ANG II in the paraventricular nucleus potentiates the cardiac sympathetic afferent reflex in rats with heart failure

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Zhu, Guo-Qing, Lie Gao, Kuashik P. Patel, Irving H. Zucker, and Wei Wang. ANG II in the paraventricular nucleus potentiates the cardiac sympathetic afferent reflex in rats with heart failure. J Appl Physiol 97: 1746–1754, 2004; doi:10.1152/japplphysiol.00573.2004.—Chronic heart failure (CHF) is characterized by sympathoexcitation, and the cardiac sympathetic afferent reflex (CSAR) is a sympathoexcitatory reflex. Our previous studies have shown that the CSAR was enhanced in CHF. In addition, central angiotensin II (ANG II) is an important modulator of this reflex. This study was performed to determine whether the CSAR evoked by stimulation of cardiac sympathetic afferent nerves (CSAN) in rats with coronary ligation-induced CHF is enhanced by ANG II in the paraventricular nucleus (PVN). Under α-chloralose and urethane anesthesia, renal sympathetic nerve activity (RSNA) was recorded. The RSNA responses to electrical stimulation (5, 10, 20, and 30 Hz) of the CSAN were evaluated. Bilateral microinjection of the AT1-receptor antagonist losartan (50 nmol) into the PVN had no significant effects in the sham group, but it abolished the enhanced RSNA response to stimulation in the CHF group. Unilateral microinjection of three doses of ANG II (0.03, 0.3, and 3 nmol) into the PVN resulted in dose-related increases in the RSNA responses to stimulation. Although ANG II also potentiated the RSNA response to electrical stimulation in sham rats, the RSNA responses to stimulation after ANG II into the PVN in rats with CHF were much greater than in sham rats. The effects of ANG II were prevented by pretreatment with losartan into the PVN in CHF rats. These results suggest that the central gain of the CSAR is enhanced in rats with coronary ligation-induced CHF and that ANG II in the PVN augments the CSAR evoked by CSAN, which is mediated by the central angiotensin AT1 receptors in rats with CHF.

cardiac sympathetic afferent reflex; paraventricular nucleus; angiotensin II; renal sympathetic nerve activity; chronic heart failure; angiotensin II; renal sympathetic nerve activity; cardiac sympathetic afferent reflex; paraventricular nucleus; angiotensin AT1 receptor

IT IS WELL KNOWN THAT THE SYMPATHETIC OUTFLOW IS INCREASED IN HUMAN AND EXPERIMENTAL HEART FAILURE, AS SUGGESTED BY AN INCREASE IN PLASMA CATECHOLAMINE LEVELS AND BY DIRECTLY RECORDED MUSCLE SYMPATHETIC NERVE ACTIVITY AND RENAL SYMPATHECTOMY FUNCTION (11, 25, 30, 40). The chronic sympathectomy state may contribute to further hemodynamic deterioration, and the degree of sympathoexcitation is prognostic for survival in the chronic heart failure (CHF) state (5, 7). However, the origin of sympathoexcitation has not yet been clearly defined. It has been reported that chronic sinoaortic denervation does not increase mean sympathetic outflow and blood pressure (10) and that the increase in plasma norepinephrine was not altered in chronically sinoaortic baroreceptor-denervated dogs with CHF (6, 18). Therefore, a blunted sympathoinhibitory reflex does not completely explain the chronic elevation in sympathetic outflow in the CHF state. It has been shown that the cardiac sympathetic afferent reflex (CSAR) is sympathoexcitatory and contributes to the sympathoexcitation in dogs with CHF (24, 32). The pathways of this reflex may be similar to those involved in signaling cardiac pain during acute ischemia (11, 23). This positive-feedback mechanism may be deleterious in the CHF state over the long term. Previous studies in our laboratory showed that the discharge of the cardiac sympathetic afferent nerves was increased in dogs with CHF (31) and that the CSAR to either electrical stimulation of cardiac sympathetic afferent nerves or epicardial application of bradykinin and capsaicin was enhanced in dogs with pacing-induced heart failure and in rats with coronary ligation-induced CHF (22, 32, 39).

