Endogenous ANG II supports lumbar sympathetic activity in conscious sodium-deprived rats: role of area postrema

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Xu, Ling, John P. Collister, John W. Osborn, and Virginia L. Brooks. Endogenous ANG II supports lumbar sympathetic activity in conscious sodium-deprived rats: role of area postrema. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R46–R55, 1998.—This study tests the hypothesis that the area postrema (AP) is necessary for endogenous ANG II to chronically maintain lumbar sympathetic nerve activity (LSNA) and heart rate (HR) in conscious sodium-deprived rats. The effect of the ANG II type 1-receptor antagonist, losartan, on LSNA and HR was determined in rats that were either AP lesioned (APX) or sham lesioned. The sham rats were divided into groups, with (SFR) or without (SAL) food restriction, to control for the decreased food intake of APX rats. Before losartan, basal mean arterial pressure (MAP), HR, and baroreflex control of LSNA and HR were similar between groups, with the exception of lower maximal reflex LSNA and higher maximal gain of the HR-MAP curve in APX rats. In all groups, losartan similarly shifted (P < 0.01) the LSNA-MAP curve to the left without altering maximal gain. Losartan also decreased (P < 0.05) minimal LSNA in all groups, and suppressed (P < 0.01) maximal LSNA (% of control) in SFR (240 ± 13 to 205 ± 15) and SAL (231 ± 21 to 197 ± 26) but not APX (193 ± 10 to 185 ± 8) rats. In general, losartan similarly shifted the HR-MAP curve to a lower MAP in all groups. The results suggest that the AP is not necessary for endogenous ANG II to chronically support LSNA and HR at basal and elevated MAP levels in sodium-deprived rats. However, the AP is required for endogenous ANG II to increase maximal reflex LSNA at low MAP levels.

angiotensin II type 1 receptor; baroreflex; heart rate; losartan; sympathetic nerve activity

recent studies suggest that chronic elevation of endogenous angiotensin II (ANG II) maintains sympathetic outflow in rats. For example, blockade of the renin-angiotensin system with ANG II antagonists or angiotensin-converting enzyme (ACE) inhibitors decreases sympathetic nerve activity in rats with chronic heart failure (8), renal hypertension (21), spontaneous hypertension (29), and sodium deprivation (9, 50, 51). A key feature of most of these studies is that the hypotension due to blockade of the renin-angiotensin system was reversed by intravenous infusion of α1-adrenergic agonists to avoid reflex increases in sympathetic outflow. Moreover, it was found that ANG II blockade shifted baroreflex control of sympathetic nerve activity or heart rate (HR) to a lower mean arterial pressure (MAP) level (8, 9, 21, 29, 51). The net result was that sympathetic nerve activity and HR after ANG II blockade were lower over a wide range of MAP. The brain has been suggested as a site at which endogenous ANG II acts to influence the autonomic nervous system (for review, see 39), but it remains unclear where in the brain the action of ANG II takes place. There are several lines of indirect evidence that the area postrema (AP), a circumventricular organ located at the floor of the fourth ventricle on the dorsal surface of the medulla of the brain, may be a site of action of ANG II in rats (12). First, the AP, like other CVOs, lacks a blood-brain barrier and therefore circulating substances, such as ANG II, can gain access to influence the sympathetic nervous system. Second, in vitro autoradiographic studies reveal many ANG II binding sites in the rat AP (17, 46), and intravenous injections of losartan, a nonpeptide ANG II type 1 (AT1)-receptor antagonist, dose-dependently competes with ANG II at the binding sites (55). Third, anterograde and retrograde anatomic tracing studies demonstrate that the rat AP is a relay station for afferents from, and efferents to, other important brain regions integrated in cardiovascular regulation, including the nucleus tractus solitarius and the parabrachial nucleus (6, 28, 42, 48). Fourth, physiological studies show that intravenously injected ANG II activates some rat AP neurons, independently of changes in arterial pressure (11). Fifth, microinjection of ANG II into the rat AP causes dose-dependent increases in arterial pressure and this increase in pressure can be attenuated by intravenously injected losartan (32). Finally, the rat AP may be involved in the development of renal and chronic ANG II induced hypertension (14, 15).

The present study was conducted to directly test the hypothesis that circulating endogenous ANG II acts at the AP to maintain sympathetic nerve activity under physiological conditions. This hypothesis was tested by determining whether the AP is necessary for endogenous ANG II to maintain lumbar sympathetic nerve activity (LSNA) and HR in conscious sodium-deprived rats with elevated levels of circulating ANG II. More specifically, we examined the effects of AP lesion on arterial pressure and baroreflex control of LSNA and HR in conscious sodium-deprived rats. We then determined whether lesions of the AP abolished the ability of losartan to suppress LSNA and HR over the entire baroreflex range of arterial pressure.

