Chronic central infusion of ANG II potentiates cardiac sympathetic afferent reflex in dogs

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Ma, Rong, Harold D. Schultz, and Wei Wang. Chronic central infusion of ANG II potentiates cardiac sympathetic afferent reflex in dogs. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H15–H22, 1999.—The aims of this study were to determine whether ANG II is involved in the central integration of the cardiac sympathetic afferent reflex (CSAR), and if this central effect of ANG II is mediated by the AT1 receptor. Experiments were undertaken in dogs that were anesthetized with α-chloralose, sinoaortic denervated, and vagotomized. The renal sympathetic nerve activity (RSNA) responses to varying frequency and voltage stimulation of cardiac sympathetic afferent nerves were used to evaluate the central sensitivity of the CSAR. In two groups of dogs, two doses (50 and 100 ng/min icv) of ANG II were acutely infused. In a third group of dogs, ANG II was chronically infused for 3 days (100 ng/min, 1 µl/h icv). We found that acute infusion into the cerebroventricle of two doses of ANG II did not affect the central sensitivity of the CSAR or the baseline hemodynamics, but the baseline RSNA increased significantly during the infusion of the higher dose of ANG II. However, chronic intracerebroventricular infusion of ANG II enhanced the central sensitivity of the CSAR significantly. In addition, chronic intracerebroventricular infusion of ANG II elicited a significant increase in water intake and in arterial pressure from the first and second day of infusion, respectively. In the group that received chronic intracerebroventricular infusion of ANG II, the administration of an AT1-receptor antagonist losartan (0.125 mg/kg icv) abolished ANG II-induced augmentation of the CSAR. These results suggest that chronic elevation of central ANG II can sensitize the CSAR via central AT1 receptors. Additional methods under ketamine (20 mg/kg im) and Rompun (2 mg/kg)

Surgical Instrumentation

In this study seven dogs were chronically infused with ANG II. These dogs were instrumented using sterile techniques under ketamine (20 mg/kg im) and Rompun (2 mg/kg)
im) anesthesia. A right cervical incision was made, and a catheter was implanted in the aorta through the omocervical artery for measurement of arterial blood pressure. The dog's skull was exposed through an incision on the midline of the scalp. After the bregma was identified, a cerebroventricular cannula was inserted 0.5 cm posterior to bregma and 0.5 cm lateral to midline. The location of the tip of the cannula was confirmed by an outflow of clear cerebrospinal fluid. A miniosmotic pump (model 2001, Alza Pharmaceuticals, Palo Alto, CA) filled with ANG II (6 ng in 1 ml at the rate of 100 ng/min) was implanted under the scalp and connected to the cerebroventricular cannula, which was fixed tightly to the skull with Super Glue adhesive and dental cement. The ANG II was infused continuously for 3 days. Control dogs (n = 4) were treated with the same procedure as ANG II-infused dogs but were infused with saline rather than ANG II. Water intake, blood pressure, and HR were measured daily in these instrumented dogs before and during infusion of either ANG II or saline.

**Acute Experiments**

At the time of the acute experiment, each dog was anesthetized with α-chloralose (100 mg/kg iv) and intubated. A femoral artery and vein were catheterized for measurement of hemodynamics (blood pressure and HR) and administered supplemental doses of anesthesia (one-tenth of initial dose of α-chloralose per hour), respectively. Arterial blood gases were measured throughout the experiment with a ABL 5 Radiometer (Radiometer Medical, Copenhagen, Denmark) and kept within the normal limits (pH 7.35–7.45, Pco2 30–40 mmHg, Po2 85–95 mmHg) by adjustments in the ventilation rate, O2 supplementation, or bicarbonate administration. A midline incision in the neck was made, and the carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified, ligated, 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 1 cm below the bifurcation to 1 cm above. Each vagus nerve was then identified in the neck, tied, and sectioned. The effectiveness of baroreceptor denervation was determined by recording the change in HR to bolus injection of nitroglycerin (25 µg/kg). This dose evoked a decrease in blood pressure between 25 and 40 mmHg. Baroreceptor denervation was assumed to be complete if HR did not change more than 5 beats/min in response to the intervention.

In all dogs with acute intracerebroventricular infusion of ANG II, the lateral cerebroventricle was cannulated using the same method as described in Surgical Instrumentation. The chest was opened through the left second intercostal space. The left ventral ansa, which contains cardiac sympathetic afferent nerves was identified, tied, and cut. A pair of stainless steel stimulating electrodes was placed on the central end of this nerve. All stimulating afferent nerves was identified, tied, and cut. A pair of stainless steel stimulating electrodes was placed on the central end of this nerve. The stimulus was delivered by a stimulator (Grass S88) and a stimulus isolation unit.