The interaction between the sympathetic nervous system and the renin-angiotensin system is well known. It has been reported that the renin-angiotensin system is activated in human and experimental CHF (30, 32, 41). Angiotensin II (ANG II) modulates sympathetic function at several loci, including the sympathetic ganglia, postganglionic synapses, and the central nervous system (1). It has been shown that angiotensin-converting enzyme inhibitors decrease plasma norepinephrine and improve arterial baroreceptor function in CHF patients (12, 14). The locus for central ANG II action is not clear in the CHF state. The hypothalamic paraventricular nucleus (PVN) is an important integrative site within the brain in controlling sympathetic outflow and thus cardiovascular function (2, 9). Activation of neurons in the PVN has been found to play a major role in the processes leading to sympathetic hyperactivity in rats with coronary ligation-induced CHF (8, 19, 20, 27, 35–37). It was reported that microinjection of ANG II into PVN increased blood pressure in normal rats (4) and that angiotensin-receptor binding in the PVN was increased in rats with chronic high-output heart failure (34). Recent experiments in our laboratory showed that intracerebroventricular administration of losartan normalized the enhanced CSAR in dogs with pacing-induced CHF (22). However, it is not known whether ANG II in the PVN is involved in the enhanced CSAR in CHF. The purpose of the present study was to determine whether the CSAR is enhanced in the rats with coronary ligation-induced CHF and whether ANG II in the PVN is involved in alterations of the central gain of the CSAR evoked by electrical stimulation of cardiac sympathetic afferent nerves.
METHODS

Male Sprague-Dawley rats weighing between 350 and 420 g were used in the experiments. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Nebraska and were carried out under the guidelines of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Model of CHF

CHF was produced by coronary artery ligation as previously described (26, 35). All rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and instrumented with sterile techniques. The trachea was cannulated to facilitate mechanical ventilation. A left thoracotomy was performed through the fifth intercostal space. After retraction of the lung, the pericardium was opened to expose the heart. The left coronary artery was ligated by using 6-0 suture near its branch point from the aorta, between the pulmonary artery outflow tract and left atrium. After these maneuvers, the heart was placed in its original position and the thorax was closed. The air within the thorax was evacuated, allowing the rats to resume spontaneous respiration and recover from anesthesia. Analgesics (Nubain-Stadol, 1 ml/kg sc) were administered after surgery. Mortality was ~30%, and death occurred mainly during the first day after ligation. The rats were caged in an environment with ambient temperature maintained at 22°C and humidity at 30–40%. Laboratory chow (Purina) and tap water were available ad libitum. The sham rats were treated the same as the CHF rats except their coronary arteries were not ligated. The final experiment was carried out 6–8 wk after coronary ligation or sham surgery.

Acute Experiments

Each rat was anesthetized with urethane (800 mg/kg ip) and α-chloralose (40 mg/kg ip). Supplemental doses of anesthesia were administered at one-tenth of the initial dose per hour. A midline incision in the neck was made, and the carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified and cut. All other nerve fibers that were visible in the area of the carotid sinus were also cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissues from 4 mm below the bifurcation to 4 mm above. The vessels were painted with 10% phenol solution to destroy any remaining nerve fibers in this area. Each vagus was then identified in the neck, tied, and sectioned. A carotid artery was catheterized for measurement of mean artery pressure (MAP) and heart rate (HR). At the end of each acute experiment, a Millar transducer-tipped catheter (Millar, Houston, TX) was advanced through the carotid artery into the left ventricle to determine left ventricular pressure. The left ventricular end-diastolic pressure and maximum of the first derivative of left ventricular pressure measurement were determined to provide an index of cardiac contractile response to the intervention. Assumed to be complete if HR did not change more than 5 beats/min over the period between each stimulus was 1–2 min.

The RSNA response to stimulation in sham and CHF rats. The RSNA response to electrical stimulation of the central end of the cardiac sympathetic afferent nerves were determined and compared in sham rats (n = 12) and CHF rats (n = 12).

Microinjection of losartan into the PVN in sham and CHF rats. Bilateral microinjections of losartan (50 nmol for each) or saline into the PVN were carried out in sham rats (n = 8) and CHF rats (n = 8). One minute later, the RSNA responses to electrical stimulation were determined and compared.

Microinjection of ANG II into the PVN in sham and CHF rats. Three doses of ANG II (0.03, 0.3, and 3 nmol) and saline were unilaterally microinjected into the PVN at random in CHF rats (n = 6). The time period between each injection was at least 10 min after complete recovery. One minute after the injection, the RSNA responses to electrical stimulation were determined.

Microinjection of ANG II into the PVN in sham and CHF rats. Unilateral microinjections of ANG II (3 nmol) or saline into the PVN were carried out in sham rats (n = 6) and CHF rats (n = 6). One minute later, the RSNA responses to electrical stimulation were determined and compared.