METHODS

Male, Sprague-Dawley rats (225–333 g, Harlan Sprague Dawley, Indianapolis, IN), at 8 wk of age, were subjected to either AP lesion or sham operation and received postsurgical care at the University of Minnesota, as previously described (4). Briefly, rats were first injected with pentobarbital sodium (32.5 mg/kg ip), and surgical anesthesia was achieved with a
second intramuscular injection of an anesthetic cocktail (acepromazine 0.2 mg/kg, butorphanol tartrate 0.2 mg/kg, ketamine 25 mg/kg). Rats were then placed in a stereotaxic apparatus, and the AP was exposed on the dorsal surface of the medulla at the caudal extent of the fourth ventricle and removed by suction with a blunt 26-gauge needle attached to a vacuum line. Sham lesion operations were identical except that the vacuum line was not attached. Because AP lesioned (APX) rats exhibit decreases in food intake for a few weeks after the lesion (4, 22, 23), a recovery period of 8 wk was allowed to restore normal growth rate. Two groups of sham rats were used in this study to control for the reduced food intake in APX rats. One group received food and water ad libitum (SAL) during the recovery period. The second group was food restricted (SFR) for the first 3 wk after sham operation to match the reduced food intake observed in APX rats. Food intake for SFR rats was limited to 50, 60, and 80% of normal food intake of SAL rats during the 1st, 2nd, and 3rd wk post-sham lesion operation, respectively.

Eight weeks after surgery for the AP lesion or sham lesion, rats were shipped to the Oregon Health Sciences University for nerve recording studies. All lesioned and sham rats were coded with ear notches, and coding information was not released to the experimenter until after studies were completed. All rats, at 16 wk of age, were then placed on sodium-deficient diet (Na< 0.02%, Harlan Teklad, Madison, WI) for 2–3 wk to increase endogenous ANG II levels before surgery for catheter and nerve electrode implantation. During the first 2 days on a sodium-deficient diet, rats received a furosemide injection (1 mg·kg$^{-1}$·day$^{-1}$ i.p.; Abbott Laboratories, N. Chicago, IL) to increase sodium excretion. All rats were housed in a room maintained on a 12:12-h light-dark cycle and were allowed to have food and distilled water ad libitum. Experiments were conducted in the same room while rats remained in their home cage.

Chemicals

All drugs were dissolved in water containing 5% dextrose. Methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN) was used for anesthesia during surgery for nerve electrode and catheter implantation. Phenytoine (PE) and nitroprusside (NP) (both from Sigma, St. Louis, MO) were infused to manipulate MAP in the baroreflex studies. Losar- tane (gift of Dr. Ronald Smith, Du Pont Merck Pharmaceutical, Wilmington, DE) was injected intravenously for blockade of AT$_1$ receptors. Methoxamine was infused intravenously to maintain MAP after losartan injection, and hexamethonium chloride was injected intravenously for ganglionic blockade at the end of the experiment to block postganglionic nerve activity (both drugs from Sigma, St. Louis, MO). All injections were given in 100-µl volume, followed by a 100-µl flush of the dead space of catheters.

Catheter and Nerve Electrode Implantation

Rats (298–496 g) were anesthetized with an initial injection of methohexital sodium (50 mg/kg ip), followed by a second injection at the same dose about 5 min later. After a venous catheter was inserted into the right jugular vein, anesthesia was maintained by intravenous methohexital sodium infusion as needed (2.7–4 µl/min, 10 mg/ml). A total of four Tygon catheters (Norton Performance Plastics, Akron, OH) were implanted for drug delivery, two of which were inserted into the right jugular vein and two placed via the left femoral vein. An additional catheter was advanced into the abdominal aorta via the left femoral artery for the measurement of MAP. For the lumbar nerve electrode implantation, a midline abdominal incision was made. After retraction of the intestines, the abdominal aorta and vena cava were gently pulled aside to expose a lumbar nerve. The nerve was then dissected free and placed on a bipolar electrode. The electrode was constructed with two Teflon-coated, three-stranded stainless steel wires (no. 7934, A & M Systems, Everett, WA) and was encased within silicon tubing (0.02 × 0.037 in., Specialty Manufacturing, Saginaw, MI). When optimal nerve activity was confirmed on the oscilloscope (model 2212, Tektronix, Beaverton, OR) by observing the rhythmic bursts of nerve traffic, the nerve and electrode were embedded in a small amount of dental gel (President Light Body, Colten, Hudson, MA).