RSNA was recorded by using the technique described in a previous study (18). In brief, the discharge was amplified with a Grass DC preamplifier (model P18D, Grass Instrument, Quincy, MA), monitored on a storage oscilloscope (model 121 N, Tektronix, Beaverton, OR), and then imported to a computer system with other parameters. Blood pressure, HR, and RSNA were digitized and analyzed by a computer (MacLab System, ADInstruments, Milford, MA). The RSNA was quantified by setting a window discriminator just above the noise level (the silence between discharge bursts). A rate meter (MacLab) counted spikes above the discriminating level and recorded the frequency.

**Experimental Protocols**

The dogs used in this study were divided into three protocols: acute infusion of low dose of ANG II (50 ng/min icv, n = 7 dogs); acute infusion of high dose of ANG II (100 ng/min icv, n = 4 dogs); and chronic infusion of ANG II for 3 days (100 ng/min at 1 µl/h icv, n = 7 dogs). Controls for the chronic infusion of ANG II group (n = 4) were infused with saline. To determine the central sensitivity of the CSAR, the percentage of increases in RSNA during given intensities of electrical stimulation of the left ventral ansa were calculated. The amplitudes of stimulus varied in the range of 5–20 V in 5-V increments with a constant frequency of 30 Hz, and the frequencies of stimulation varied at 1, 5, 10, 20, 30, 40, and 50 Hz with a constant voltage of 20 V. The pulse width was kept at 1 ms and each stimulus lasted 30 s. All stimuli were delivered at random sequence in any experimental protocol. The time period between each stimulus was at least 2 min.

Acute infusion of ANG II. ANG II was acutely infused into the cerebroventricle through the cannula by a pump that had been set at the rate of either 50 or 100 ng/min (Harvard Apparatus, South Natick, MA). The central sensitivity of the CSAR before infusion of ANG II was measured as the control in each group. CSAR central sensitivity was measured again 20 min after onset of infusion on the background of continu- ous infusion of ANG II and compared with the control values.

Chronic infusion of ANG II. Water intake, mean arterial pressure (MAP), and HR were measured from the first day of infusion in both chronically ANG II-infused dogs (n = 7) and saline-infused dogs (n = 4) in a conscious, unrestrained state. Terminal experiments were carried out on the fourth day of infusion. At the time of the terminal experiment, RSNA responses to varying voltages and frequencies of stimulation were examined in chronically ANG II-infused and saline-infused dogs and compared between the two groups.

Effect of losartan on central sensitivity of CSAR in chronic intracerebroventricular infusion of ANG II. To determine whether the effect of chronic infusion of ANG II was mediated by an AT1 receptor in this study, a specific AT 1-receptor antagonist losartan was applied through the cerebroventricular cannula in all chronically ANG II-infused dogs (0.125 mg/kg in 0.1 ml). RSNA responses to stimulation were examined 20 min after losartan administration and compared with the responses before administration of losartan.

**Statistical Analysis**

The last 10 s of the RSNA before initiation and termination of the cardiac sympathetic afferent stimulation were sampled and averaged. The RSNA was expressed as the percent change from control (before stimulation). The percent changes in RSNA (induced by given voltages and frequencies of stimulation) were plotted against frequencies and voltages in each group and were used as an index of the central sensitiv- ity of the CSAR. Student's t-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 responses before and after an infusion of ANG II and administration of losartan) in the same animal. All statistical analyses were done using computer software (SigmaStat, Jandel). All data are expressed as means ± SE. P < 0.05 was considered statistically signifi- cant.

**RESULTS**

RSNA responses to varying voltages and frequencies of stimulation of cardiac sympathetic afferent nerves...
were used to evaluate the central sensitivity of the CSAR in the present study. Effect of acute infusion of ANG II on central sensitivity of CSAR. ANG II was infused at 50 and 100 ng/min in two groups of dogs (n = 7 and 4 dogs, respectively). The central sensitivity of the CSAR was measured and compared before and during infusion of ANG II in each group. RSNA rose when the left ventral ansa was stimulated. Before the infusion of ANG II, a significant increase in RSNA began at 10 V with a 30-Hz stimulus and at 20 V with a 20-Hz stimulus in either group (Fig. 1). In most dogs, RSNA increased immediately on delivery of stimuli and reached a maximal level within 10 s (see Fig. 4).

