evoked by EX in the setting of CHF. Thus we reasoned that maintenance of plasma ANG II (“ANG II clamp”) in CHF-EX rabbits would prevent many of these beneficial effects of EX. It is worth noting that such changes are observed in the resting state after a 4-wk EX protocol. Alterations in these parameters “during” acute exercise before and after the EX period in CHF have not been examined in this or in previous studies with this model.

METHODS

Animals

Experiments were carried out on 23 male New Zealand White rabbits weighing between 3 and 4 kg. All 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.

Address for reprint requests and other correspondence: I. H. Zucker, Dept. of Cellular and Integrative Physiology, 985850 Nebraska Medical Center, Omaha, NE 68198-5850 (e-mail: izucker@umc.edu).

Submitted 5 June 2007; accepted in final form 12 December 2007

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.
Surgery and Instrumentation

All rabbits underwent sterile thoracic instrumentation as described previously (29). A platinum wire pacing electrode was sutured to the epicardium of the left ventricle in all rabbits. A ground electrode was secured to the left atrium. All wires were tunneled beneath the skin and exited in the midscapular area. The chest was closed and evacuated; in the same setting, a radiotelemetry unit (Data Sciences International) was implanted into the right femoral artery with the tip of its catheter in the descending aorta to monitor blood pressure and heart rate (HR) in the conscious state. Rabbits were allowed to recover from surgery for 2 wk before they were entered into the study.

Induction of Heart Failure

A rapid pacing model of CHF was prepared as described previously (30). In brief, after recovery from surgery, animals were paced at a rate of 360–380 beats/min with the use of a small, light-weight pacing unit of our own design. The pacing rate was adjusted and monitored by frequent echocardiograms (Acuson Sequoia 512 C). In general, each rabbit was paced at 360 beats/min for the first week to determine whether it would tolerate this protocol. After the first week, the pacing rate was increased to 380 beats/min and continued at this rate for the remainder of the protocol. The rabbits were continuously paced for 3 wk. Cardiac dimensions (left ventricular end-diastolic diameter, left ventricular end-systolic diameter, fractional shortening, and ejection fraction) and other hemodynamic parameters were monitored on a weekly basis. In addition to left ventricular dimension changes, clinical signs of CHF such as ascites, pulmonary congestion, and cachexia were appreciated as symptoms of this CHF model.

RSNA

The renal sympathetic nerve recording electrodes were implanted as described earlier (28, 42). In brief, the left kidney was approached via a flank incision, and the renal artery and nerves were identified. After a small portion of several nerve fibers was isolated from the surrounding tissues, Teflon-coated (except at the distal 1–2 mm) wire electrodes were wrapped around the 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 (QuickSil, World Precision Instruments). The electrode wires were then tunneled beneath the skin and exited in the midscapular area. Experiments were carried out in the conscious state 2–3 days after renal nerve electrode implantation.

Exercise Protocol

Rabbits were EX on a motor-driven treadmill for a total of 30–40 min/day, 6 days/wk for a total period of 4 wk. A warm-up period of 5 min at 5 m/min was followed by peak exercise (15–18 m/min) for 30 min, which was followed by a cool down of an additional 5 min at 5 m/min whenever possible. Rabbits were started on the EX protocol 1 wk before initiation of pacing to acquaint them with the treadmill and the general protocol.

Measurement of Plasma ANG II Levels

Blood samples were collected from the central ear artery after local anesthesia with 2% lidocaine. Care was taken to ensure that the rabbits were not excited during blood sampling. Three milliliters of arterial blood were drawn into iced heparinized tubes at the beginning of the experiment. Each tube contained 1,250 KIU aprotinin in 2.5 ml of blood. The blood was centrifuged at 4,000 rpm at 4°C. The plasma was frozen at −70°C until assayed. ANG II was measured with a radioimmunoassay as described previously (44). Plasma ANG II levels were measured, and the values before and after development of CHF were compared. The sensitivity of the assay was 2 pg/tube.

