Forebrain renin-angiotensin system has a tonic excitatory influence on renal sympathetic nerve activity

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Wei, Shun-Guang, and Robert B. Felder. Forebrain renin-angiotensin system has a tonic excitatory influence on renal sympathetic nerve activity. Am J Physiol Heart Circ Physiol 282: H890–H895, 2002.—All elements of the renin-angiotensin system (RAS) are present in the forebrain, particularly in circumventricular organs surrounding the third cerebral ventricle. We tested the hypothesis that forebrain angiotensin-converting enzyme (ACE) has a tonic excitatory influence on sympathetic drive. Neurally intact and sinoaortic-denervated pentobarbital-anesthetized rats were treated with forebrain-directed intracarotid artery (ICA) versus intravenous injections of angiotensin I (ANG I) and of the ACE inhibitor captopril. In intact rats, ICA ANG I elicited a rise in arterial pressure and a concomitant reduction in renal sympathetic nerve activity (RSNA; ICA captopril elicited the opposite responses). In barodenervated rats, ICA ANG I increased and ICA captopril decreased arterial pressure and RSNA in parallel; intravenous ANG I had no effect on RSNA. The findings suggest that the intrinsic forebrain RAS has a tonic excitatory influence on sympathetic drive that is overshadowed in normal rats by baroreflex mechanisms, but may assume a more prominent role in pathophysiological states (e.g., heart failure) in which baroreflex mechanisms are impaired and RAS activity is augmented.

THE RENIN-ANGIOTENSIN SYSTEM (RAS) in the rat forebrain is prominently involved in the regulation of extracellular fluid volume and sympathetic drive (3, 9). Previous work examining the central effects of the RAS on sympathetic drive has focused primarily on the effects of angiotensin II (ANG II) mediated by angiotensin type 1 (AT1) receptors. However, tissue concentrations of angiotensin-converting enzyme (ACE) are high in the forebrain, particularly in the circumventricular organs (12) relative to peripheral tissues, such as the lung, (13) which are involved in the conversion of circulating ANG I to ANG II. Investigators examining dipsogenic responses routinely utilize forebrain ACE as an experimental tool by injecting ANG I or by stimulating its production to examine the effects of ANG II mediated by forebrain AT1 receptors (16).

The present study tested the hypothesis that the activity of forebrain ACE has an excitatory influence on sympathetic drive. The findings demonstrate a previously unrecognized tonic excitatory influence of the intrinsic forebrain RAS on renal sympathetic drive, an effect that may act in concert with the behavioral influences of central RAS to promote volume accumulation. Although the results suggest that the influence of forebrain RAS on sympathetic drive is relatively small under normal conditions, it may assume greater importance in the altered neurohumoral milieu of pathophysiological states like heart failure.

MATERIALS AND METHODS

Experiments were performed on 20 adult male Sprague-Dawley rats (250–350 g) obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals were housed in the Animal Care Facility at the Department of Veterans Affairs Medical Center in Iowa City. All experimental procedures were approved by the institution’s Animal Care and Use Committee and conformed to the guidelines of the American Veterinary Association.

General preparation. While the rats were under pentobarbital anesthesia (50 mg/kg ip), a polyethylene (PE)-10 catheter connected to PE-50 tubing filled with heparinized saline (50 U/ml) was inserted into the abdominal aorta through the left femoral artery for direct measurement of arterial pressure (AP). A PE-50 catheter was inserted into the left femoral vein for intravenous injection of drugs. The catheters were tunneled to the back of the neck. The trachea was then cannulated low in the neck; the rat breathed spontaneously and was not mechanically ventilated. Body temperature was maintained at 37 ± 1°C with a heating pad and heat lamp. A rostrally directed PE-20 cannula was inserted into the left common carotid artery, with the tip placed in the bifurcation for intracarotid artery (ICA) injection. Supplemental anesthesia (10 mg/kg) was given when necessary, as evidenced by marked changes in AP, heart rate (HR), and renal sympathetic nerve activity (RSNA), either spontaneously or in response to a pinch of the hind paw. The arterial catheter was connected to a pressure transducer (Statham P23dB; Gould; Cleveland, OH) to measure arterial pressure.