Figure 1 shows the CSAR-induced RSNA before and during central infusion of two doses of ANG II acutely. Even though both low and high doses of ANG II had a tendency to increase the RSNA responses to stimulation of the cardiac sympathetic nerves, neither dose evoked a significant change in the sensitivity of this reflex. The changes in MAP, HR, and RSNA elicited by ANG II alone were measured about 20 min after the initiation of ANG II infusion and are shown in Table 1.

Table 1. Effect of acute intracerebroventricular infusion of ANG II on baseline MAP, HR, and RSNA

<table>
<thead>
<tr>
<th>ANG II, ng/min</th>
<th>50</th>
<th>100</th>
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<tbody>
<tr>
<td>MAP, %</td>
<td>1.0 ± 3.2</td>
<td>0.3 ± 2.3</td>
</tr>
<tr>
<td>HR, %</td>
<td>−0.2 ± 0.8</td>
<td>−2.4 ± 1.1</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>22.5 ± 9.9</td>
<td>35.2 ± 5.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as percent change from control before infusion. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity. *P < 0.05 compared with control. With the exception of RSNA in response to the higher dose of ANG II, the baseline values were not changed significantly.

Effect of chronic central infusion of ANG II on central sensitivity of CSAR. Seven dogs were infused with ANG II (100 ng/min at 1 µl/h) for 3 consecutive days. Water intake was increased significantly from the first day onward, whereas MAP was significantly elevated on the second and third days. However, HR was not affected significantly throughout the 3-day infusion (Fig. 2). In saline-infused dogs (n = 4 dogs), water intake rose significantly on the first day after surgery (32.3 ± 2.0 vs. 45.8 ± 3.7 ml·kg⁻¹·day⁻¹, before infusion vs. the first day after infusion, P < 0.05) and then returned to preinfusion levels. Baseline hemodynamics did not change during the entire infusion period in this group.

Intracerebroventricular infusion of ANG II for 3 days clearly enhanced the central sensitivity of the CSAR as indicated in Fig. 3. First, RSNA responses to stimulation increased significantly from 10 Hz with the application of a constant voltage of 20 V in RSNA-frequency-response curve compared with baseline values (Fig. 3A). RSNA response to stimulation increased significantly from 5 V with a constant frequency of 30 Hz in RSNA-voltage response curve compared with baseline (Fig. 3B) in chronic ANG II infusion group, whereas these values in the control group were 30 Hz, 20 V and 10 V, 30 Hz, respectively. Second, in a comparison with the control group the same parameters of stimulation at 20 Hz, 30 Hz, and 50 Hz with 20 V (Fig. 3A) and 20 V, and 20 V with 30 Hz (Fig. 3B) elicited significantly greater RSNA increases in the chronic ANG II infusion group.

Figure 4 is a representative recording taken from one acute infusion and one chronic infusion of an ANG II-
infused dog, respectively. As this particular case demonstrates, the same intensity of stimulation induced a greater increase in the RSNA in the dog with chronic intracerebroventricular infusion of ANG II than in the dog with acute infusion. For further comparison of the response difference between acute and chronic groups, changes in RSNA at 30 Hz and 20 V in the two groups were plotted and shown in Fig. 5. The RSNA was significantly elevated in dogs with chronic infusion of ANG II but not in dogs with acute infusion of ANG II.

Effect of intracerebroventricular administration of losartan on enhanced central sensitivity of CSAR induced by chronic intracerebroventricular infusion of ANG II. In seven dogs with chronic infusion of ANG II, losartan (0.125 mg/kg) was administered into the cerebroventricle, and RSNA responses to stimulation were examined. These data were summarized in Fig. 6. Losartan abolished the elevated RSNA responses to stimulation of the left ventral ansa induced by ANG II. This inhibition of losartan at 30 Hz and 20 V is also shown in Fig. 5. However, losartan did not affect baseline MAP (−2.74 ± 2.69%), HR (−0.62 ± 0.91%), or RSNA (5.73 ± 8.92%) (all values are expressed as percent change from the values before administration of losartan).

DISCUSSION

The primary findings in this study were that 1) chronic intracerebroventricular infusion of ANG II potentiated the CSAR-induced RSNA, 2) these enhanced responses were abolished by intracerebroventricular administration of losartan, and 3) acute infusion of ANG II (at 50 and 100 ng/min icv) did not affect the central sensitivity of the CSAR.

