Central gain of the cardiac sympathetic afferent reflex in dogs with heart failure

Rong Ma, Irving H. Zucker, and Wei Wang

Department of Physiology and Biophysics, University of Nebraska College of Medicine, Omaha, Nebraska 68198-4575

Ma, Rong, Irving H. Zucker, and Wei Wang. Central gain of the cardiac sympathetic afferent reflex in dogs with heart failure. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2664–H2670, 1997.—Previous studies from our laboratory have shown that the cardiac sympathetic afferent reflex is enhanced in dogs with experimental heart failure. The aim of the present study was to determine if the central gain of the cardiac sympathetic afferent reflex was also enhanced in dogs with heart failure. Fifteen dogs with pacing-induced heart failure were used in this study. Seventeen sham-operated dogs served as control. At the time of the acute experiment the dogs were anesthetized with α-chloralose. Arterial blood pressure, heart rate, and renal sympathetic nerve activity were recorded. After sinoaortic denervation and cervical vagotomy, a thoracotomy was performed in the second intercostal space. The left stellate ganglion was identified, and the left cardiac sympathetic nerves were cut. The central end of the left cardiac sympathetic nerves was placed on bipolar stimulating electrodes. The renal sympathetic nerve activity responses to electrical stimulation (30 Hz, 1 ms with varying voltages from 1 to 10 V; or 10 V, 1 ms with varying frequencies from 1 to 30 Hz) of the afferent cardiac sympathetic nerves were compared between sham and heart failure groups. Reflex renal sympathetic nerve activity responses to stimulation of the cardiac sympathetic nerves were significantly greater in the heart failure group compared with that in the sham group (21.4 ± 3.2 vs. 9.8 ± 2.9% at 10 V, 30 Hz and 27.7 ± 4.5 vs. 9.9 ± 3.4% at 30 Hz, 10 V, heart failure vs. sham group, respectively; for both relationships, P < 0.05). This enhanced central gain of the cardiac sympathetic afferent reflex in the heart failure group was significantly attenuated after intravenous and cerebroventricular injection of the angiotensin II receptor antagonist losartan (5 mg/kg iv and 0.125 mg/kg in 0.1 ml iv). These data suggest that the central gain of the cardiac sympathetic afferent reflex is enhanced in dogs with heart failure and central angiotensin II plays an important role in this enhanced response.

Central gain of the cardiac sympathetic afferent reflex in dogs with heart failure.

CONGESTIVE HEART FAILURE (CHF) is characterized by increased sympathetic outflow and decreased parasympathetic outflow (6, 12). Although the mechanisms for the sympathoexcitation and parasympathoinhibition are unknown, one formulation attributes the elevated sympathetic tone and depressed vagal tone to decreased activity from arterial and cardiopulmonary baroreceptors (9, 26). However, blunted vagal and arterial baroreflexes are not the sole mechanism for the high level of sympathetic activity in CHF (2, 18). It is well known that the cardiac sympathetic afferent reflex (CSAR) also contributes to an increase in sympathetic outflow (21, 22, 28). In this way, excitatory sympathetic reflexes initiated by hemodynamic changes and by the relative ischemia of CHF may contribute to the observed increase in sympathetic efferent activity. A previous experiment from our laboratory suggested that the CSAR was enhanced in dogs with CHF (28) and that the increased sensitivity of cardiac sympathetic afferent endings was involved in the elevated sympathoexcitatory reflex (27). Whether other components in addition to sympathetic afferent endings play a role in the enhanced CSAR in CHF is unclear. Therefore, one goal of the present experiment was to examine the central sensitivity of the CSAR in dogs with CHF.

It is well known that circulating concentrations of angiotensin II (ANG II) are increased in severe CHF (10) and may be involved in the enhanced sympathetic outflow in CHF (3, 7, 8, 19). The sympathoexcitation induced by ANG II may be related to a central modulation of cardiovascular reflexes. Accordingly, the second objective of the current study was to test the hypothesis that ANG II contributes to the alteration of the central gain of this reflex in CHF.