Western Blot Analysis of AT1 Receptors

On termination of the experiment, rabbits were anesthetized, and brains were rapidly removed and frozen on dry ice. Tissues from the rostral portion of the ventrolateral medulla (RVLM), hypothalamus, and cerebral cortex were blocked off as previously described (16) and homogenized with a homogenizer in radiolmmunoprecipitation assay buffer. Protein extraction from homogenates was used for Western blot analysis for the rabbit AT1 receptor. The protein concentration was measured with a protein assay kit (Pierce, Rockford, IL). Samples were adjusted to the same concentration of protein, mixed with equal volumes of 2X 4% SDS sample buffer, and then boiled for 5 min, followed by loading on the 7.5% SDS-PAGE gel (5 μg protein/30 μl per well) for electrophoresis using a Bio-Rad mini gel apparatus at 40 mA/each gel for 45 min. The fractionized proteins on the gel were then electrophoretically transferred onto the PVDF membrane (Millipore) at 300 mA for 90 min. The membrane was probed with primary antibody (rabbit anti-human AT1-receptor polyclonal antibody). Five brains from normal rabbits were similarly analyzed and served as a control for AT1-receptor expression. No hemodynamic data for these rabbits were obtained.

RT-PCR Analysis of AT1 Receptors

As indicated above, tissue punches were taken from the RVLM, hypothalamus, and cerebral cortex as described previously (16). In
addition, the hypothalamus was blocked off, and a portion of the cerebral cortex was removed. Total RNA of these tissues was isolated by means of the RNeasy Mini-Kit total RNA isolation system (Qiagen), after which cDNA was synthesized by means of Maloney murine leukemia virus reverse transcriptase (Invitrogen Life Technologies). PCR amplification was performed by means of a PTC-100 programmable thermal controller (MJ Research) as follows: 1 cycle at 95°C for 15 min, followed by 35 cycles of 94°C for 45 s, 55°C for 1 min. The primer pairs were based on the cDNA sequences of rabbit AT1 receptor (GenBank S59041), with \( 5'\)-GCCAGCCAGCA-3' and \( 5'\)-TTTGGGAACAGCTTGGCGGT-3' as an internal control. The primer pairs were those shown in CHF rabbits from a previous study from our laboratory (26). From our prior findings, that our EX protocol “normalizes” plasma ANG II levels, we used the same determined dose of ANG II infusion in the CHF-EX rabbits. ANG II infusion was started 2 wk after the initiation of pacing. This time period was chosen because our preliminary experiments showed that plasma ANG II levels rise at approximately this time after pacing in the sedentary animals.

On the day of the experiment, the rabbit was placed in a Plexiglas box, and the pacemaker was turned off 30 min before data collection. After the animal had adjusted to the environment and all hemodynamic parameters were stabilized, baseline recordings of RSNA, arterial blood pressure, and HR were taken for several minutes. Maximal RSNA was determined in each rabbit by observing its response to 50 ml of cigarette smoke delivered into the external nares with a syringe (42).

Evaluation of baroreflex function was carried out as described earlier (27, 28). Arterial baroreflex control of HR and RSNA was determined by the response of these parameters to an infusion of sodium nitroprusside (SNP; 100 \( \mu g/kg \) iv) and phenylephrine (PE; 80 \( \mu g/kg \) iv) at a rate of 0.5 ml/min. This infusion rate changed pressure in either direction at a rate of 1–2 mmHg/s. When mean arterial pressure reached its nadir (usually 40–50 mmHg), the SNP infusion was stopped and the animal was allowed to return to baseline hemo-

### Table 1. Baseline hemodynamics and echo data from different rabbit groups before and after continuous ventricular pacing for 3 wk

