Effects of exercise training on SFO-mediated sympathoexcitation during chronic heart failure

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Llewellyn TL, Sharma NM, Zheng H, Patel KP. Effects of exercise training on SFO-mediated sympathoexcitation during chronic heart failure. Am J Physiol Heart Circ Physiol 306: H121–H131, 2014. First published October 25, 2013; doi:10.1152/ajpheart.00534.2013.—Exercise training (ExT) has been shown to reduce sympathetic drive during heart failure (HF). The subfornical organ (SFO) is involved in the neural control of sympathetic drive. We hypothesized that an activated SFO contributes to enhanced sympathetic activity in HF. We also postulated that ExT would reduce the activation of the SFO and its contribution to the sympathetic drive during HF. Sprague-Dawley rats were subjected to coronary artery ligation to induce HF. Rats were assigned to ExT for 3–4 wk. Rats with HF had a 2.5-fold increase in FosB-positive cells in the SFO compared with sham-operated rats, and this was normalized by ExT. Microinjection of ANG II (100 pmol) into the SFO resulted in a greater increase in renal sympathetic nerve activity (RSNA); blood pressure, and heart rate in the HF group than in the sham-operated group. These responses were normalized after ExT (change in RSNA: 23 ± 3% vs. 8 ± 2%). ExT also abolished the decrease in RSNA in HF rats after the microinjection of losartan (200 pmol) into the SFO (−21 ± 4% vs. −2 ± 3%). Finally, there was elevated mRNA (5-fold) and protein expression (43%) of ANG II type 1 receptors in the SFO of rats with HF, which were reversed after ExT. These data suggest that the enhanced activity of the SFO by elevated tonic ANG II contributes to the enhanced sympathoexcitation exhibited in HF. The decrease in ANG II type 1 receptor expression in the SFO by ExT may be responsible for reversing the neuronal activation in the SFO and SFO-mediated sympathoexcitation in rats with HF.

FosB; renal sympathetic nerve activity; angiotensin II; paraventricular nucleus; subfornical organ

CARDIOVASCULAR DISEASE is the leading cause of death in the United States (28), with 7% of these deaths caused by heart failure (HF) (23). The American Heart Association reports that 5.3 million adults over the age of 20 yr have HF (23). The increasing incidence of HF suggests the need for better management as well as understanding the underlying mechanisms involved. Exercise training (ExT) is an established therapy for HF that improves the health, quality of life, and clinical outcome of patients with this disease (3). However, the precise mechanisms involved in the beneficial effects of ExT remain unclear.

Enhanced sympathetic nerve activity is a risk factor that influences the progression of HF and mortality in patients. Although most therapeutic pharmaceutical strategies target the peripheral symptoms of the disease, they may not influence the enhanced sympathetic nerve activity. Indeed, studies (6, 17) have detailed the role of cardiac and hemodynamic mechanisms involved in the elevated sympathetic drive in HF, but these mechanisms do not fully account for the total sympathoexcitation observed in HF.

In the central nervous system, the paraventricular nucleus (PVN) of the hypothalamus mediates sympathetic nerve activity and influences the cardiovascular system (1, 18, 19). Our recent study (15) has shown that the PVN is activated in rats with HF in conjunction with enhanced glutamatergic tone within the PVN. Specifically, the neurons of the PVN exhibited an increased FosB expression, which is a marker of chronic neuronal activity (30). In addition, renal sympathetic nerve activity (RSNA) responses were enhanced in rats with HF after the microinjection of N-methyl-D-aspartate (NMDA) into the PVN. The activation of the PVN during HF and the exaggerated sympathoexcitatory response to NMDA were both normalized after ExT (15). However, the mechanisms that drive the enhanced activation of the PVN during HF and mediate the ExT effect remain to be examined.