It is well known that ANG II exerts effects on numerous central sites to induce sympathoexcitatory effects. Microinjections of ANG II into the SFO and OVLT elicit an elevation in blood pressure (4, 21). A previous study (3) also shows that ANG II applied into...
PVN resulted in an increase in MAP. ANG II infused into RVLM shifted the RSNA-MAP response curve to the right, whereas injection of saralasin, an ANG II-receptor antagonist, into this area reduced the upper plateau, the range, the range-dependent gain of the baroreflexes, and the resting level of the RSNA (27). The data from several groups have also shown that ANG II induces dose-dependent pressor and/or tachycardia responses by acting on the NTS and AP (7, 17, 22). Taking these studies together, it is evident that ANG II produces sympathetic excitatory effects by acting on multiple central sites and by modulating cardiovascular reflexes, such as baroreflexes (37).

The CSAR has been defined as a sympathoexcitatory reflex that is tonically operative in controlling cardiovascular functions (19). It has been hypothesized that this positive-feedback mechanism cooperates with arterial and cardiopulmonary baroreflexes to maintain cardiovascular homeostasis (19). Additionally, it has been proposed that the alteration of this reflex is partly involved in certain disease states, such as congestive heart failure (20). Previous studies (18, 35) have shown the CSAR enhancement in dogs with pacing-induced heart failure and that this partly contributed to the elevated sympathetic outflow. However, agents involved in the central integration of this reflex have been unknown. Because ANG II has been acknowledged to excite the sympathetic nervous system and modulate cardiovascular reflexes (baroreflexes) by acting on some brain sites, it is assumed that the central components of the CSAR are also the targets on which ANG II acts.

We tested this hypothesis in this study by measuring the influence of exogenous ANG II infused into the lateral cerebroventricle on the CSAR. The current data shows that two doses of ANG II (50 and 100 ng/min, respectively) did not raise the central sensitivity of the CSAR significantly. Combined with previously reported data, which showed that intracerebroventricular administration of losartan did not affect the central sensitiv-
ity of the CSAR in normal dogs (18), the current results suggest that acute elevation of central ANG II may not affect the central integration of the CSAR. It could be argued that the doses of ANG II used in this study were not high enough to facilitate this reflex. This possibility may not exist because 1) the doses used in the current study were comparable to those in other studies that have clearly demonstrated sympathoexcitatory effects (14, 38, 39); 2) the baseline RSNA was significantly elevated 20 min after infusion of ANG II (100 ng/min; Table 1) in this study, indicating excitation of sympathetic nervous system by this dose of ANG II; and 3) in addition, we tested the CSAR under infusion of a higher dose of ANG II (500 ng/min), which is sufficient to modulate baroreceptor reflex (2, 13) in two additional dogs, and no effect on this reflex was observed. Therefore, we speculated that even though acute infusion of ANG II (100 ng/min) raised the sympathetic drive in the resting state (baseline RSNA), it may not affect the specific signal transduction of the CSAR in the central nervous system. It should be mentioned that all dogs used in this study have been baroreceptor denervated (sinoaortic denervated and vagotomized). The CSAR has been suggested to exhibit an inhibition on baroreflexes (19). It is also known that modulation of baroreflexes is an important mechanism of ANG II-induced sympathoexcitatory effects. Hence, it is likely that ANG II modulates the CSAR by influencing integration of the CSAR with baroreflexes. Therefore, the efficacy of ANG II on the CSAR may have been underestimated in the animal model (baroreceptor denervation) used in the present study.

One major difference in the results of this study and other studies (38, 39) probably is that we found no change in blood pressure and HR during acute infusion of ANG II. This discrepancy could be explained by different experimental interventions. As mentioned above, the baroreflexes in all dogs used in this study have been eliminated. Removal of these sympathoinhibitory reflexes resulted in much higher sympathetic tone, which was indicated by an increase in baseline MAP and HR after the intervention. In this case, the effector organs of sympathetic nervous system have probably been accommodated or adapted to sympathetic stimuli (24). Thus, although acute infusion of ANG II elevated sympathetic drive (RSNA, at 100 ng/min, Table 1), blood vessel response to the drive has been weakened and, consequently, MAP did not have significant change. In addition to that, bilateral vagotomy and unilateral cardiac sympathectomy (left side) could also contribute to no change in HR after infusion of ANG II.