<table>
<thead>
<tr>
<th></th>
<th>Prepace (n = 29)</th>
<th>CHF (n = 9)</th>
<th>CHF-EX (n = 10)</th>
<th>CHF-EX + ANG II (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>3.7±0.1</td>
<td>3.7±0.1</td>
<td>3.7±0.1</td>
<td>3.8±0.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>215.6±6.2†</td>
<td>243.1±6.5</td>
<td>217.8±2.7*</td>
<td>230.7±9.2</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>72.2±2.4</td>
<td>64.7±3.9</td>
<td>64.9±2.6</td>
<td>72.2±3.7</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>0.8±0.1†</td>
<td>1.7±0.1</td>
<td>1.6±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>9.9±1.5</td>
<td>9.9±1.5</td>
<td>6.4±1.4</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>77.4±1.5‡</td>
<td>56.1±4.2</td>
<td>50.9±2.2</td>
<td>55.5±2.9</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>39.7±1.8‡</td>
<td>22.9±3.6</td>
<td>20.4±2.5</td>
<td>21.1±3.9</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>17.9±0.5</td>
<td>17.5±0.5</td>
<td>17.5±0.5</td>
<td>17.6±0.4</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>12.9±0.7</td>
<td>13.3±0.5</td>
<td>13.3±0.5</td>
<td>12.9±0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n = no. of rabbits. CHF, chronic heart failure; EX, exercise training; MAP, mean arterial pressure; LVEDD, left ventricular end-diastolic pressure; LVESD, left ventricular end-systolic diameter; LV/BW, left ventricle-to-body weight ratio. *P < 0.05 compared with CHF group. †P < 0.01 compared with CHF. ‡P < 0.001 compared with all CHF groups.
dynamic levels before the PE infusion was started and continued until mean arterial pressure reached ~110 mmHg. The BRS was analyzed over the pressure range from the lowest to the highest every 2 s from the threshold to the saturation point. Individual linear regression curves were applied on the SNP and the PE responses. Baroreflex control of HR was assessed before and after the 3-wk pacing protocol, whereas the baroreflex control of RSNA was assessed at the end of the 3-wk pacing protocol because of the difficulty in maintaining RSNA for long periods of time. These experiments were carried out on the day after the last EX session (i.e., ~24 h after EX).

Data Analysis

RSNA determination. All parameters were recorded with a data-acquisition and analysis system (PowerLab model 8S; ADInstruments). Hemodynamic parameters were digitized at 1,000 samples/s. RSNA was digitized at 2,000 samples/s after preamplification with a Grass P15 preamplifier with the bandwidth set between 3 Hz and 1 kHz. The raw nerve activity was rectified and integrated. In addition, the frequency of nerve activity was determined by setting a cursor just above the noise level so that all spikes that crossed the cursor were counted. Both frequency and integrated nerve activity were recorded continuously along with the raw nerve activity. Integration was carried out using the PowerLab system with the time constant set at 0.2 s. The baseline sympathetic nerve activity was expressed as a percentage of maximum-inducible RSNA (induced with smoke).

Echocardiography. Echocardiographic measurements were made with an Acuson Sequoia 512C echocardiograph with a 4-MHz probe to determine left ventricular function during the development of heart failure. The point of reference for the diameter measurement was determined in the two-dimensional mode. The parameters of left ventricular function were determined in the M-mode.

Euthanasia. Before rabbits were euthanized, measurements of left ventricular end-diastolic pressure (LVEDP) were carried out in all groups under anesthesia using a cocktail of 20 mg/kg ketamine, 2.5 mg/kg xylazine, and 0.2 mg/kg acepromazine.

Fig. 3. Original recordings of arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), RSNA, RSNA frequency, and RSNA integrated nerve activity in conscious CHF-EX (A) and CHF-EX-ANG II (B) animals showing baseline parameters and the response to intravenous sodium nitroprusside (SNP) infusion (arrows).
Probability value of appropriate. Post hoc analysis consisted of the Bonferroni test. A by a one-way or two-way ANOVA for repeated measures whenever within groups was used; differences between groups were determined.

RESULTS

Statistical Analysis

Data are expressed as means ± SE. A paired t-test for comparisons within groups was used; differences between groups were determined by a one-way or two-way ANOVA for repeated measures whenever appropriate. Post hoc analysis consisted of the Bonferroni test. A probability value of $P < 0.05$ was considered statistically significant.

Baseline Hemodynamics

Table 1 compares baseline hemodynamic parameters in normal, CHF, CHF-EX, and CHF-EX + ANG II groups. Because all animals were in the same state before pacing, their data have been grouped in the prepaced state. There were no statistical differences in any prepaced variable between groups. EX significantly lowered resting HR compared with that shown in CHF non-EX rabbits ($243.1 ± 6.5$ vs. $217.8 ± 2.7$ beats/min; $P < 0.05$). CHF rabbits in either group showed significant increases in resting HR, left ventricular weight/body weight, LVEDP, left ventricular end-diastolic diameter, and left ventricular end-systolic diameter and exhibited significant reductions in ejection fraction and fractional shortening. Although EX lowered resting HR, there was no significant effect on apparent cardiac function. Note that LVEDP was measured in the anesthetized state with the aforementioned anesthesia, whereas all other hemodynamic parameters were measured in the conscious state. Because no normal rabbits were included in the physiological experimental protocol, we do not have data on LVEDP in this group. However, data from other studies from our laboratory have shown LVEDP in normal rabbits at comparable time periods to be between $0.7 ± 1.3$ and $4.6 ± 1.5$ mmHg (16, 26).