Other studies (2, 24, 44) have demonstrated the importance of the subfornical organ (SFO) in sympathetic output and cardiovascular regulation. The SFO is a highly vascularized circumventricular organ that lacks a functional blood-brain barrier (37). Therefore, it provides an interface between peripherally circulating molecules, such as ANG II, and the brain. Plasma ANG II levels have been shown to increase in HF and to be restored after ExT (21, 48). Therefore, the SFO provides a potential link for cross-talk between peripheral and central cardiovascular regulation. In fact, our group and others (2, 22, 25, 44) have demonstrated that the SFO is neuroanatomically connected to the PVN. Furthermore, electrical stimulation of the SFO produces action potentials in neurons in the PVN that can be blocked by AP5 (NMDA receptor blocker), suggesting that the SFO influences the PVN by a glutamatergic mechanism (24). Recently, we (22) have shown that ANG II stimulation in the SFO induces sympathoexcitation via the PVN by a glutamatergic mechanism. We (15, 48) have also shown that the PVN is activated in rats with HF in conjunction with enhanced glutamatergic tone within the PVN.

The mechanisms that drive the enhanced activation of the PVN during HF and mediate the ExT effect remain to be examined. We hypothesized that the SFO is activated in HF and makes critical contributions to the regulation of sympathetic drive in HF. Specifically, we postulated that the ANG II response and tonic endogenous activation of ANG II type 1 (AT1) receptors in the SFO are enhanced in HF and that ExT would reverse these mechanisms. Furthermore, we predicted that AT1 receptor protein expression would be enhanced in the SFO during HF and that ExT would restore its expression leading to the normalization of sympathetic tone.
MATERIALS AND METHODS

**Induction of HF.** Male Sprague-Dawley rats weighing 180–200 g (Sasco Breeding Laboratories, Omaha, NE) were fed and housed according to institutional guidelines. The Institutional Animal Care and Use Committee of the University of Nebraska Medical Center approved all protocols. Rats were randomly assigned to sham-operated (sham) and HF groups. Left coronary artery ligation surgery was performed to induce HF. Each rat was anesthetized with pentobarbital sodium (50 mg/kg ip), and the trachea was intubated to assist ventilation. A left thoracotomy was performed, and the left coronary artery was ligated between the pulmonary artery outflow tract and the left atrium. Sham rats received only the thoracotomy and heart manipulation. The thorax was closed, the tracheal tube was removed, and the rat was allowed to recover from anesthesia.

Six weeks after surgery, echocardiography parameters were measured under light isoflurane anesthesia in a subset of rats. The left ventricular (LV) end-systolic dimension (LVESD) and LV end-diastolic dimension (LVEDD) were visualized, measured, and used to calculate fractional shortening and ejection fraction. Before euthanization, LV end-diastolic pressure (LVEDP) was measured (PowerLab, AD Instruments, Colorado Springs, CO) using a conductance catheter (Millar) inserted into the LV via the right carotid artery. To anatomically assess the extent of HF, infarct size of the LV was calculated with high- and low-frequency cutoffs of 1,000 and 100 Hz, respectively (Grass amplifier). The rectified output from the amplifier (RC filtered, time constant: 0.5 s) was displayed using the PowerLab system (8si, AD Instruments) to record and integrate the raw renal nerve discharge. Background noise was determined at the end of the experiment after the administration of hexamethonium (30 mg/kg iv). Overall integrated renal nerve activity was calculated by subtracting the background noise from the recorded value.

**ANG II and losartan microinjection into the SFO.** Anesthetized rats were placed in a stereotoxic apparatus (David Kopf Instruments, Tujunga, CA) for microinjections into the SFO. A longitudinal incision was made on the head to expose the bregma, and a small burr hole was made in the skull to access the SFO. The coordinates of the SFO were 0.9 mm posterior to the bregma, 5.6 mm ventral to the dura, and on the midline (33). A thin needle (outer diameter: 0.2 mm) connected to a 0.5-µl microsyringe (Hamilton, Reno, NV) was lowered into the SFO. RSNA, MAP, and HR were recorded before and after the microinjection of 100–200 pmol (50–100 nl) of exogenous ANG II (Sigma-Aldrich, St. Louis, MO) or losartan (Merck) dissolved in artificial cerebrospinal fluid into the SFO. Injections were randomized and given at 30–45-min intervals. Vehicle injections of artificial cerebrospinal fluid were given to control for volume responses. Three to four microinjections were made into the SFO in each experiment. Microinjection sites were confirmed at the end of each experiment by histology. Sympathoexcitation after drug injection was calculated as the percent change in RSNA from baseline and as absolute changes in MAP and HR from baseline. Baseline and peak responses were averaged over a 30-s time interval.