Pretreatment with losartan into the PVN in CHF rats. This series of experiments were carried out in 10 CHF rats and included three interventions: 1) unilateral microinjection of saline (100 nl) into the PVN as control, 2) unilateral microinjection of losartan (50 nmol) followed by ANG II (3 nmol) into the PVN, and 3) unilateral microinjection of ANG II (3 nmol) into the PVN. One minute later...
after each microinjection, the RSNA responses to electrical stimulation were determined and compared. The time period between interventions 1 and 2 was at least 15 min, and the period between intervention 2 and 3 was at least 120 min, well after the acute effects of losartan returned to normal.

**Drugs**

ANG II was obtained from Sigma Chemical. Losartan was a gift from Merck. All drugs were made fresh on the day of the experiment.

**Infarct Size Determination**

At the conclusion of the acute experiment, the heart was dissected free of adjacent tissues and lungs. The ventricles were separated from the atria, and the right ventricular free wall was dissected from the septum. The atria and both ventricles were rinsed, blotted, and weighed. The left ventricle was opened with an incision along the septum from base to apex. Incisions were made in the left ventricle so that the tissue could be pressed flat. The circumferences of the left ventricle and the region of infarcted tissue were outlined on a clear photograph taken by a digital camera. Infarct size was calculated and expressed as a percentage of left ventricular surface area on the basis of the surface areas measured by the SigmaScan program (SPSS Science, Chicago, IL).

**Data and Statistical Analysis**

The RSNA was expressed as the percent change from control (before stimulation). The percent changes in the RSNA induced by cardiac sympathetic afferent nerve stimulation were plotted in each group and were used as an index of the central sensitivity of the CSAR. The slope of the linear relationship between the RSNA response and frequency of stimulation was also calculated by linear regression. Baseline parameters were determined by averaging 10 s of the integrated RSNA, MAP, and HR immediately before cardiac sympathetic afferent stimulation. The last 10 s of the stimulus response were compared with the baseline. A two-way repeated-measures ANOVA followed by the Newman-Keuls test for post hoc analysis was used when multiple comparisons were made. All statistical analyses were done using computer software (SigmaStat, SPSS). All data are expressed as means ± SE. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Baseline Hemodynamics After Coronary Artery Ligation**

The baseline hemodynamics, heart weight, and infarction size were measured at 6–8 wk after coronary ligation or sham surgery (Table 1). Coronary-ligated rats showed an average infarct size of 33.7 ± 1.9%. The heart weight and the ratio of heart weight to body weight were significantly increased in CHF rats, suggesting compensatory hypertrophy of the noninfarcted region of the myocardium. The baseline systolic arterial pressure, pulse pressure, left ventricle peak systolic pressure, and maximum of the first derivative of left ventricular pressure were decreased, and the left ventricular end-diastolic pressure was increased significantly in CHF rats. There were no statistical differences in baseline MAP, diastolic arterial pressure, and HR between the sham and CHF rats. These histological and functional data show the presence of myocardial damage and suggest a decreased contractile function in CHF rats.

**RSNA Responses to Electrical Stimulation in Sham and CHF Rats**

The RSNA responses to varying frequencies of stimulation of the cardiac sympathetic afferent nerves were used to evaluate the central gain of the CSAR in 12 sham rats and 12 CHF rats. A significant increase was found at 10, 20, and 30 Hz of stimulation in CHF rats. In most rats, RSNA increased immediately after stimulation were delivered and reached its maxi-

![Fig. 1](https://example.com/fig1.png)
The slope of the RSNA responses to varying frequency of stimulation was also increased significantly after unilateral microinjection of high and middle doses of ANG II into the PVN.

Response to ANG II in the PVN After Pretreatment With Losartan

To determine the contribution of AT_1 receptors to the enhanced response to stimulation in CHF rats, losartan was unilaterally microinjected into the PVN before measurement of the effect of administration of ANG II into the PVN. As shown in Fig. 5, losartan abolished the effect of ANG II. There were no significant differences between the saline group and losartan plus ANG II group.

Unilateral Microinjection of ANG II into the PVN in Sham and CHF Rats

Three doses of ANG II (0.03, 0.3, and 3 nmol) or saline were microinjected into the PVN in rats with CHF. Figure 6 shows dose-related responses. The RSNA responses were significantly enhanced after microinjection of the 0.3- and 3-nmol doses of ANG II in rats with CHF. To compare with sham rats, the effects of unilateral microinjection of ANG II (3 nmol) into the PVN were determined in six sham rats and six CHF rats.