Plasma ANG II Levels

After 3 wk of pacing, as illustrated in Fig. 2, CHF rabbits showed significantly elevated levels of plasma ANG II vs. the prepaced levels ($19.4 ± 3.5$ vs. $134 ± 46.1$ pg/ml, $P < 0.05$; Fig. 2, top), whereas in CHF-EX rabbits there was a complete abolition of this hyperangiotensinemia ($25.1 ± 7.2$ vs. $18.7 ± 2.4$ pg/ml, $P > 0.05$; Fig. 2, middle). In the CHF-EX + ANG II infusion group, ANG II levels again exhibited a significant elevation compared with that shown at prepaced levels ($20.9 ± 6.2$ vs. $150.2 ± 85.1$ pg/ml, $P < 0.05$; Fig. 2, bottom) but not compared with ANG II levels in the CHF group ($134 ± 46.1$ vs. $150.2 ± 85.1$ pg/ml, $P > 0.05$). No significant difference in the plasma ANG II level was observed among all groups ($P > 0.05$).

Baseline RSNA

Figure 3 shows original recordings of RSNA in conscious rabbits. Mean data for baseline RSNA (expressed as a percentage of the maximum activity induced by cigarette smoke) are shown in Fig. 4. EX significantly lowered RSNA in CHF compared with that shown in the CHF non-EX group, whereas “clamping” ANG II levels in CHF-EX resulted in a significantly higher baseline RSNA.

Arterial Baroreflex Control of RSNA

Figure 3 shows the neural and blood pressure responses during SNP infusion used to evaluate one component of the BRS. The increases in RSNA and HR appear to be blunted in the ANG II-infused rabbit (compare Fig. 3B vs. Fig. 3A).

As shown in Fig. 5, EX in CHF rabbits enhanced the sensitivity of the hypotensive (SNP) component of baroreflex control of RSNA compared with either CHF rabbits or CHF-EX rabbits with ANG II infusion (Fig. 5, left). Although EX in CHF rabbits did not have a significant effect on the hypertensive (PE) component of baroreflex control of RSNA compared with CHF rabbits, ANG II infusion in the CHF-EX rabbits resulted in a significant blunting of the sensitivity of the baroreflex compared with CHF-EX rabbits (Fig. 5, right).

Arterial Baroreflex Control of HR

Figure 6 shows the mean BRS data in the various groups. In the CHF (Fig. 6A) and the CHF-EX-ANG II (Fig. 6C) rabbits, there was a depressed BRS compared with prepaced data for both SNP and PE. On the other hand, EX (Fig. 6B) prevented this depression in the BRS.

Comparisons in postpace CHF and CHF-EX + ANG II rabbits failed to show statistical significance ($P > 0.05$).

AT1-Receptor Expression

As shown by AT1-receptor mRNA expression (Fig. 7, top) and Western blot (Fig. 7, bottom), a similar pattern of significant upregulation in the RVLM of CHF rabbits occurred compared with that shown in normal rabbits ($P < 0.01$). The normal brain tissue shown in Fig. 7 was obtained for AT1-receptor expression from rabbits for sake of comparison; however, no hemodynamic data were included in this group. Similar data were observed in the hypothalamus (data not shown). EX nearly normalized this upregulation ($P < 0.05$). When CHF results were compared with CHF-EX results, systemic ANG II infusion is shown to be capable of reversing this effect and AT1-receptor expression was again ($P < 0.05$) upregulated in CHF-EX + ANG II compared with CHF-EX animals. RT-PCR and Western blot for AT1 receptors from the cerebral cortex showed no significant differences among groups (data not shown).