**Brain histology and immunohistochemistry for FosB and the AT1 receptor.** A separate cohort of the four groups of rats was anesthetized with pentobarbital (65 mg/kg ip) and perfused transcardially with 150 ml of heparinized saline followed by 300 ml of freshly prepared 4% paraformaldehyde in 0.1 M of sodium phosphate buffer. The brain was removed from each rat, postfixed at 4°C for 4 h in 4% paraformaldehyde, and then placed in 30% sucrose for 72 h. The brain was sectioned (30 µm) in the coronal plane with a cryostat. After being washed with PBS, sections were blocked with 10% goat serum and then incubated with goat anti-FosB primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 days at 4°C. After being washed with PBS, sections were incubated with biotinylated anti-goat secondary antibody for 2 h. Sections were treated with avidin-biotin complex (1:200, Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA) for 1 h and then rinsed in PBS for 30 min. Sections were stained with diaminobenzidine solution (Vector Laboratories) for 10 min, washed with PBS, and mounted for visualization with light microscopy (>100, Leica, Buffalo Grove, IL). ImageJ software (NIH) was used to quantify FosB-positive cells in the SFO. The number of nuclei stained with FosB within a 200-µm radius from the center of the SFO was counted, blindly, using the Find Maxima function in ImageJ, which was validated by manual counting of the nuclei. Four to five sections of the SFO were averaged for each rat.

**Immunofluorescence was used to assess the localization of AT1 receptor expression in the SFO.** SFO sections were obtained as described above and incubated with 10% goat serum and 0.02% Triton X-100 in PBS for 1 h at room temperature. Tissues were then incubated with anti-rabbit AT1 receptor primary antibodies (1:500, Santa Cruz Biotechnology) overnight at 4°C. After being washed with PBS, sections were incubated with Cy3-conjugated goat anti-rabbit secondary antibody for 2 h. Sections were mounted on slides, and coverslips were placed with fluoromounting-G (SouthernBiotech). The distribution of immunofluorescence within the SFO was assessed by visualizing a digital camera (Qimaging). Quantification of the intensity of the fluorescence was done using ImageJ software (NIH).

**Plasma levels of ANG II.** At the time of death, whole blood was collected via the left renal vein from rats from all four groups. The needle was coated in EDTA (80 mg/ml), and the collection vial
Table 1. Primer sequences for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference Sequence</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tr>
<td>ANG II type 1 receptor</td>
<td>NM_030985</td>
<td>5'-TATCACAAGTGCGGCAGCCTGCA-3'</td>
<td>5'-TGTTAACGGCCAGGCTATG-3'</td>
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<tr>
<td>Angiotensin-converting enzyme</td>
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<td>5'-CACCTGACAGCTGACGTGGC-3'</td>
<td>5'-CTAGGAAAGCAGGACCACGAC-3'</td>
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<tr>
<td>Ribosomal protein L19</td>
<td>NM_031103</td>
<td>5'-CCTCACTGAAACCAACGAAA-3'</td>
<td>5'-ATGGAGACTCAGGCGTTC-3'</td>
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Table 2. Effects of ExT on characteristics of HF