METHODS

Surgical instrumentation. Thirty-two mongrel dogs of either sex, weighing between 20 and 30 kg, were used in these experiments. All experiments were approved by the University of Nebraska International Animal Care and Use Committee and were carried out under the Guidelines for the Care and Use of Experimental Animals of the National Institutes of Health and the American Physiological Society. In general, all dogs were instrumented using sterile techniques under pentobarbital sodium anesthesia (30 mg/kg iv initially plus ½α initial dose). Through a right thoracotomy (the 4th intercostal space), catheters were implanted in the left atrium or left ventricle through a branch of a pulmonary vein. A catheter was also implanted in the aorta through the omocervical artery. Catheters were used for measurement of the respective vessel or chamber pressure. An epicardial pacing lead (Medtronic model 6917–357) was placed in the myocardium near the base of the right ventricle. Postoperatively, dogs were treated with Tylan 50 (8 mg/kg im for 3 days). Approximately 1 wk was allowed for the dogs to recover from surgery before pacing was begun.

Model of CHF. The model of low cardiac output heart failure used in the present study was that of chronic ventricular pacing in the dog (30). In brief, after control measurements were made in the conscious state, the dogs were paced (right ventricular) at 250 beats/min using a Medtronic 8340 pacemaker (Medtronic, Minneapolis, MN) that had been modified to pace at this rate.

Acute experiments. When dogs were paced for 3–4 wk and their left atrial and left ventricular end-diastolic pressures were significantly elevated (>15 mmHg), acute experiments were carried out. Each dog was anesthetized with α-chloralose (100 mg/kg iv) and intubated. A femoral artery was catheterized for measurement of systolic, diastolic, pulse, and
mean arterial pressure (MAP). A femoral vein was cannulated for administration of supplemental doses of anesthesia (% of initial dose of α-chloralose/h) and drugs. Arterial blood gases were measured throughout the experiment and were kept within normal limits (pH 7.35–7.45; P_O2 30–40 mmHg; P_CO2 85–95 mmHg).

Through a midline incision in the neck, the carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified, ligated, and cut. All other visible nerve fibers in the area of the carotid sinus were cut. The carotid bifurcation and the common carotid arteries were stripped of adventitial tissue from ~1 cm below the bifurcation to 1 cm above. Each vagus was then identified in the neck, tied, and sectioned. The effectiveness of baroreceptor denervation was determined by recording the change in heart rate (HR) to bolus injections of nitroglycerin (25 μg/kg). These doses evoked changes in blood pressure of between 25 and 40 mmHg. Baroreceptor denervation was assumed to be complete if the HR did not change by >5 beats/min to the intervention.

A midline scalp incision was made, and the skull was exposed. After the bregma was identified, a lateral cerebroventricular cannula was inserted 0.5 cm posterior to bregma and 0.5 cm lateral to midline. The location of the tip of the cerebroventricular cannula was confirmed by outflow of clear cerebrospinal fluid.

Through the left second intercostal space, the chest was opened. The left stellate ganglion was identified. The branches innervating the heart were tied and cut as distal as possible. A pair of stainless steel stimulating electrodes was placed on the central end of these branches. The stimulus was delivered by a stimulator (Grass S88) and stimulus isolation unit.

A left flank incision was made, and a retroperitoneal dissection was used to expose the renal artery and nerves. The renal sympathetic nerves were identified, and a branch was carefully dissected free of the surrounding connective tissue. The nerve was immersed in a warm mineral oil bath and was placed on a pair of platinum-iridium recording electrodes. The signal was amplified with a Grass current preamplifier (model P18D; Grass Instrument, Quincy, MA) with low frequency cutoff set at 30 or 100 Hz and high frequency cutoff set at 1 or 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N; Tektronix, Beaverton, OR). The raw nerve activity, arterial pressure, and HR were recorded on an electrostatic strip chart recorder (model ES 1000B; Gould, Glen Burnie, MD). Hemodynamics and nerve activity were also digitized and analyzed by a computer (MacLab System; ADInstruments, Milford, MA). The renal sympathetic nerve activity (RSNA) was quantified by setting a window discriminator just above the noise level (the silence between discharge bursts). A rate meter (MacLab) counted all spikes above the discriminating level and recorded the frequency.

