Exercise Training Enhances Baroreflex Sensitivity by an Angiotensin II Dependent Mechanism in Chronic Heart Failure

Tarek M. Mousa, MD; Dongmei Liu, MD, PhD; Kurtis G. Cornish, PhD; Irving H. Zucker, PhD
Department of Cellular and Integrative Physiology
University of Nebraska Medical Center
Omaha, NE 68198-5850

Running title: Exercise Training in Heart Failure

Address for Correspondence:
Irving H. Zucker, Ph.D.
Department of Cellular and Integrative Physiology
985850 Nebraska Medical Center
Omaha, NE 68198-5850
(402) 559-7161
FAX: (402) 559-4438
Email: izucker@unmc.edu

Total word count: 7406

Abstract

Exercise training (EX) has become an important modality capable of enhancing the quality of life and survival of patients with chronic heart failure (CHF). While we have shown that 4 weeks of EX in CHF evokes a reduction in renal sympathetic nerve activity (RSNA), angiotensin II (Ang II) plasma levels, and an enhancement in baroreflex sensitivity (BRS) at rest it is unclear if these phenomena are causally related. CHF was induced in rabbits by ventricular pacing (360-380 bpm) for 3 weeks. CHF rabbits were EX for 4 wks at 15-18 m/min, 6 days/wk, 30-40 min/day. Three groups of rabbits were studied; CHF, CHF-EX, and CHF-EX where 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 in CHF rabbits. CHF non-EX and CHF-EX rabbits with Ang II infusion had a significantly depressed BRS slope (p<0.01 for SNP and p<0.001 for PE) and a higher baseline RSNA when compared to CHF-EX animals. EX down-regulated 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 following EX is due to the concomitant reduction in Ang II and angiotensin receptors in the central nervous system.

Keywords: sympathetic nerve activity; renal function; blood pressure; training
Introduction

Sympatho-excitation is a well documented consequence of severe, chronic heart failure (CHF) (14). Initially the sympatho-humoral excitation that occurs in the CHF state is beneficial, as it acts as a protective mechanism to maintain arterial blood pressure, and hence, peripheral tissue perfusion in the face of a reduced cardiac output. However, chronic stimulation of neuro-humoral pathways ultimately becomes deleterious and results not only in a progressive downward spiral of cardiovascular depression but also has been shown to be prognostic for increased complications and in some cases, death (15). Most of the modern therapeutic interventions for CHF have targeted the sympathetic nervous system and the renin-angiotensin system using beta blockers, ACE 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 have shown that a long term EX program prolongs life and reduces adverse effects including hospitalization in patients with CHF (2; 38). Nevertheless, the mechanism(s) by which EX is beneficial to patients with CHF is/are 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) has been reported to be reduced (32; 43; 49; 51) in subjects following a course of EX. There is a paucity of data on the role of EX in the modulation of neuro-humoral function in CHF. Recent data from our laboratory have shown 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 to CHF non-EX rabbits (26). Furthermore, EX augmented vagal efferent tone in CHF rabbits (30).

While 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 if 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 following 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 sympatho-excitation 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 week 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 using this model.

Methods

Animals

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

Figure 1 illustrates the timeline of the experimental protocol.

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, Inc.) was implanted into the right femoral artery with the tip of its catheter in the descending aorta in order to monitor blood pressure and the heart rate in the conscious state. Rabbits were allowed to recover from surgery for 2 wks before entering into the study.

Induction of heart failure
The rapid pacing model of heart failure was prepared as described previously (30). In brief, after recovery from surgery, animals were paced at a rate of 360-380 beats/min by using 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 wks. Cardiac dimensions (left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), fractional shortening (FS), and ejection fraction (EF)) 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.