Table 1. Heart weight, infarct size, and baseline hemodynamics after 6–8 wk of coronary ligation or sham surgery in rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CHF</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>BW, g</td>
<td>412.4±5.2</td>
<td>407.6±4.3</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.32±0.03</td>
<td>1.65±0.03*</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>3.21±0.06</td>
<td>4.06±0.08*</td>
</tr>
<tr>
<td>IS, %LV area</td>
<td>0</td>
<td>33.7±1.9*</td>
</tr>
<tr>
<td>SAP, mmHg</td>
<td>115.2±4.0</td>
<td>95.9±2.4*</td>
</tr>
<tr>
<td>DAP, mmHg</td>
<td>66.9±3.5</td>
<td>70.5±2.7</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>48.3±3.2</td>
<td>25.4±1.9*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>85.0±3.4</td>
<td>82.0±2.5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>334.6±18.2</td>
<td>388.9±9.4*</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>126.2±5.3</td>
<td>99.5±4.1*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>1.6±0.7</td>
<td>15.5±1.1*</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td>3.497±94</td>
<td>1.894±97*</td>
</tr>
</tbody>
</table>

Values are as means ± SE; n, no. of animals. BW, body weight; HW, heart weight; IS, Infarct size; LV, left ventricle; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PP, pulse pressure; MAP, mean arterial pressure; LVSP, LV peak systolic pressure; LVEDP, LV end-diastolic pressure; dP/dtmax, maximum of the first differentiation of LV pressure. *P < 0.05 compared with sham rats.
(Fig. 7). ANG II not only augmented the RSNA responses to stimulation in sham rats but also enhanced the RSNA responses to stimulation in CHF rats compared with control (saline), respectively. The significant enhancement appeared at 20 and 30 Hz in both sham and CHF rats. Although the RSNA responses were enhanced in CHF rats, much greater responses to stimulation after microinjection of ANG II into the PVN in CHF rats were observed compared with sham rats after microinjection of ANG II. Similarly, the slope of the RSNA responses to varying frequency of stimulation was increased significantly after microinjection of ANG II into the PVN in both sham and CHF rats. The slope of the RSNA responses to stimulation was significantly greater in CHF rats than in sham rats either before or after administration of ANG II.

As shown in Table 2, microinjection of ANG II into the PVN significantly increased the baseline RSNA in sham and CHF rats. However, the baseline RSNA change was greater in CHF rats than in sham rats (22.0 ± 3.4 vs. 10.7 ± 2.1%; P < 0.05). ANG II also significantly increased baseline MAP in sham and CHF rats; however, the baseline MAP change was not significantly different between the two groups.

### DISCUSSION

The primary findings in this study were that 1) the CSAR evoked by stimulating cardiac sympathetic afferent nerves was enhanced in rats with CHF, 2) bilateral microinjection of the angiotensin AT1-receptor antagonist losartan into the PVN normalized the enhanced CSAR in CHF rats, 3) unilateral microinjection of ANG II into the PVN augmented the enhanced CSAR in sham and CHF rats, and 4) pretreatment with losartan abolished the effects of ANG II.

In these studies, we used the coronary ligation technique to produce CHF. This model has been extensively used to investigate CHF in rats (15, 28, 35). Coronary-ligated rats showed an average infarct size of 33.7% of the left ventricle. The heart weight and heart weight-to-body weight ratio were significantly greater in CHF rats than in the sham rats, suggesting compensatory hypertrophy of noninfarcted regions of the myocardium. In rats with coronary ligation, systolic arterial pressure, pulse pressure, left ventricular peak systolic pressure, and maximum of the first derivative of left ventricular pressure were decreased, and left ventricular end-diastolic pressure was increased. These changes indicated that the rats with coronary ligation had decreased myocardial contractile function and CHF.

The mechanism by which sympathetic function is enhanced in the CHF state has been a topic of intense investigation for many years. The precise cause is still not completely understood because of its multifactorial nature. The chronic elevation in sympathetic outflow in this disease cannot be completely explained by blunted sympathoinhibitory reflexes, because chronic sinoaortic denervation does not increase sympathetic outflow or arterial pressure (6, 10, 18). A previous study from our laboratory indicated that the CSAR was en-

### Table 2. Baseline change after microinjection of ANG II and losartan into PVN

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CHF</th>
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<tr>
<td></td>
<td>n</td>
<td>ΔRSNA, %</td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>0.8±1.8</td>
</tr>
<tr>
<td>Losartan (50 nmol)</td>
<td>8</td>
<td>-1.2±1.6</td>
</tr>
<tr>
<td>Saline</td>
<td>6</td>
<td>-1.2±2.8</td>
</tr>
<tr>
<td>ANG II (3 nmol)</td>
<td>6</td>
<td>10.7±2.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. ANG II, angiotensin II; PVN, paraventricular nucleus; ΔRSNA, change in renal sympathetic nerve activity; ΔMAP, change in mean arterial pressure. *P < 0.05 compared with preadministration. †P < 0.05 compared with sham.
Fig. 4. Tracings showing the effect of unilateral microinjection of angiotensin II (ANG II; 3 nmol in 100 nl) on the RSNA response to cardiac sympathetic afferent stimulation (10 V, 0.5 ms, and 30 Hz) in a CHF rat. ABP, arterial blood pressure; MAP, mean arterial pressure. Microinjection of ANG II into the paraventricular nucleus enhanced the RSNA response to stimulation.