Experimental protocols. In sinoaortic denervated and vagotomized dogs, MAP, RSNA, and HR were measured as baseline ~30 min after surgery but before each protocol in sham and CHF groups. To compare the central gain of the CSAR between CHF and sham dogs, the percentage increase in RSNA during electrical stimulation of the cardiac sympathetic afferent nerves was determined. The intensity of the stimulus was varied from 1 to 10 V in 1-V increments with a constant frequency of 30 Hz, or the frequency of stimulation was delivered at 1, 2, 5, 10, 20, and 30 Hz at a constant voltage (10 V). The pulse width was kept at 1 ms, and each stimulus lasted 30 s.

There were two sets of experiments in this study. The first was to compare the central gain of the CSAR between sham dogs and dogs with CHF. The RSNA responses to stimulation were examined and compared between the two groups of dogs. The second was to determine the role of ANG II in the regulation of the central gain of the CSAR and the site at which ANG II acted. Therefore, the sham and CHF dogs used in the first set of experiment were divided into two groups. In one group (7 sham and 6 CHF dogs), losartan (5 mg/kg) was given intravenously, and the same stimuli as described above were applied 20 min later. In the other group, the responses of sympathetic outflow to varying voltages and frequencies of stimulation of the cardiac sympathetic nerve were also examined in seven sham and six CHF dogs at 20 min after a low dose of losartan (0.125 mg/kg in 0.1 ml) was administered intracerebroventricularly. The other three sham and CHF dogs in this group were used as time and vehicle controls.

Statistical analysis. The last 10 s of the renal sympathetic nerve discharge before initiation and termination of the cardiac sympathetic afferent stimulation were sampled and averaged. RSNA is expressed and calculated as the percent change from control (before stimulation). The percent changes in RSNA were plotted against frequencies and voltages to analyze the central sensitivity of the CSAR in sham and CHF dogs. Analysis of variance for repeated measures followed by post hoc analysis using Duncan's test was used for determining the level of significance of mean data between the two groups of animals. A paired t-test was used when comparing a control response with the response after an intervention (administration of losartan) in the same animal. Linear regression was used for analyzing responses of RSNA at different voltages and frequencies of stimulation. All statistical analyses were done using computer software (Sigmastat; Jandel). All data are expressed as means ± SE. A P value of <0.05 was considered statistically significant.

RESULTS

Hemodynamics of anesthetized, intact sham, and heart failure dogs. Left ventricular end-diastolic pressure, left ventricular systolic pressure, MAP, and HR were measured in anesthetized, intact sham, and CHF dogs (~30 min after cessation of pacing). These data are shown in Table 1. Left ventricular systolic pressure was significantly decreased, and left ventricular end-diastolic pressure and HR were significantly increased in dogs with CHF. MAP tended to be reduced but did not reach statistical significance.

Renal nerve discharge responses to electrical stimulation of cardiac sympathetic afferent nerves in heart failure and sham dogs. Stimulation of cardiac sympathetic afferent nerves elicited an increase in RSNA. A representative recording is shown in Fig. 1. The increase in RSNA in response to cardiac sympathetic

Table 1. Hemodynamics of anesthetized intact sham and CHF dogs

<table>
<thead>
<tr>
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<th>Sham (n = 17)</th>
<th>CHF (n = 15)</th>
<th>P Value</th>
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<tr>
<td>LVSP, mmHg</td>
<td>138.9 ± 7.6</td>
<td>107.7 ± 1.4</td>
<td>&lt;0.05</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>3.3 ± 0.7</td>
<td>25.1 ± 3.2</td>
<td>&lt;0.01</td>
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<tr>
<td>MAP, mmHg</td>
<td>105.3 ± 5.3</td>
<td>96.0 ± 4.2</td>
<td>NS</td>
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<tr>
<td>HR, beats/min</td>
<td>120.8 ± 5.8</td>
<td>155.3 ± 3.3</td>
<td>&lt;0.01</td>
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Values are means ± SE; n, no. of dogs. CHF, congestive heart failure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; HR, heart rate; NS, no significance.
afferent stimulation was greater in the CHF dog than that in the sham dog, even though the baseline RSNA appeared to be greater in the CHF dog.