Renal sympathetic nerve activity

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 isolating a small portion of several nerve fibers from the surrounding tissues, teflon-coated (except at the distal 1 to 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, Inc.). The electrode wires were then tunneled beneath the skin and exited in the midscapular area. Experiments were carried out in the conscious state 2 to 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 wks. 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 their exercise training protocol one week prior to initiation of pacing in order to acquaint them with the treadmill and the exercise 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 insure that the rabbits were not excited during blood sampling. Three milliliters of arterial blood was drawn into iced heparinized tubes at the beginning of the experiment. Each tube contained 1250 KIU aprotinin in 2.5 ml of blood. The blood was centrifuged at 4000 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**

Upon termination of the experiment, the rabbit was anesthetized and the brains 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 were homogenized with a homogenizer in radioimmunoprecipitation assay buffer. Protein extraction from homogenates was used for Western blot analysis for the rabbit AT1 receptor. The protein concentration was measured using 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 following by loading on the 7.5% SDS-PAGE gel (5µg protein/30µL per well) for electrophoresis using Bio-Rad mini gel apparatus at 40 mA/each gel for 45 min. Then the fractionized proteins on the gel were 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 minutes, followed by 35 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1 minute. The primer pairs were based on the cDNA sequences of rabbit AT1 receptor (GenBank-S59041), with β–Actin (GenBank-AF309819) as an internal control. The primer pairs were 5’-TTTGGGAACAGCTTGGCGGT-3’ and 5’-GCCAGCCAGCGCCAAATAA-3’ for AT-1 receptor. The bands were analyzed using UVP BioImaging Systems. As mentioned above, brains from normal rabbits were obtained for AT1 receptors expression analysis.

**Experimental protocol**
Three groups of rabbits were studied. Group I (n=9) was a CHF group. Group II (n=10) was a CHF EX group; Group III (n=10) was a CHF EX group that received an Ang II infusion via a subcutaneous osmotic minipump (Alzet) delivering Ang II at a dose of 50 ng/kg/min at a constant rate of 5 µl/hr. This infusion kept plasma Ang II close to levels seen in CHF non-EX rabbits. This dose of Ang II infusion was determined previously from preliminary experiments where we used different doses of Ang II delivered via an osmotic minipump in normal rabbits and measured plasma Ang II levels. We determined the dose of Ang II that would maintain a plasma Ang II level compared to those seen in CHF rabbits from previously published data from our laboratory (26). Based on our prior findings, that were later confirmed in this study, that our EX protocol “normalizes” plasma Ang II level we used the same determined dose of Ang II infusion in the CHF-EX rabbits. Ang II infusion was started 2 weeks after the initiation of pacing. This time period was chosen because our preliminary experiments showed that plasma Ang II levels began to rise at approximately this time following 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 minutes prior to data collection. After the animal had adjusted to the environment and all hemodynamic parameters were stabilized, baseline recordings of RSNA, arterial blood pressure (ABP), and heart rate (HR) were taken for several minutes. Maximal RSNA was determined in each rabbit by observing its response to 50 cc 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 µg/kg iv) and phenylephrine (PE;80 µg/kg iv) at a rate of 0.5 ml/min. This infusion rate changed pressure in either direction at a rate of 1-2 mm
Hg/second. When mean arterial pressure (MAP) reached its nadir (usually 40-50 mmHg) the SNP infusion was stopped and the animal was allowed to return to baseline hemodynamics before the PE infusion was started and continued until MAP reached approximately 110 mmHg. The baroreflex sensitivity (BRS) was analyzed over the pressure range from the lowest to the highest every 2 seconds from the threshold to the saturation point. Individual linear regression curves were applied on the SNP and the PE responses. Baroreflex control of heart rate 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 following the last EX session (ie. ~ 24 hrs after EX).

Data analysis

RSNA determination

All parameters were recorded with a data acquisition and analysis system (Powerlab model 8S; ADInstruments, Inc.). Hemodynamic parameters were digitized at 1000 samples/second. RSNA was digitized at 2000 samples/second 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 to 0.2 seconds. The baseline sympathetic nerve activity was expressed as a percent of maximum-inducible RSNA (induced with smoke).

Echocardiography
Echocardiographic measurements were made using an Acuson Sequoia 512 C 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 2D-mode. The parameters of left ventricular function were determined in the M-mode.

_Euthanasia_

Before euthanizing the rabbits, measurements of left ventricular end diastolic pressure (LVEDP) was carried out in all groups under anesthesia using a cocktail of 20 mg/kg ketamine, 2.5 mg/kg xylazine, and 0.05 mg/kg atropine. Euthanasia was carried out using intravenous pentobarbital. The heart was removed and weighed. Left ventricular weight/body weight (LV/BW) was calculated and used as an index of hypertrophy.

**Statistical Analysis**

Data are expressed as means ± SEM. A paired t test for comparisons within groups was used; differences between groups were determined by a 1-way or a 2-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.