<table>
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<tr>
<th>Characteristic</th>
<th>Sham Sed Group</th>
<th>HF Sed Group</th>
<th>Sham ExT Group</th>
<th>HF ExT Group</th>
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<tbody>
<tr>
<td>Infarct size, % of LV</td>
<td>0</td>
<td>34.1 ± 2*</td>
<td>0</td>
<td>32.6 ± 2*</td>
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<td>LV end-diastolic pressure, mmHg</td>
<td>4.1 ± 0.5</td>
<td>24.3 ± 3.9*</td>
<td>3.6 ± 0.4</td>
<td>9.6 ± 1.5†</td>
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<td>Mean arterial pressure, mmHg</td>
<td>122.6 ± 6.7</td>
<td>109.6 ± 6.1</td>
<td>104.3 ± 9.4</td>
<td>85.1 ± 5</td>
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<tr>
<td>Ejection fraction, %</td>
<td>69.2 ± 0.9</td>
<td>40.9 ± 3*</td>
<td>75.15 ± 1.6</td>
<td>42.25 ± 3.6*</td>
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<tr>
<td>Fractional shortening, %</td>
<td>40.2 ± 0.7</td>
<td>21.4 ± 1.9*</td>
<td>45.39 ± 1.3</td>
<td>22.48 ± 2.4*</td>
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<td>LV end-diastolic dimension, mm</td>
<td>7.4 ± 0.4</td>
<td>10.3 ± 0.7*</td>
<td>7.2 ± 0.8</td>
<td>10.1 ± 1.2*</td>
</tr>
<tr>
<td>LV end-systolic dimension, mm</td>
<td>4.4 ± 0.4</td>
<td>8.3 ± 0.8*</td>
<td>4.0 ± 0.7</td>
<td>7.9 ± 1.5*</td>
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<tr>
<td>Urinary norepinephrine, μg/day</td>
<td>3.6 ± 0.3</td>
<td>8.1 ± 0.3*</td>
<td>3.5 ± 0.2</td>
<td>3.8 ± 0.1†</td>
</tr>
<tr>
<td>Citrate synthase activity, μmol·g⁻¹·min⁻¹</td>
<td>11.5 ± 1.5</td>
<td>12.8 ± 0.7</td>
<td>18.2 ± 2.9*</td>
<td>16.3 ± 1.8†</td>
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Values are means ± SE; n = 8 rats/group. ExT, exercise training; HF, heart failure; Sham, sham operation; Sed, sedentary; LV, left ventricle. *P < 0.05 vs. the sham Sed group; †P < 0.05 vs. the HF Sed group.
sham group. LVEDP was also elevated in the HF Sed group compared with the sham Sed group, suggesting impaired LV function. ExT improved LVEDP during HF but did not normalize it to sham Sed levels. Further characterization by echocardiography revealed that ejection fraction was reduced in the HF Sed group compared with the sham Sed group, and ExT did not improve this marker for HF. A similar pattern was seen for fractional shortening of the LV.

An index of overall sympathetic activation, measured by urinary norepinephrine excretion, was increased in the HF Sed group compared with the sham Sed group and was normalized by ExT. Finally, to characterize the training effect from exercise, citrate synthase activity in the soleus muscle was measured. Both sham ExT and HF ExT groups exhibited a similar increase in citrate synthase activity compared with their respective Sed control groups.

Fig. 1. Effects of exercise training (ExT) on FosB immunohistochemistry in the subfornical organ (SFO) during heart failure (HF). 

A and B: representative images of FosB immunostaining from sham-operated (sham) sedentary (Sed) and HF Sed rats (A) and sham Ext and HF Ext rats (B). Arrows indicate examples of FosB-positive staining. Bars = 100 μm.
**FosB immunohistochemistry and protein expression in the SFO.** The number of cells in the SFO that stained positive for the neuronal activation marker FosB was increased in the HF Sed group (101 ± 9 cells) compared with the sham Sed group (29 ± 2 cells; Figs. 1 and 2A). This enhanced activation was attenuated in HF ExT rats (36 ± 7 cells). Western blot data, using the more specific FosB antibody, showed a similar pattern for activation of the SFO; ∆FosB expression was increased 33% in the HF Sed group (Fig. 2B). Interestingly, both sham and HF groups demonstrated little FosB expression in the SFO after ExT. Altogether, these data show that the SFO is in an activated state during HF, perhaps contributing to the enhanced sympathetic activity. Interestingly, after ExT, the activation in the SFO is attenuated, paralleling the decrease in
overall sympathetic activation as measured by urinary norepinephrine excretion.

Ang II and losartan microinjection into the SFO. Stimulation of the SFO by microinjection of ANG II increased RSNA in both sham Sed and HF Sed groups. The percent change in RSNA from basal values was enhanced in the HF Sed group (22.7 ± 3.1%) compared with the Sham Sed group (9.0 ± 1.3%; Figs. 3 and 4). Similarly, MAP and HR responses to ANG II were elevated in the HF Sed condition (13 ± 4 vs. 3 ± 1 mmHg and 17 ± 3 vs. 5 ± 1 beats/min, respectively). However, after ExT, these parameters were all normalized to those observed in sham Sed rats. These data show that in HF, there is an exaggerated response to ANG II stimulation in the SFO, suggesting a hyperactive state in this region. Further, ExT restored the response to ANG II stimulation, suggesting that the ExT mechanism reestablishes the ANG II response in the SFO, similar to the sham Sed group.