The RSNA responses to varying voltages and frequencies of stimulation are shown in Fig. 2. The RSNA responses from 6 to 10 V (at 30 Hz; Fig. 2A, 18.7 ± 4.2 vs. 6.6 ± 1.9% at 6 V, CHF vs. sham dogs, respectively P < 0.05) or 30 Hz (at 10 V; Fig. 2B, 27.7 ± 4.5 vs. 9.9 ± 3.4, CHF vs. sham dogs, respectively, P < 0.05) were significantly enhanced in the CHF group. The linear slopes of the RSNA responses to varying voltages and frequencies of stimulation were significantly different between CHF and sham dogs (3.1 ± 0.5 vs. 1.3 ± 0.05%ΔRSNA/V and 0.8 ± 0.1 vs. 0.3 ± 0.1%ΔRSNA/Hz, CHF vs. sham dogs, respectively, P < 0.05 for both relationships). In the sham group, the RSNA increased significantly from baseline at 30 Hz in response to varying frequencies of stimulation (Fig. 2B). In the same group, the significant increase in RSNA started at 5 V in response to varying voltages of stimulation (Fig. 2A). However, in the CHF group, the two parameters were 10 Hz and 4 V (Fig. 2). In most of the dogs, RSNA started to increase at 5–10 s after the onset of stimulation and stayed at maximal levels from 10 to 20 s after beginning stimulation.

Effect of losartan on baseline MAP and RSNA in sham and heart failure dogs. Intravenous administration of losartan significantly reduced MAP in both sham and CHF dogs (15.7 ± 7.5% in sham dogs, P < 0.05, and 18.5 ± 6.2% in CHF dogs, P < 0.05). However, intracerebroventricular administration of losartan

Fig. 1. Representative recordings from a sham (A) and chronic heart failure (CHF; B) dog before and after electrical stimulation of cardiac sympathetic afferent nerves with 7 V, 1 ms, and 30 Hz. Arrows indicate when electrical stimulation was started. Traces indicate arterial blood pressure (ABP), raw renal sympathetic nerve activity (RSNA), and frequencies of RSNA (Integrated RSNA).

Fig. 2. Response of RSNA to varying intensities (A, voltage; B, frequency) of cardiac afferent nerve stimulation in CHF (n = 6) and sham dogs (n = 7). HF, heart failure; Ctrl, control. *Significant difference in RSNA responses between sham and CHF animals. †RSNA responses significantly different compared with baseline RSNA.
caused small and insignificant changes in MAP in both groups of dogs (2.5 ± 0.8% in sham dogs and 7.6 ± 1.9% in CHF dogs, P > 0.05). Baseline RSNA was not significantly reduced in either sham or CHF dogs after intravenous administration of losartan (31.8 ± 7.7 vs. 25.3 ± 5.4 spike/s for sham dogs and 47.1 ± 7.3 vs. 44.7 ± 7.0 spike/s for CHF dogs). Similarly, central administration of losartan did not reduce RSNA (22.3 ± 3.1 vs. 20.8 ± 2.6 spike/s in sham dogs and 36.4 ± 6.1 vs. 34.5 ± 5.7 spike/s in CHF dogs, P > 0.05 in each group). HR was not affected by losartan in either group of dogs.

Table 2 shows the baseline MAP, HR, and RSNA before each protocol. Only in the intravenous group was baseline MAP significantly lower after administration of losartan.

Effect of intravenous administration of losartan on RSNA responses to stimulation of cardiac sympathetic afferent nerves. The responses of RSNA to stimulation of cardiac sympathetic afferent nerves were compared before and after intravenous administration of losartan (5 mg/kg). As seen in Fig. 3, intravenous administration of losartan significantly attenuated the responses of RSNA to stimulation of cardiac sympathetic afferent nerves in CHF dogs. However, losartan did not affect the response of RSNA to stimulation of cardiac sympathetic afferent nerves in the sham dogs (Fig. 3). In addition, the intravenous administration of losartan significantly inhibited the linear slopes of RSNA responses to stimulation in CHF but not in sham dogs (Table 3).