**Results**

**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 prior to pacing, their data has been grouped in the pre-paced state. There were no statistical differences in any pre-pace variable between groups. EX significantly lowered resting HR compared to CHF non-EX rabbits (243.1±6.5 vs 217.8±2.7, p<0.05). CHF rabbits in either group showed a significant increase in
resting HR, LV/BW, LVEDP, LVEDD, and LVESD, and exhibited a significant reduction in EF & FS. Although EX lowered resting HR, there was no significant effect on apparent cardiac function. Note that LVEDP was measured in the anesthetized state using the aforementioned anesthesia whereas all other hemodynamic parameters were measured in the conscious state. Since no normal rabbits were included in the physiologic 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 mm Hg (16; 26)

**Plasma Ang II levels**

After 3 weeks of pacing, as illustrated in figure 2, CHF rabbits showed significantly elevated levels of plasma Ang II compared to the pre pace levels (19.4±3.5 pg/ml vs 134±46.1 pg/ml, p<0.05; panel A), whereas in CHF-EX there was a complete abolition of this hyperangiotensinemia (25.1±7.2 vs 18.7±2.4, p>0.05; panel B). In the CHF-EX group with Ang II infusion, Ang II levels again exhibited a significant elevation compared to the pre pace levels (20.9±6.2 vs 150.2±85.1, p<0.05; panel C) but not when compared to Ang II levels in the CHF group (134±46.1 vs 150.2±85.1, 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 an original recording of RSNA in conscious rabbits. Mean data for baseline RSNA (expressed as a percent of maximum nerve activity in response to oropharyngeal stimulation with smoke) are shown in figure 4. EX significantly lowered RSNA in CHF compared to 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 increase in RSNA and HR appears to be blunted in the Ang II infused rabbit (compare panel B to panel A).

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

**Arterial baroreflex control of heart rate**

Figure 6 shows the mean BRS data in the various groups. In the CHF (panel A) as well as in the CHF-EX-Ang II (panel C) rabbits there was a depressed BRS compared to pre-paced data for both SNP & PE. On the other hand, EX (panel B) prevented this depression in the BRS. Comparison across post pace rabbits in the CHF and the CHF-EX-Ang II groups failed to show statistical significance (p>0.05).

**AT1 receptor expression**

As shown in figure 7, the AT1 receptor mRNA expression (panel A) and the Western blot (panel B) showed a similar pattern of significant up-regulation in the RVLM in the CHF group compared to normal rabbits (p<0.01). The normal brain tissue shown in figure 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 up-regulation (p<0.05). When comparing CHF to CHF-EX, systemic
Ang II infusion was capable of reversing this effect and AT1 receptor expression was again (p<0.05) up regulated in CHF-EX-Ang II compared to CHF-EX. 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, observations that were reproduced in the current study. The previous findings strongly suggested a relationship among these parameters, yet 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 following EX mediates the reduced RSNA and improved baroreflex function and hence, plays 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, but 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 it has been shown that EX prevented the rise in plasma Ang II in CHF rabbits compared to sedentary CHF rabbits (26; 30) however, EX in normal rabbits did not alter plasma Ang II, baseline RSNA, or BRS.

In a study carried out in normal rabbits subjected to EX for 8 wks albeit at a higher exercise level than that of the rabbits in the present study, DiCarlo and Bishop (11) showed decreases in BRS for both HR and RSNA. Studies carried out in hypertensive rats showed improvement in baroreflex function after EX (4; 22) even in their normotensive 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 most important, 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). The latter contributing importantly to a decreased baseline HR following 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 exercise trained normal individuals.

The fact that infusion of Ang II prevented the apparent reduction in RSNA due to EX is significant since 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 stress (9) 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. While completely speculative, perhaps it is due to an improvement in renal blood flow due to a generalized enhancement in endothelial function, a reduction in baseline sympathetic nerve activity leading to decreased renal arterial vasoconstriction and renin release, or a change in the angiotensin converting enzyme (ACE) activity. More studies into the mechanism by which EX reduces plasma Ang II concentration are warranted.