Sinoaortic baroreceptor denervation. Baroreceptors were denervated by cutting the carotid sinus and aortic depressor nerves bilaterally. A 2-cm midline incision was made on the ventral surface of the neck. After the omohyoideus muscle...
was retracted and the carotid arteries were exposed bilaterally, the aortic depressor nerves were identified by their characteristic anatomy and were cut near their junctions with the superior laryngeal nerves, or the superior laryngeal nerves were transected bilaterally at their convergence with the vagus nerve. Because aortic baroreceptor fibers in rats sometimes travel in the cervical sympathetic and recurrent laryngeal nerves were transected bilaterally near their junctions with the superior laryngeal nerves, or the superior laryngeal nerves were identified by their origin in the carotid bifurcation and insertion into the glossopharyngeal nerves and were then sectioned bilaterally near their insertion into the glossopharyngeal nerves. Sham sinoaortic baroreceptor denervation (SAD) was performed by the exposure of the baroreceptor nerves, which were left intact. Baroreceptor denervation was confirmed by the lack of change in HR and RSNA when mean arterial pressure (MAP) was increased or decreased by the intravenous injection of phenylephrine (5 μg/kg) or sodium nitroprusside (10 μg/kg), respectively. Both baroreceptor-denervated and neurally intact animals were allowed to recover for 2 h after the surgical preparation before being tested for the effects of ANG I and captopril. The duration of the recording procedure was comparable in the two groups, as was the amount of supplemental anesthesia.

RSNA recording. The left kidney was exposed through a flank incision. One of the nerves to the left kidney was dissected free from surrounding tissue and placed on bipolar silver wire recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve signal was determined using a window discriminator (20-ms time constant, BAK Electronics; Germantown, MD) to rectify and integrate the raw signal. The raw RSNA was passed to a Paynter filter (20-ms time constant, BAK Electronics; Germantown, MD) to rectify and integrate the raw signal. In addition, the frequency (spikes/s) of multifiber discharge in the raw renal nerve signal was determined using a window discriminator (model DIS-1, BAK) that generated a standard transistor-transistor logic pulse for each spike exceeding a selected voltage, which was set just above the noise level. The AP, integrated RSNA, and windowed RSNA signals were passed to a Cambridge Electronics Design laboratory interface (CED, model 1401; Cambridge, UK) linked to a personal computer. Heart rate (in beats/min) was then derived from the frequency of the arterial pressure pulses. The raw RSNA and blood pressure were also recorded on videotape with the use of a pulse code modulation-recording adapter (Vetter; Rebersburg, PA) for analysis off-line.

Data analysis. Data were analyzed with Spike2 software (CED). The integrated renal nerve voltage signal was further processed to assess burst frequency, using a program written with CED software that identified the nadir between bursts of activity. Peak responses of MAP (mmHg), HR (beats/min), and RSNA (mV, spikes/s, and bursts/s) over 20-s intervals were compared with baseline values averaged over 60-s intervals immediately preceding each intervention. RSNA responses shown in figures are changes in spike frequency (8), and systemically by the intravenous route. All drugs were administered by bolus injection (ICA; 5-μl drug, followed by 15-μl aCSF flush; intravenous 5-μl drug, followed by 45-μl flush). aCSF (20 μl) was injected ICA as a control.

Protocols. After recovery from the surgical preparation, baroreceptor-denervated (n = 8) rats were studied for AP, HR, and RSNA responses to bolus ICA or intravenous injections of ANG I (25, 50, and 100 ng/kg) and captopril (50 μg/kg). Each injection was preceded by a measurement of baseline AP and RSNA, and followed by a recovery period of ~20 min, allowing measured variables to return to baseline levels. In a separate group of barodenervated rats (n = 4), the responses to increasing intravenous doses of captopril (0.1–3.2 mg/kg) were tested using the same bolus injection and recovery protocol. At the completion of the protocols, animals were euthanized with an overdose of anesthesia.

Values are means ± SE; n, no. of rats. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity. *P < 0.05, compared with intact rats.