DISCUSSION

In a previous study from our laboratory (26), we showed a significant effect of EX on ANG II levels, baseline RSNA, and baroreflex function in an experimental model of CHF, obser-
vations that were reproduced in the present study. The previous findings strongly suggested a relationship among these parameters, yet they did not differentiate between a primary effect of ANG II on lowering sympathetic nerve activity in CHF-EX rabbits and a secondary effect of RSNA to prevent the increase in plasma ANG II. In this study, we maintained plasma ANG II ("ANG II clamp") during EX in CHF rabbits at a level similar to that seen in CHF non-EX rabbits. This procedure prevented the reduction in RSNA and the normalization of baroreflex function in EX rabbits. These data provide evidence that reduced ANG II levels after EX mediate the reduced RSNA and improved baroreflex function and hence play a significant role in the beneficial effects of EX in the CHF state.

There is clear evidence that the renin-ANG II system plays an important role in the pathogenesis and progression of the CHF state (14, 15). Not only does ANG II participate in the cardiac hypertrophic process, it also plays an important role in the sympathetic neural activation that characterizes CHF (45) and eventually has a detrimental effect that contributes to the downward spiral of ventricular function in CHF (7, 39).

High levels of plasma ANG II have been shown to positively correlate with the severity of CHF (18) and are elevated late in the rapid pacing model of CHF (31). In two studies published from our laboratory (26, 30), EX prevented the rise in plasma ANG II in CHF rabbits compared with that seen in sedentary CHF rabbits; however, EX in normal rabbits did not alter plasma ANG II, baseline RSNA, or BRS.

In a study in normal rabbits subjected to EX for 8 wk, albeit at an exercise level higher than for rabbits in the present study, DiCarlo and Bishop (11) showed decreases in BRS for both HR and RSNA. In hypertensive rats, baroreflex function was improved after EX (4, 22), even in the normotensive rat counterparts. It is not clear why there was little effect of EX in normal rabbits in our previous studies (26, 30). This may be indicative of the low resting RSNA and plasma ANG II levels in the normal conscious state.

EX in patients with CHF increases endurance, quality of life (35, 55), and, of most importance, survival (2, 40, 41). In addition, EX has been shown to mediate several important effects on autonomic outflow, such as a reduction in sympathetic nerve activity (26, 47) and an increase in cardiac vagal outflow (50), with the latter contributing importantly to a decreased baseline HR after EX. This finding may bear on the observation of Williamson and Raven (56) in which they showed that there was a leftward shift of the R-R interval-carotid sinus pressure relationship without an alteration in baroreflex gain in normal individuals with EX.

The fact that infusion of ANG II prevented the apparent reduction in RSNA due to EX is significant because a close correlation exists between RSNA and renal venous norepinephrine concentration (37), and changes in sympathetic outflow to different vascular beds are, for the most part, directionally similar (53) at rest. Furthermore, studies by Coats et al. (6), Tyni-Lenne et al. (52) in humans, and by Liu et al. (26, 30) in rabbits strongly suggest a role for EX in reducing sympathetic activity and increasing vagal tone in CHF.

The mechanism(s) for the apparent beneficial effects of EX in CHF is still not well understood. EX increases oxygen consumption in patients with CHF, minimizes ventricular wall...
Interestingly, these conditioning-related changes observed with EX in the CHF state may be important in addition to the neurohumoral adjustments that we and others have reported (3). The precise mechanism by which EX normalizes plasma ANG II is not clear. Although completely speculative, perhaps other areas of the brain may influence the vagal baroreflex response in rats with CHF (13). Moreover, there is evidence that it is associated with an attenuated stress (9), and improves endothelial function (1, 19, 54) and skeletal muscle metabolic function (36).

Indeed, these conditioning-related changes observed with EX in the CHF state may be important in addition to the neurohumoral adjustments that we and others have reported (3). The precise mechanism by which EX normalizes plasma ANG II is not clear. Although completely speculative, perhaps other areas of the brain may influence the vagal baroreflex response in rats with CHF (13). Moreover, there is evidence that it is associated with an attenuated stress (9), and improves endothelial function (1, 19, 54) and skeletal muscle metabolic function (36).

Fig. 7. Top: representative and mean data showing the effect of exercise training on AT1-receptor (AT1R) mRNA expression in the rostral portion of the ventrolateral medulla (RVLM). M, marker; N, normal; H, chronic heart failure. *P < 0.05. Bottom: representative and mean data of Western blots showing that EX downregulates AT1-receptor protein expression in the RVLM of CHF rabbits, which was reversed by systemic ANG II infusion. *P < 0.05, **P < 0.01.

preparation reduced the baroreflex response to PE, whereas metoprolol had little effect, suggesting that the cardiac baroreflex effects of ANG II are mediated by a vagal mechanism.