Whereas, Ang II has been shown to reduce BRS (45; 48); (24), there is evidence that it is associated with an attenuated vagal baroreflex response in rats with CHF (13). Moreover, central Ang II acting in the nucleus of tractus solitarii and, perhaps other areas of the brain may influence the vagal component of the BRS (20), a finding that was supported by Reid and Chou (46) by showing that atropine in a conscious rabbit 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 current study, the finding that EX did not enhance the PE component of the baroreflex control of RSNA while Ang II infusion blunted it is not completely clear. However, one explanation may be that this is due to a lower baseline RSNA before PE infusion in the
CHF-EX animals compared to CHF-EX-Ang II infused animals. Another explanation could be that Ang II infusion increased sympathetic nerve activity in a non-specific 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 compared to the CHF non-EX group. A recent study by Rondon et al. (47) showed that EX improves baroreflex control of RSNA for both increases and decreases in BP whereas we did not see an effect for decreases in BP in the present study. However, in that study they assessed the BRS using the “spontaneous technique” where data was incorporated into software that detected at least 3 or more adjacent systolic blood pressure impulses whose intervals were plotted against their subsequent amplitude and the regression average slope was termed the spontaneous BRS. In short, the technique they used to assess the BRS included both the upward slope and the downward slope sequences collectively.

In severe heart failure AT1 receptors are increased in areas such as the RVLM and paraventricular nucleus (PVN)(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 current 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 up-regulated with systemic Ang II infusion points to the fact that high levels of circulating Ang II acts as the stimulus for an up-regulation 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 suggest 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). We have recently shown that intracerebroventricular delivery of the AT₁ receptor blocker losartan, blocks receptor up-regulation in the brain of CHF rabbits. This suggests that central Ang II mediates AT₁ receptor up-regulation(25). Data from our laboratory strongly suggest that increases in Ang II concentration mediates an up-regulation 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 leads to an increase 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 over express the AT₁ receptor selectively in the brain the response to icv 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 up-regulated in the brain of animals with CHF and following icv infusion of Ang II(16; 17). Therefore, we believe that prevention of the AT₁ receptor up-regulation 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.
References


Acknowledgements

This study was supported, in part, by a grant from the National Heart Lung and Blood Institute (PO-1 HL062222). Dr. Mousa was supported by a postdoctoral fellowship from the American Heart Association, Heartland Affiliate. The authors would like to acknowledge the expert technical assistance of Johnnie F. Hackley, Pamela Curry, and Jodi Hallgren.
Figure legends

**Figure 1.** Experimental protocol: Exercise training (EX) started 1 week before starting pacing. Before pacing started an echo was done, plasma angiotensin II (Ang II) was measured, and baroreflex sensitivity (BRS) assessed. Pacing continued throughout 3 weeks (at 360 bpm over the first week then 380 thereafter) along with EX. In the CHF-EX-Ang II group, Ang II was infused over the third week of pacing. At the end of the 3 weeks of pacing and EX an echo was done, plasma Ang II was measured, renal sympathetic nerve activity (RSNA) & BRS assessed, left ventricular end diastolic pressure (LVEDP) measured, and central angiotensin II type 1 (AT₁) receptors measured.

**Figure 2.** Plasma Ang II in CHF (panel A), CHF-EX (panel B), and CHF-EX-Ang II (panel C) rabbits in the pre and post paced states. *p<0.05 compared to pre pace*

**Figure 3.** An original recording of arterial pressure (AP), mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), RSNA frequency, and RSNA integrated nerve activity in conscious CHF-EX (panel A) and CHF-EX-Ang II (panel B) animals showing baseline parameters and the response to intravenous SNP infusion (arrows).

**Figure 4.** Baseline RSNA expressed as a percent of the maximum activity induced by cigarette smoke. †p<0.01 compared to CHF-EX, ‡p<0.001 compared to CHF-EX
**Figure 5.** Linear slopes of baroreflex control of RSNA in response to SNP & PE in CHF, CHF-EX, and CHF-EX-Ang II groups. ‡ p<0.001 compared to CHF & CHF-EX-Ang II, *p<0.05 compared to CHF-EX

**Figure 6.** Linear slopes of baroreflex control of HR in response to SNP & PE before and after inducing CHF (panel A), CHF-EX (panel B), and CHF-EX-Ang II (panel C). *p<0.01, **p<0.001 compared to pre pace

**Figure 7.** *Panel A.* Representative and mean data showing the effect of exercise training on AT₁ receptor mRNA expression in the RVLM. M: marker; N: normal; CHF: heart failure; EX: exercise training; Ang II: Ang II infusion. *p<0.05