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RSNA, mV</th>
<th>RSNA, bursts/s</th>
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<tr>
<td>Intact</td>
<td>8</td>
<td>107.3 ± 3.6</td>
<td>347 ± 7</td>
<td>9.1 ± 0.4</td>
<td>3.8 ± 0.2</td>
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<tr>
<td>Denervation</td>
<td>8</td>
<td>118.4 ± 4.8*</td>
<td>366 ± 11*</td>
<td>10.3 ± 0.6*</td>
<td>4.1 ± 0.2</td>
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Table 1. Baseline values
Fig. 2. Effects of increasing doses of ANG I, administered ICA, on HR, RSNA (as % change from baseline in spikes/s) and mean arterial pressure (MAP) in neurally intact (A; n = 8) and baroreceptor-denervated (B; n = 8) rats. Responses are unidirectional in the denervated rats; the intact rats demonstrate a baroreflex-mediated inhibition of HR and RSNA. bpm, beats/min. *P < 0.05, compared with control values. †P < 0.05, compared with 25 ng/kg dose values.

(spiess/s), reported as a percentage of baseline control. Student’s t-test was used to determine statistical significance between paired data for a single comparison. Statistical significance among multiple comparisons was determined by analysis of variance for repeated measures. Differences were considered significant at P < 0.05. All values are expressed as the means ± SE.

RESULTS

Effects of baroreceptor denervation on baseline variables. Table 1 shows the baseline values for MAP, HR, and RSNA (measured as integrated voltage, burst frequency, and spike frequency) in the neurally intact and baroreceptor-denervated rats. The baseline values of all three variables were higher in the barodenervated rats.

Effect of ICA administration of ANG I. Figure 1 shows a representative recording of the responses to ICA administered ANG I in intact and baroreceptor-denervated rats. In the intact animal (Fig. 1A), ANG I elicited an increase in AP and a decrease in RSNA and HR. The maximum response occurred ~30 s after the ICA bolus of ANG I. In the baroreceptor-denervated rat (Fig. 1B), ICA ANG I increased AP and RSNA, with little change in HR. In either case, all variables had returned to baseline 10–15 min after ANG I injection.

Figure 2 shows dose-response data for the ICA injection of ANG I (25, 50, and 100 ng/kg) in intact (n = 8) and barodenervated rats (n = 8). In the intact rats (Fig. 2A), there was a dose-dependent increase in MAP, and a corresponding dose-dependent decrease in RSNA. The effect of 100 ng/kg ANG I on MAP and RSNA was significantly (P < 0.05) greater than the effect of 25 ng/kg. In contrast, in the barodenervated rats, MAP, HR, and RSNA all increased in a dose-dependent manner. MAP increased significantly (P < 0.05) at each dose increment, and the RSNA response to the 100 ng/kg dose was significantly (P < 0.05) greater than to the 25 ng/kg dose. The MAP response to ANG I was greater (P < 0.05) in the baroreceptor-denervated rats than the intact rats at each dose tested. Administration of ICA aCSF had no effect on the recorded variables: intact rats (n = 5, aCSF vs. baseline), MAP, 109.6 ± 4.2 versus 108.9 ± 4.2 mmHg, HR, 351.6 ± 8.1 versus 351.1 ± 8 beats/min, RSNA, −0.63 ± 0.03% change; denervated rats (n = 6), MAP, 119.2 ± 5.3 versus 118.8 ± 5.3 mmHg, HR, 367.6 ± 13.0 versus 367.3 ± 13.1 beats/min, and RSNA, 0.54 ± 0.04% change.

ICA versus intravenous ANG I. The responses to ICA ANG I (100 ng/kg) were compared with responses to the same dose of ANG I administered intravenously. In intact rats (Fig. 3A), intravenous ANG I elicited changes in MAP, HR, and RSNA very similar to those induced by ICA ANG I. In baroreceptor-denervated animals (Fig. 3B), however, the MAP response to intravenous ANG I was similar to the ICA response, but the increase in HR or RSNA was not observed.
Effect of ICA administration of captopril. Figure 4 shows a representative recording of the effects of ICA captopril (50 μg/kg) on AP, HR, and RSNA. In the intact rats (Fig. 4A), ICA captopril elicited a reduction in AP and an increase in RSNA and HR. In the denervated rats (Fig. 4B), ICA captopril induced a decrease in AP, HR, and RSNA.