Effect of cerebroventricular administration of losartan on RSNA responses to stimulation of cardiac sympathetic afferent nerves. Losartan administered intracerebroventricularly (0.125 mg/kg in 0.1 ml) caused a similar effect on the RSNA responses to stimulation as did intravenous losartan. In the CHF group, the RSNA responses to stimulation were significantly inhibited by
Table 3. Effect of losartan on slopes of RSNA responses to stimulation of cardiac sympathetic afferent nerves in sham and CHF dogs

<table>
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<tr>
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<th>Sham</th>
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<tr>
<td></td>
<td>Losartan</td>
<td>Losartan</td>
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<td></td>
<td>(iv)</td>
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<tr>
<td>%ΔRSNA/Hz</td>
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<tr>
<td>Before</td>
<td>0.26 ± 0.05</td>
<td>0.22 ± 0.18</td>
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<tr>
<td>After</td>
<td>0.20 ± 0.06</td>
<td>0.20 ± 0.08</td>
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<tr>
<td>%ΔRSNA/V</td>
<td></td>
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<tr>
<td>Before</td>
<td>1.27 ± 0.05</td>
<td>0.53 ± 0.06</td>
</tr>
<tr>
<td>After</td>
<td>0.75 ± 0.06</td>
<td>0.69 ± 0.06</td>
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Values are means ± SE; n = 7 dogs in each group for sham, and n = 6 dogs in each group for CHF. ΔRSNA, change in RSNA; before, before administration of losartan; after, after administration of losartan. *P < 0.05 and †P < 0.01 compared with the value before administration of losartan in each group.

Losartan from 6 to 10 V at 30 Hz and at 30 Hz, 10 V (Fig. 4). Similar to intravenous administration, cerebroventricular injection of losartan did not affect the RSNA responses to stimulation in sham dogs (Fig. 4).

The effects of central administration of losartan on the linear slopes of RSNA responses to cardiac afferent nerve stimulation in CHF and sham groups are also shown in Table 3. Losartan significantly depressed the slope of the voltage response in CHF dogs. However, the slopes of RSNA responses to varying frequencies of stimulation in CHF dogs and to either varying voltage or frequency stimulation in sham dogs were not significantly affected.

For the time and vehicle controls, the same volume of saline as losartan given centrally (0.1 ml) was administered intracerebroventricularly in three sham and CHF dogs. The changes in RSNA on response to electrical stimulation were 6.9 ± 0.4 and 17.9 ± 4.8% at 30 Hz, 10 V (P < 0.05) in sham and CHF dogs, respectively, and 4.0 ± 0.9 and 12.3 ± 2.9% at 6 V, 30 Hz (P < 0.05) in sham and CHF dogs, respectively. Following this protocol, the effect of intravenous administration of the central dose of losartan (0.125 mg/kg) on the CSAR was examined in the same dogs. The RSNA increased by 6.2 ± 1.7% at 30 Hz, 10 V and by 3.6 ± 1.5% at 6 V, 30 Hz in sham dogs. For dogs with CHF, the RSNA increased by 14.1 ± 1.9% at 30 Hz, 10 V and by 11.5 ± 5.2% at 6 V, 30 Hz. Compared with before administration of losartan (0.125 mg/kg iv), the RSNA responses to stimulation did not change significantly after peripheral administration of this dose of losartan.

**DISCUSSION**

Cardiovascular sympathetic afferent fibers have been widely accepted as part of the neural afferent pathway that participates in the regulation of cardiovascular functions (20). These sympathetic afferent fibers mediate reflexes that are mainly excitatory and exhibit positive feedback characteristics. The CSAR has been
described by many investigators (17, 21, 29). This excitatory sympathetic afferent reflex is activated by an increase in cardiac pressure and dimension and by various substances that may be augmented in the myocardium during ischemia or CHF (17, 21, 22). Previous data from our laboratory showed that the CSAR response to epicardial application of bradykinin and capsaicin was enhanced in dogs with pacing-induced heart failure (28). In addition, another study from our laboratory showed that the discharge of cardiac sympathetic afferent nerves was increased in dogs with CHF (27). These results supported the hypothesis that the CSAR is enhanced in the CHF state. The enhanced sensitivity of cardiac sympathetic afferent receptors is likely one of the mechanisms by which this reflex is augmented.