*Panel B.* Representative and mean data of Western blots showing that EX down-regulates AT₁ receptor protein expression in the RVLM of CHF rabbits that was reversed and its reversal by systemic Ang II infusion. *p<0.05
Table 1

Baseline hemodynamics and echo data from different groups pre and post continuous ventricular pacing for 3 weeks

<table>
<thead>
<tr>
<th></th>
<th>PrePace (n=29)</th>
<th>CHF (n=9)</th>
<th>CHF-EX (n=10)</th>
<th>CHF-EX-AngII (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt (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>HR (bpm)</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 (mm Hg)</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 (mm Hg)</td>
<td>9.9±1.5</td>
<td>6.4±1.4</td>
<td>7.6±0.9</td>
<td></td>
</tr>
<tr>
<td>EF (%)</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>FS (%)</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>14.9±0.4‡</td>
<td>17.9±0.5</td>
<td>17.5±0.5</td>
<td>17.6±0.4</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>8.5±0.3‡</td>
<td>12.9±0.7</td>
<td>13.3±0.5</td>
<td>12.9±0.5</td>
</tr>
</tbody>
</table>

* p<0.05 compared to CHF † p<0.01 compared to CHF ‡ p<0.001 compared to all CHF

HR, Heart rate; MAP, Mean arterial pressure; LVEDP, Left ventricular end diastolic pressure; EF, Ejection fraction; FS, Fractional shortening; LVEDD, Left ventricular end diastolic diameter; LVESD, Left ventricular end systolic diameter; LV/BW, Left
Figure 1

1st wk
2nd wk
3rd wk
4th wk

Start pace
Start EX

360 bpm
380 bpm
380 bpm

30-40 min/day, 6 days/week, 15-18 m/min

Echo
Plasma Ang II
BRS

Echo
Plasma Ang II
RSNA
BRS
LVEDP
AT₁ receptors

Ang II infusion
Figure 2

A

\[ \text{pg/ml} \]

\[ \begin{align*}
\text{CHF} & : & 0 & \quad \text{Pre Pace} \\
& & \text{Bar} & \quad \text{Post Pace}
\end{align*} \]

B

\[ \text{pg/ml} \]

\[ \begin{align*}
\text{CHF-EX} & : & 0 & \quad \text{Pre Pace} \\
& & \text{Bar} & \quad \text{Post Pace}
\end{align*} \]

C

\[ \text{pg/ml} \]

\[ \begin{align*}
\text{CHF-EX-Ang II} & : & 0 & \quad \text{Pre Pace} \\
& & \text{Bar} & \quad \text{Post Pace}
\end{align*} \]

\[ * p < 0.05 \text{ compared to pre pace} \]
Figure 3

A. CHF-EX

B. CHF-EX-Ang II

[Graphs showing blood pressure, heart rate, and other physiological parameters for CHF-EX and CHF-EX-Ang II conditions, with SNP as a point of reference.]
Figure 4

Baseline RSNA (% of max)

CHF | CHF-EX | CHF-EX-Ang II

† ‡
Figure 5

[Diagram showing the BRS gain (% max/mmHg) for SNP and PE in CHF, CHF-EX, and CHF-EX-Ang II groups.]

- SNP: CHF < CHF-EX < CHF-EX-Ang II
- PE: CHF-EX-Ang II > CHF-EX > CHF

Significance indicated by asterisks (*).
Figure 6

A

CHF

BRS gain (bpm/mmHg)

SNP

PE

*

B

CHF-EX

BRS gain (bpm/mmHg)

SNP

PE

C

CHF-EX-Ang II

BRS gain (bpm/mmHg)

SNP

PE

* *

Legend:
- Pre pace
- Post pace

Legend for B and C:
- * P < 0.05
- ** P < 0.01

Legend for A:
- * P < 0.05
Figure 7

A

600bp →

100bp →

AT1 receptor

B-Actin

B

AT1R

ß-Tubulin

Protein Expression (AT1R ß-tubulin)

Normal  CHF  CHF + Ex  CHF + Ex + AgII

Normal  CHF  CHF + Ex  CHF + Ex + AgII

mRNA Expression (AT1R ß-tubulin)

Normal  CHF  CHF + Ex  CHF + Ex + AgII