To elucidate the endogenous tonic contribution of ANG II, the AT1 receptor blocker losartan was microinjected into the SFO in all four groups of rats. After losartan microinjection,
there was a significantly greater decrease in RSNA (−21.6 ± 4.9% of the basal value), MAP (−17 ± 4 mmHg), and HR (−28 ± 6 beats/min) from baseline in the HF Sed group, whereas there was little change from baseline in the sham Sed group (Figs. 5 and 6). After ExT, responses to losartan in the HF group were similar to sham groups. These data suggest that there is enhanced ANG II-mediated endogenous tone in the HF Sed state in the SFO. Furthermore, ExT restored the sympathoexcitatory and hemodynamic responses to losartan and ANG II, indicating that ExT improves sympathoexcitation via the SFO during HF by decreasing the endogenous ANG II-mediated tone or activity in the SFO.

Expression of the ANG II system during HF. Circulating levels of ANG II were increased in the HF Sed group nearly twofold (53.2 ± 8.3 vs. 29.5 ± 1.9 pg/ml; Fig. 7A). ANG II levels were restored to sham levels after ExT in HF rats (29.3 ± 1.8 pg/ml), which has been previously reported (21, 48). Interestingly, ACE mRNA expression in the SFO was unchanged during HF and after ExT (sham Sed group: 1, HF Sed group: 1.03 ± 0.18, sham ExT group: 0.71 ± 0.23, and HF ExT group: 0.67 ± 0.22, n = 4 rats/group), suggesting that circulating ANG II, rather than centrally produced ANG II, is responsible for ANG II-mediated activation of the SFO during HF. In the SFO of HF Sed rats, AT1 receptor mRNA expression was increased fivefold (Fig. 7B) and protein expression was increased 43% compared with sham Sed control rats (Fig. 7C). Additionally, immunofluorescence corroborated the enhanced AT1 receptor expression in the SFO during HF and its reduced expression after ExT (Fig. 8). These results validate the functional data and suggest that the SFO is overactive during HF due to the increased AT1 receptor expression. After ExT, mRNA and protein expression of the AT1 receptor in the SFO were restored to sham Sed levels.

DISCUSSION

Our study demonstrates that neuronal activity in the SFO is elevated during HF with a concomitant increase in systemic sympathoexcitatory output, as indicated by the elevated excretion of norepinephrine in the urine. Additionally, sympathoexcitatory and hemodynamic responses to both endogenous and exogenous ANG II in the SFO were exaggerated in HF, suggesting that the SFO contributes to the increased sympathoexcitatory

exhibited in HF via an ANG II mechanism. The AT1 receptor, which is well known to excite neurons upon ANG II binding (39), is increased in terms of mRNA and protein expression in the SFO during HF. We propose that the elevated AT1 receptor expression contributes to the neuronal activation of the SFO during HF. These results demonstrate the critical role for the SFO in relaying humoral signals such as ANG II, which are elevated in HF, to downstream cardiovascular centers, such as the PVN, during HF. Our study also revealed the therapeutic effects of ExT during HF and provide insights into how the SFO contributes to improving the elevated sympathetic tone during HF, likely through an ANG II/AT1 receptor-dependent mechanism.

The present study found that the SFO is activated in HF. In HF Sed rats, there were threefold more FosB-positive cells in the SFO compared with sham Sed rats. In addition, HF Sed rats exhibited greater protein expression of ∆FosB, which is a stable splice variant of FosB that is specifically upregulated during chronic neuronal activation (29, 30). These findings support the hypothesis that the increased activation of the neurons in the SFO during HF functionally alters sympathetic drive. Another study (40) has reported SFO activation using Fra-like immunofluorescence at only 2 wk but not after 4 wk after the induction of HF. Furthermore, ANG II infusion has also been shown to activate the SFO (5, 11). These studies are consistent with our findings because circulating ANG II levels are elevated in HF (32, 48). In addition, a time course study (5) examining inducible transcription factors in the SFO found that expression of the neuronal activity marker cFOS was rapidly increased after an intracerebroventricular injection of ANG II. Therefore, as the SFO can sense circulating peptides, it could be inferred that the enhanced FosB expression is related to the increased levels of plasma ANG II during HF.