In the present experiments, the RSNA response to electrical stimulation of cardiac sympathetic nerves was examined in both CHF and sham dogs. Because the stimulus was delivered to the afferent limb (bypassing the cardiac receptors) and the response was recorded in the efferent limb of the CSAR arc, the ratio of changes in RSNA to changes in stimulation parameters (voltages and frequencies) represents the central gain of this reflex. Because all dogs used in the present study were sinoaortic denervated and vagotomized, the possibility of contribution from arterial and cardiopulmonary baroreceptor reflexes secondary to changes in arterial and cardiac pressures was eliminated. Under those conditions, the current study showed that the RSNA increased during stimulation of cardiac sympathetic afferent nerves in both CHF and sham dogs. These responses were voltage and frequency dependent in both groups of dogs. However, the increase in RSNA responses to different voltages and frequencies of stimulation was greater in the CHF dogs compared with the sham dogs. In addition, the linear slopes of the responses were significantly steeper in CHF dogs than in sham dogs. These data suggest that the central gain of the CSAR is enhanced in the CHF dogs.

It has been shown that there is an increased level of ANG II in the peripheral circulation in the CHF state (10, 13). Chronic increases in circulating ANG II increased sympathetic nerve activity, which was related to arterial baroreflex resetting (16, 19, 25). However, this chronic arterial baroreflex resetting is largely independent of the effect of ANG II to increase arterial pressure (3). This indicates that ANG II has a direct effect on the arterial baroreflex. It is likely that ANG II exerts its action centrally in CHF (8). In addition, it has also been shown that ANG II in the central nervous system affects sympathetic outflow and cardiovascular functions (1, 11, 25). In view of the evidence that ANG II in the circulation and in the central nervous system excites sympathetic nerve activity, we speculated that increased ANG II may contribute to the enhanced central gain of the CSAR in the CHF state. If this is true, then blockade of ANG II receptors should attenuate the augmented CSAR in dogs with CHF. In the present study, intravenous injection of losartan, a highly specific AT1 receptor blocker, significantly depressed the responses of RSNA to stimulation of the cardiac sympathetic afferent nerves in CHF dogs. However, losartan had no effect in the sham dogs. These data suggest that ANG II plays an important role in the enhanced central gain of the CSAR in the CHF state.

Because losartan can cross the blood-brain barrier (5, 31), it was not possible to identify whether the results due to intravenous administration of losartan were due to peripheral or central effects. Therefore, losartan was also injected into the lateral cerebral ventricle at 1/40 the dose of the intravenous administration. Central administration of losartan had similar effects on the central gain of the CSAR as did peripheral administration. It is unlikely that the effect of cerebroventricular injection of losartan was due to a peripheral effect of losartan that may have leaked from the cerebrospinal fluid through the blood-brain barrier, since neither the arterial blood pressure nor the RSNA responses to stimulation were affected by intravenous administration of the same dose of losartan as given centrally. These data suggest that ANG II is acting in the central nervous system to increase the CSAR control of RSNA.

In the present study, intravenous administration of losartan (5 mg/kg) significantly reduced baseline MAP without significantly affecting basal RSNA in both sham and CHF dogs. The reduction of baseline MAP induced by intravenous losartan could be simply interpreted as blocking the vasoconstrictor action of ANG II. However, the influence of losartan on RSNA is complicated. Two possible mechanisms may be involved. One is blockade of the action of ANG II, which can increase RSNA by attenuation of the baroreflex (3, 4, 8, 23). The other is losartan-induced reduction of MAP, which increases RSNA by unloading baroreceptors. The interaction between these two opposite influences of losartan on RSNA, both of which are mediated by baroreflex, may result in no significant change in RSNA after intravenous administration of losartan. Therefore, it is not surprising that the intravenous losartan did not affect baseline RSNA in the present study.

Intracerebroventricular administration of losartan (0.125 mg/kg) only evoked small and insignificant changes in MAP in both groups of dogs. The most likely explanation for this phenomenon is that neural and humoral factors interact with one another in the central nervous system to regulate sympathetic and parasympathetic outflow. ANG II is only one factor contributing to this regulation. In the animal model used in the present study, removal of the baroreflex may activate sympatoexcitatory factors that are not modulated by the renin-angiotensin system. Therefore, blockade of ANG II by losartan may have only a slight influence on resting sympathetic outflow or may be compensated for by other neural and humoral factors. The study by DiBona et al. (8) showed results similar to ours in that central losartan enhanced baroreflex function in rats with coronary artery ligation-induced CHF. This study also supports that ANG II is initially involved in the central regulation of sympathetic outflow in CHF. Removal of the arterial baroreflex in our
experiments may have resulted in the failure of central losartan to attenuate RSNA.