This dose of captopril also prevented the changes in MAP, HR, and RSNA induced by ANG I in both groups of rats. In the intact rats, after ICA captopril administration, there was no significant change (P > 0.05) in MAP (100.4 ± 3.3 vs. 103.8 ± 3.4 mmHg), HR (340.1 ± 11.4 vs. 338.9 ± 11.1 beats/min), or RSNA (−2.78 ± 0.63%) in response to ICA ANG I (100 ng/kg). Similarly, in the denervated rats, after ICA captopril administration, MAP (112.0 ± 5.3 vs. 116.4 ± 5.2 mmHg), HR (356.3 ± 12.0 vs. 357.6 ± 11.9 beats/min), and RSNA (0.96 ± 1.42%) did not change (P > 0.05) in response to ICA ANG I (100 ng/kg).

ICA versus intravenous captopril. The responses to ICA captopril (50 μg/kg) were compared with responses to the same dose of captopril administered intravenously. In intact rats (Fig. 5A), ICA and intravenous captopril elicited very similar decreases in MAP and increases in HR and RSNA. In baroreceptor-denervated animals (Fig. 5B); however, ICA captopril decreased MAP, RSNA, and HR. Intravenous administration of captopril decreased MAP, but there was no change in HR or RSNA.

Effects of systemic captopril on sympathetic drive. In a separate group of baroreceptor-denervated rats (n = 4), increasing doses of captopril (0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg) were administered intravenously to determine the systemic dose required to cause a central inhibition of sympathetic outflow (Fig. 6). Intravenous captopril doses of at least 0.4 mg/kg were required to achieve any significant (P < 0.05) reduction in RSNA, and higher doses were required to achieve a substantial (20–30%) reduction in RSNA. A similar dose-response relationship was observed for HR and MAP (Fig. 6).

DISCUSSION

This study demonstrates the novel finding that intrinsic forebrain RAS has a tonic excitatory influence on sympathetic drive to the kidney, as evidenced by a reduction in RSNA in response to blocking angiotensin-converting enzyme activity in the forebrain region of the baroreceptor-denervated rat. The results from the neurally intact rats demonstrate that baroreceptor reflexes exert a powerful restraining influence on sympathetic regulation by the forebrain region.

It is well recognized that activation of AT1 receptors in forebrain CVOs by ANG II leads to a pressor response and increased sympathetic drive. These receptors are accessible to circulating ANG II, but might also be stimulated by ANG II produced locally by the intrinsic RAS. Forebrain CVOs have high tissue concentrations of ACE, even compared with peripheral tissues such as the lung that convert circulating ANG I to ANG II (13). In the present study, we have de-
In our study, the effects of ANG I and captopril on RSNA were baroreceptor mediated in the intact animals; i.e., there was a reciprocal relationship between MAP and RSNA. After baroreceptor denervation, this relationship was lost and parallel responses in MAP and RSNA were observed, consistent with a central effect of these substances on sympathetic drive. The captopril data suggest a previously unrecognized tonic excitatory influence of forebrain RAS on RSNA. In normal rats, this influence is overridden by prominent baroreceptor modulation. The data also indicate that relatively high doses of systemically administered ACE inhibitor are necessary to elicit a centrally mediated suppression of sympathetic drive, even after the baroreceptors are denervated.

**Perspectives.** In pathophysiological states such as congestive heart failure, in which the normal baroreflex restraints are diminished (18), forebrain RAS may play an important role in setting the excessive level of sympathetic drive. The influence of the forebrain may be augmented by standard heart failure treatment with diuretics and ACE inhibitors, which fosters high levels of circulating ANG I (4) without substantially blocking the formation of ANG II (4) or aldosterone (10). All three of these circulating components of the RAS can act on receptors in the forebrain to augment sympathetic drive [see (Ref. 7) for discussion of aldosterone effects on sympathetic drive]. Although orally administered ACE inhibitors are theoretically capable of blocking both central (13) and peripheral ACE, it is likely that the clinical goal of reducing systemic vascular resistance is reached at doses too low to substantially block the actions ACE in forebrain, and particularly in the subfornical organ, where ACE is present in very high concentrations (5). Thus the beneficial peripheral effects of afterload reduction and improved cardiac output may be achieved at the expense of a centrally mediated increase in sympathetic drive that contributes to the progression of myocardial injury (2) and lethal arrhythmias (14). The present study highlights the need for further research into the role of the forebrain in pathological conditions like heart failure that are characterized by enhanced sympathetic drive.

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**REFERENCES**