In our previous study (18), we found that central administration of losartan attenuated the enhanced central gain of the CSAR in dogs with heart failure with the same intervention as that in this study (sinoaortic denervated and vagotomized dogs), suggesting that ANG II participated in the central integration of the CSAR in the heart failure state. Because development of heart failure is a chronic process and ANG II is gradually elevated during this chronic development, we speculated that central ANG II may affect the CSAR by a long-term effect. On the basis of this point, the effect of chronic central infusion of ANG II on the central sensitivity of the CSAR was examined. As was expected, the 3-day infusion of ANG II potentiated the central sensitivity of this reflex significantly. The difference in the effect on the CSAR between acute and chronic infusion of ANG II indicates that ANG II may modulate the CSAR by a long-term effect. We inferred that the chronic elevation of ANG II in the central nervous system resulted in structural and/or functional alterations in the neurons that are involved in the central pathways of the CSAR and raised the neuronal excitability and/or facilitated the synaptic transmission in the pathways of this reflex.

Consistent with the results of most studies, chronic infusion of ANG II elicited a dipsogenic response from the first day and throughout the 3 days, whereas MAP appeared to be significantly increased from the second day forward in the conscious dogs in this study. As is
well known, the primary sites for ANG II-induced dipsogenicity are located in the CVO (36, 37). ANG II infused into the cerebroventricle in the current study was readily sensed by the CVO and resulted in the significant increase in water intake. It has repeatedly been shown that intracerebroventricular injections of angiotensins produce a reliable pressor response (37). Microinjection of ANG II into the SFO (21) and OVLT (4) also elicits elevation in blood pressure. This response appears to be dependent on both sympathoexcitation and vasopressin release. Studies showed that OVLT and SFO projected to the anterior hypothalamus. From the anterior hypothalamus one pathway coursed to the brain stem, whereas the second pathway projected to the PVN and SON that project to the posterior pituitary and brain stem, such as the RVLM and the NTS (37). In another CVO, the area postrema was also shown to have reciprocal connections with brain stem (15). These networks constitute the components that mediate the pressor effect induced by ANG II. The increase in MAP during infusion of ANG II occurred in this study could be attributed to activation of one or more parts of this network. Unlike elevation of water intake and MAP, HR was not altered by infusion of ANG II. Several possibilities could exist to contribute to this result. First, the existence of baroreceptor reflexes that were activated secondary to the increase in MAP induced by ANG II might compromise the change in HR. Although ANG II attenuated the function of baroreflexes (5, 7, 16, 22, 27, 32), loading baroreceptors due to ANG II pressor effect could buffer, more or less, the possible excitatory effect on HR. Second, with regard to autonomic nervous system, the responses to ANG II were mainly mediated by the sympathetic nervous system (26, 31, 37). Because HR is controlled by both the sympathetic and parasympathetic nervous system, the effect of ANG II on HR is consequently lighter than that on blood pressure, which is mostly determined by sympathetic nervous system.

The physiological effects of ANG II on autonomic function appear to be mediated by ANG II type 1 (AT1) receptors (36, 37). Studies (1, 28, 29, 36) suggest that AT1 receptors are concentrated in the CVO, NTS, RVLM, and hypothalamic nuclei (PVN and SON). In accordance with those studies, intracerebroventricular injection of losartan completely abolished the enhanced central sensitivity of the CSAR induced by chronic infusions of ANG II in the current study, suggesting that the ANG II-induced facilitation on the CSAR is mediated by the AT1 receptor. In a previous study (18), it was reported that the central gain of the CSAR was enhanced in dogs with heart failure, and this enhancement was abolished by intracerebroventricular administration of losartan, whereas losartan did not affect the central gain of the CSAR in normal dogs. ANG II level is known to be elevated during the heart failure state (11). The similar results that the CSAR was enhanced and central administration of losartan abolished this enhancement in both studies imply that ANG II might be involved in cardiovascular disturbances in the disease state like heart failure by modulation of the CSAR.

One concern in this study is whether ANG II infused into the brain has leaked into the circulation, and, consequently, the effect of chronic infusion of ANG II was attributed to its peripheral and not central, action. However, in the present study the low dose of losartan applied intracerebroventricularly in the present study abolished this enhanced CSAR response completely. Because the dose of losartan used in this study (0.125 mg/kg) was much lower than the dose that blocks the peripheral effects (vasoconstriction) of ANG II (8, 10, 12), it was presumed that the sensitization of the CSAR induced by ANG II was primarily, if not exclusively, attributed to its central effect.

In summary, chronic central infusion of ANG II potentiated the central sensitivity of the CSAR, and this effect was abolished by the central administration of losartan, whereas acute infusion of ANG II did not have a significant effect on the central sensitivity of this reflex. These results suggest that ANG II may facilitate the CSAR by a long-term effect via central AT1 receptors.

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