It is known that there are numerous ANG II binding sites in medullary regions and in the hypothalamus that have been shown to influence cardiovascular function. Microinjection of ANG II into the rostral regions of the ventrolateral medulla has been shown to elicit dose-dependent pressor responses in spontaneously hypertensive rats and Wistar-Kyoto rats (24). Ganglionic blockade with hexamethonium bromide prevented the effect of ANG II injection in the rostral regions of the ventrolateral medulla (24). This study provides evidence that ANG II acts as an excitatory agent at sites within the ventrolateral medulla that determine the vasomotor control of blood pressure in both normotensive and hypertensive rats. Moreover, suppressor infusion of ANG II in the nucleus of the solitary tract has been shown to inhibit the reflex bradycardia after intravenous administration of the vasoconstrictor phenylephrine in normotensive rats (4). These data suggested that ANG II within the nucleus of the solitary tract can inhibit the function of baroreceptor reflexes in normotensive animals, indicating that the endogenous peptide may perform an inhibitory role in the baroreflex arc. Although the central pathway of the CSAR is unclear, the evidence suggests that several regions, including the spinal cord, brain stem, and the hypothalamus, are involved in this reflex (14). Therefore, in the present experiment, it is also possible that intravenous or intracerebroventricular losartan blocked AT1 receptors in either of these regions to inhibit the enhanced RSNA responses to stimulation of the cardiac sympathetic afferent nerves. It has been known that the area postrema (AP), a circumventricular organ, plays an important role in the acute baroreflex resetting by ANG II (23). However, a recent study by Gorbea-Oppliger and Fink (15) has shown that intracerebroventricular administration of the active metabolite of losartan, EXP-3174, did not block the pressor effect of ANG II injected into the area postrema. This suggests that intracerebroventricular losartan does not block the AT1 receptor in the area postrema. This finding also suggests that the area postrema may not play an important role in the alteration of the enhanced CSAR in the CHF state.

Limitations of the study. The present study provides the first evidence that the central gain of the CSAR is enhanced in heart failure and that central ANG II is involved in the elevated sensitivity. However, several limitations of this study should be discussed. First, our experiments were carried out in sinoaortic denervated dogs. This animal model raises a crucial question, which is, what influence did sinoaortic denervation have on the CSAR? Baroreceptor reflexes interact with the CSAR (20). Although the central mechanism for this interaction is not known, it is logical to speculate that the baroreceptor reflexes inhibit the expression of the CSAR centrally. Therefore, removal of the inhibitory action from baroreceptor reflexes by sinoaortic denervation in this study should increase the sensitivity of the CSAR. Because the baroreceptor reflexes are attenuated in the CHF state (6), sinoaortic denervation should have a greater influence on the CSAR in the normal dog than in the CHF dog. Even under those conditions, the RSNA responses to stimulation of cardiac sympathetic afferent nerves were significantly greater in CHF. In the present study, all responses were obtained in anesthetized dogs in which the buffering influences exerted by vagal and carotid sinus afferent fibers no longer existed. It is not clear from these experiments whether similar findings would be observed in the fully innervated animal and whether a substantial activation of the cardiovascular sympathetic afferent fibers occurs in the conscious state via the CSAR in the setting of CHF.

Another concern is whether the central renin-angiotensin system is activated in CHF. The results of this experiment appear to indicate that the activity of the renin-angiotensin system in the central nervous system is higher in dogs with CHF than that in sham dogs. However, we lack direct evidence for supporting this possibility. In future experiments, it will be important to measure ANG II concentrations in cerebrospinal fluid in the CHF state.

In summary, dogs with CHF exhibited enhanced RSNA responses to electrical stimulation of the cardiac sympathetic afferent nerves. Administration (iv or icv) of the AT1 receptor antagonist losartan attenuated the RSNA response to stimulation in the CHF group. These results indicate that the central gain of the CSAR is enhanced and that ANG II in the central nervous system contributes significantly to the elevated central gain of this reflex in the CHF state. It is tempting to speculate that tonic activation of this reflex contributes to the sympathoexcitatory state in CHF.

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Address for reprint requests: W. Wang, Dept. of Physiology and Biophysics, University of Nebraska Medical Center, 600 S. 42nd St., Omaha, NE 68198-4575.

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