In the present study, why EX did not enhance the PE component of the baroreflex control of RSNA but ANG II infusion blunted it is not completely clear. However, one explanation may be that this is due to a baseline RSNA before PE infusion in the CHF-EX animals lower than that shown in CHF-EX + ANG II-infused animals. Another explanation could be that ANG II infusion increased sympathetic nerve activity in a nonspecific manner, contributing to a greater blunting of BRS. This is supported by the finding that the baroreflex control of HR was blunted to a greater degree with ANG II infusion in CHF-EX than in CHF non-EX animals. A recent study by Rondon et al. (47) showed that EX improved baroreflex control of RSNA for both increases and decreases in blood pressure; however, we did not see an effect for decreases in blood pressure in the present study. Rondon et al., however, assessed the BRS using the “spontaneous technique,” where data was incorporated into software that detected at least three or more adjacent systolic blood pressure impulses whose intervals were plotted against their subsequent amplitudes and the regression average slope was termed the spontaneous BRS. In short, the technique that they used to assess the BRS included both the upward slope and the downward slope sequences. In severe heart failure, AT1 receptors are increased in areas such as the RVLM and paraventricular nucleus (59). Similar data have been presented by Yoshimura et al. (57) in rats with high-output heart failure. These findings are reproduced by the results of the present study. Our findings of the effect of EX on AT1-receptor protein and mRNA expression are unique and, to our knowledge, have not been shown before. They positively correlate with plasma ANG II, and the finding that these receptors were upregulated with systemic ANG II infusion points to the fact that high levels of circulating ANG II act as the stimulus for an upregulation in brain AT1 receptors, especially in the RVLM and hypothalamus where central sympathetic flow is processed and mediated. Although the augmented expression of AT1 receptors in the RVLM and hypothalamus suggests that systemic ANG II reached the brain, the most definitive evidence would have been measurement of ANG II itself in brain tissue. Nevertheless, there is evidence that systemic circulating ANG II can cross certain areas in which the blood-brain barrier is absent or weak, namely, the area postrema, the subfornical organ, and the organum vasculosum of the lamina terminalis (33). Our group (25) has recently shown that intracerebroventricular delivery of the AT1-receptor blocker losartan blocks receptor upregulation in the brain of CHF rabbits. This suggests that central ANG II mediates AT1-receptor upregulation (25). Data from our laboratory strongly suggest that increases in ANG II concentration mediates an upregulation of AT1-receptor expression (17) through activation of the transcription factor activator protein-1 (25).

The mechanisms by which alterations in AT1-receptor expression in the central nervous system lead to increases in sympathetic outflow are complicated, but we can speculate on the mechanism based on previous work in the literature and data from our laboratory. In transgenic mice that overexpress the AT1 receptor selectively in the brain, the response to intracerebroventricular ANG II is enhanced (23). These data suggest (although were not proven in that study) that AT1
receptors can mediate augmented sympathetic outflow. Evidence by Campese et al. (5) and by Zimmerman et al. (58) suggest that this increase is mediated by superoxide. We have previously shown that several of the subunits for NAD(P)H oxidase are also upregulated in the brain of animals with CHF and following intracerebroventricular infusion of ANG II (16, 17). Therefore, we believe that prevention of AT1-receptor upregulation in CHF is beneficial in mediating a decrease in sympathetic outflow by inhibition of a superoxide-NAD(P)H oxidase mechanism.

In summary, these data are consistent with the idea that EX lowers sympathetic nerve activity and normalizes the baroreflex control of both HR and RSNA in the CHF state through a reduction in ANG II levels and/or central ANG II receptors. The results of this study provide further insight into the mechanisms responsible for the beneficial effects of EX in the CHF state.

ACKNOWLEDGMENTS
The authors acknowledge the expert technical assistance of Johnnie F. Hackley, Pamela Curry, and Jodi Hallgren.

GRANTS
This study was supported, in part, by National Heart, Lung, and Blood Institute Grant PO-1 HL-062222. T. Mousa was supported by a postdoctoral fellowship from the American Heart Association, Heartland Affiliate.

REFERENCES


J Appl Physiol • VOL. 104 • MARCH 2008 • www.jap.org