Fig. 5. Effects of microinjection of saline, ANG II (3 nmol), and losartan (50 nmol) into the paraventricular nucleus on the RSNA responses to varying frequencies of stimulation of cardiac sympathetic afferent nerves in CHF rats. A: percent change. B: slope. Values are means ± SE. RSNA response was significantly enhanced after microinjection of ANG II into the paraventricular nucleus. Pretreatment with losartan completely abolished the effects of ANG II. *P < 0.05 compared with saline. †P < 0.05 compared with losartan + ANG II.

Fig. 6. Effects of microinjection of 3 doses of ANG II (0.03, 0.3, and 3 nmol) into the paraventricular nucleus on the RSNA responses to varying frequencies of stimulation of cardiac sympathetic afferent nerves in CHF rats. A: percent change. B: slope. Values are means ± SE. RSNA responses were significantly enhanced after microinjection of the 0.3- and 3-nmol doses of ANG II. *Significant difference compared with saline, †P < 0.05; ††P < 0.05 compared with control.
It has been shown that ANG II in the central nervous system affects sympathetic outflow and cardiovascular function (16, 29). Previous studies in our laboratory indicated that intravenous and intracerebroventricular administration of losartan significantly attenuated the augmented CSAR in dogs with CHF (22) and that chronic intracerebroventricular infusion of ANG II enhanced the central sensitivity of the CSAR significantly in normal dogs. The latter response was abolished by losartan (21). These results suggest that elevation of central ANG II can sensitize the CSAR via central AT$_1$ receptors and that central ANG II plays an important role in the enhanced responses in dogs with heart failure. However, the specific sites where ANG II acts in the central integration of this reflex are still not known.

The PVN is an important integrative site within the brain to control cardiovascular function (2, 9). It is known that the PVN contains neurons that project to the intermediolateral cell column of the thoracolumbar spinal cord and the rostral ventrolateral medulla, areas involved in controlling sympathetic nerve activity and blood pressure (3, 9). Microinjection of ANG II into the PVN resulted in an increase in RSNA, MAP, and HR in rats, and the response was significantly attenuated after systemic administration of losartan (4). ANG II receptors are densely distributed in the PVN (33). Furthermore, ANG II-mediated excitatory projections to the RVLM has been reported recently (13). In view of our studies, we suggest that ANG II may also be a mediator of the enhanced CSAR in this hypothalamic nucleus. Therefore, we tested this hypothesis by determining the effects of microinjection of ANG II and losartan into the PVN on the CSAR in CHF and sham rats.

Cardiac sympathetic afferent reflex responses were significantly augmented by exogenous ANG II injected unilaterally into the PVN. To block tonic ANG II in the PVN, bilateral microinjections of the AT$_1$-receptor antagonist losartan was carried out. In the present experiments, bilateral microinjection of losartan into the PVN had no effect in sham rats, but it normalized the enhanced RSNA response to electrical stimulation in rats with CHF. Although the RSNA response to sympathetic afferent stimulation was enhanced in CHF rats, unilateral microinjection of ANG II into the PVN further potentiated this response. The effects of ANG II were abolished by pretreatment with losartan. These results suggest that the enhanced central gain of the CSAR in the CHF state is related to the elevated sensitivity or increased number of AT$_1$ receptors in the PVN. Recently, Yoshimura et al. (34) reported that ANG II receptors in the PVN was increased in rats with chronic high-output heart failure. Similar results were found in dogs with pacing-induced CHF (31, 32).

Fig. 7. Comparison of the effects of microinjection of ANG II (3 nmol into paraventricular nucleus) on the RSNA responses to varying frequencies of stimulation of cardiac sympathetic afferent nerves in CHF rats with sham rats. A and B: percent change. Values are means ± SE. HF, heart failure (CHF). Losartan abolished the enhanced RSNA responses evoked by ANG II. *Significant difference compared with sham, $P < 0.05$. †Significant difference compared with control.
receptors were involved in setting the tonic level of sympathetic outflow in this CHF model, we would observe a significant decrease in RSNA after losartan. Indeed, we demonstrated that angiotensin II receptors were involved in setting the tonic level of sympathetic nerve efferents originating in the subfornical organ.