Additionally, HF Sed rats exhibited an increased sympathoexcitatory and hemodynamic response to acute activation of the SFO by ANG II microinjection. In light of the FosB results, which suggest that the SFO is activated during HF, may be due to the exaggerated sympathoexcitatory response to ANG II. Consistent with these findings, there was a sharp decrease in sympathetic activity in response to losartan in the SFO in HF Sed rats, whereas this response was minimal or absent in the sham Sed group. These results imply that there is a tonic high
have shown that ANG II stimulation in the SFO induces sympathoexcitation via the PVN by a glutamatergic mechanism. Therefore, the ANG II-induced activation of the SFO influences the neuronal activity in the PVN and thereby regulates sympathetic activity. Others have elucidated some of the cellular mechanisms within neurons that contribute to ANG II-mediated effects in HF and other cardiovascular diseases, such as hypertension. First, it has been demonstrated that superoxide is involved in increasing neuronal activity via ANG II inhibition of neuronal K⁺ channels (45). Additionally, ANG II-mediated hypertension can be abolished by scavenging intracellular superoxide in the SFO (35, 49). The enhanced inflammatory milieu during HF and high-ANG II states may also act upon the SFO and contribute to its neuronal activation (8, 12, 43). The superoxide and inflammatory mechanisms likely contribute to the neuronal activation in the SFO and the downstream sympathetic activation observed during high-ANG II and HF conditions.

Consistent with our findings of an overactive sympathoexcitatory state in the SFO during HF, the AT₁ receptor was upregulated during HF in the SFO. Previous work has also shown that AT₁ receptor mRNA and protein (42, 46), and AT₁ receptor binding densities (46) are elevated in the SFO during HF. The AT₁ receptor has also been found to be elevated in other important cardiovascular regulatory regions during HF, such as the PVN and rostral ventrolateral medulla (20, 47). Due to its permeable blood-brain barrier, elevated ANG II in circulating blood during HF could increase sympathetic activation via its AT₁ receptor in the SFO. Interestingly, ANG II exhibits positive feedback on its own receptor, upregulating its expression during HF. It has been shown that ANG II upregulates AT₁ receptors in cultured neurons via an NF-κB mechanism (26) and in the SFO and PVN through an ANG II-dependent MAPK pathway (41, 42). Here, we also found that ACE mRNA expression was unchanged in the SFO during HF, suggesting that local ANG II production in the SFO may not be influencing SFO activation to the same extent as circulating ANG II. Ultimately, the elevated AT₁ receptor expression in the SFO is improved by ExT, and further research is warranted to determine the precise source of the ANG II.

Although ExT did not improve cardiac function significantly, overall sympathetic drive was attenuated, as indicated by a decrease in excretion of urinary norepinephrine. Reducing norepinephrine is very important, as HF patients with lower levels of plasma norepinephrine are given a better prognosis (7). These results suggest that ExT may alter the activity of neurons in central cardiovascular regulatory sites, such as the SFO, thereby contributing to the improvement in sympathetic tone. Indeed, our results show that ANG II-mediated sympathetic and hemodynamic responses were improved as well as AT₁ receptor expression in the SFO. We and others have previously shown that ExT improves ANG II signaling in downstream regions of the brain, such as the PVN (10, 13, 48), and rostral ventrolateral medulla (13, 50). Our present evidence adds that the SFO also displays significant ANG II signaling and rostral ventrolateral medulla (13, 50). Our present evidence adds that the SFO also displays significant ANG II signaling dependency during HF and in mediating the effects of ExT. However, it was not explicitly established here whether the downstream centers are dependent on changes in the SFO in HF. Additionally, components of the nonclassical axis of the ANG system were not investigated here but have been linked to the central effects of ExT and losartan in the treatment of HF (13, 50). Future studies should investigate the influence of

Fig. 7. A and B: effects of ExT on plasma levels of ANG II in four groups (A; n = 5 rats/group) and mRNA expression of the ANG II type 1 (AT₁) receptor (AT₁R) in the SFO in four groups (B; n = 4 rats/group). C: representative Western blots (top) and summary data (bottom) of AT₁R protein expression in the SFO in four groups (sham Sed, HF Sed, sham ExT, and HF ExT). n = 6 rats/group. GAPDH was used as a loading control. *P < 0.05, sham Sed group vs. HF Sed group; †P < 0.05, HF Sed group vs. HF ExT group.
ACE2 and the Mas receptor, as they may be involved in mediating the ExT-induced changes in the SFO during HF. The present study offers insights into the mechanism of the positive effects of ExT during HF. To date, the mechanisms for the normalization of sympathetic outflow by ExT during HF have not been fully elucidated. We (15, 48) have previously shown that glutamatergic and angiotensinergic activation in the PVN are restored after ExT during HF; however, it was not clear what upstream mechanisms may be mediating the improvement after ExT. Here, we found that ExT normalizes the activation of the SFO during HF, likely through an ANG II-dependent mechanism. ExT reversed FosB activation of the SFO during HF as well as ANG II-mediated sympathoexcitation. Additionally, there was little response to losartan microinjection after ExT in HF rats, suggesting the restoration of ANG II tonicity in the SFO by ExT.

The ANG system was also normalized to sham Sed levels after ExT. Specifically, plasma ANG II and AT1 receptor mRNA and protein expression were reduced in the HF ExT group. The mechanism responsible for decreasing ANG II after ExT during HF has still not been elucidated. While there are local ANG systems present in the brain, our finding that there was no change in ACE expression in the SFO during HF or after ExT suggests that local ANG II production in the SFO may not contribute to SFO activation but, rather, circulating ANG II. Indeed, current evidence indicates that plasma renin levels are reduced after ExT in normal subjects (9), cardiac ANG II levels and cardiac ACE activity are decreased after

![Figure 8](http://ajpheart.physiology.org/)

**Fig. 8.** Effects of ExT on immunofluorescence staining of the AT1R in the SFO during HF. **A:** representative images from each of the four groups (sham Sed, HF Sed, sham ExT, and HF ExT). **B:** quantification of AT1R immunofluorescence intensity. *n* = 4 rats/group. Bars = 100 μm. *P* < 0.05, sham Sed group vs. HF Sed group; †P < 0.05, HF Sed group vs. HF ExT group.
ExT in rats with HF (34), and ACE expression is decreased, whereas ACE2 expression is increased in the rostral ventrolateral medulla of rabbits with HF after ExT (13). It is also possible that other neurohumoral factors are altered with ExT and influence the SFO, such as cytokines and the endothelin system, which have also been shown to be involved in HF (14, 43). Additionally, further investigation is needed to determine if neuronal mechanisms in the SFO are improved, such as oxidative and inflammatory stress, which is present during high-ANG II and HF states.

Our study describes the role of the SFO as a sensory organ involved in sympathetic drive during HF. Because the SFO lacks a functional blood-brain barrier, it has a close interaction with the circulation and is influenced by peripheral factors such as circulating ANG II. Here, we found that the SFO is activated in HF, and we propose that the high levels of peripheral ANG II contribute to the chronic activation of the SFO. It is known that activation of the SFO by ANG II leads to glutamatergic release at downstream centers like the PVN (22), and, because the SFO positively influences the activity of the PVN, it likely contributes to the elevated sympathetic drive during HF. Therefore, the SFO participates in setting the elevated sympathetic activity during HF or hypertension through sensing neurohumoral factors such as ANG II, cytokines, endothelin-1, and many others (37).

In conclusion, the increased activation of the SFO during HF contributes to the enhanced sympathetic drive exhibited in HF, and the SFO mediates the ExT effect of restoring sympathetic activity during HF. Additionally, we conclude that the restoration of plasma ANG II levels during ExT reverses SFO activation as well as systemic sympathetic activity and SFO-mediated sympathetic drive observed in the HF condition.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.L.L., H.Z., and K.P.P. conceived and design of research; T.L.L., T.L.L., and N.M.S. analyzed data; T.L.L., T.L.L., and K.P.P. interpreted results of experiments; T.L.L., N.M.S., and K.P.P. prepared figures; T.L.L. and K.P.P. drafted manuscript; T.L.L., H.Z., and K.P.P. edited and revised manuscript; T.L.L., N.M.S., H.Z., and K.P.P. approved final version of manuscript.

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