Catheters and the electrode lead were tunneled subcutaneously to the back of the neck and exteriorized. All incisions were closed with silk suture. The rats were returned to their home cage and allowed 20–40 h for recovery.

Hemodynamic and Nerve Activity Recordings

MAP was monitored via the femoral arterial catheter connected to a Statham pressure transducer and a Grass preamplifier (7P1). HR was measured using a Grass tachograph (7P4) triggered by the amplified arterial pressure pulse. Rat LSNA was measured using a Grass differential preamplifier (P511) with a bandpass filter of 30 Hz to 10 kHz. The gain (25,000–70,000×) of the preamplifier was adjusted so that the amplitude of maximal nerve activity output did not exceed the linear input range (+1.5 V peak-peak) of the Grass integrator (7P10), which was used for integration of raw nerve activity. The amplified nerve activity traffic was observed on the storage oscilloscope and was whole wave rectified and integrated with a rest time of 1 s. Together with MAP and HR, integrated LSNA was recorded on chart paper using a Grass polygraph (7D). Nerve activity was first quantified by averaging the integrated activity just before reset over 12 s (12 peaks) during stable and quasistable periods (slow or no change in measured parameters) or 3–4 s (3–4 peaks) during transient periods (e.g., baroreceptor reflex curve) (51). In addition, the noise level was quantified at the end of the experiment by averaging the integrated output over 12 s (12 peaks) after efferent nerve activity was eliminated by the combined use of a bolus injection of hexamethonium and infusion of methoxamine. The noise output was then subtracted from the average integrated nerve activity to provide a measure of net LSNA. For each animal, the net LSNA was normalized using two methods. First, LSNA was normalized to basal nerve activity in the control period and was expressed as a percentage of control. Basal nerve activity was defined as the average of resting activity at two time points within 10 min before the first baroreflex curve was generated. Second, LSNA was normalized to the maximal nerve activity during the control period and was expressed as a percentage of maximum. Maximal LSNA was the peak LSNA induced by NP infusion during baroreflex curve generation.

Baroreceptor Reflex Curves

MAP was varied by intravenous infusion of increasing doses of either PE (0.68–27 µl/min, 1 mg/ml) to increase MAP up to ~170 mmHg or NP (1.35–68 µl/min, 1 mg/ml iv) to decrease MAP to ~50 mmHg. The corresponding response of LSNA and HR, together with MAP, were recorded. The ramp increase or decrease of MAP was completed in ~2 min. Infusions of PE or NP were performed randomly. MAP, LSNA and HR were allowed to return to baseline (~30 min) before a subsequent ramp of MAP was made.
On the morning of the experiment, NP and PE for the baroreflex study were loaded into separate catheters to fill the dead space. After MAP, HR and LSNA were stable (in ≈ 30 min), these variables were recorded for 40–60 min. Starting around noon, time course and baroreflex function studies were begun. After basal parameters were obtained and prelosartan baroreflex control of HR and LSNA was determined, losartan was injected (10 mg/kg iv), and immediately intravenous infusion of methoxamine (5–33 µg/min) was started to prevent postlosartan hypotension. MAP, HR, and LSNA were recorded for at least 40 min after losartan administration, because, in our previous study (50), it took at least 40 min for the depressor effect of losartan to stabilize. The postlosartan baroreflex control of HR and LSNA was then determined. At the beginning and end of each experiment, the pressor response to an Ang II bolus (100 ng/kg iv) was tested by measuring the peak change in MAP.

Verification of AP Lesions

After the experiments, rats were anesthetized with methohexitol sodium and then perfused via the left cardiac ventricle with saline followed by 4% paraformaldehyde. The brain was then removed and soaked in 4% paraformaldehyde at 4°C. The following morning, the brain was rinsed with PBS and then transferred to a bottle containing 30% sucrose solution. The brain was then stored in the bottle and shipped back to the University of Minnesota for histological verification of the AP lesion in APX rats, as described previously (4).

To determine whether the AP lesion damaged the neighboring NTS, MAP lability was also examined and compared between APX and sham rats (30, 36). MAP was recorded for at least 40 min before baroreflex function studies in individual rats and sampled every 30 s to generate at least 80 readings. The SD of MAP was calculated in individual rats. The SDs of MAP in individual rats and sampled every 30 s to generate at least 80 readings.

Histological verification of AP lesion was confirmed in all APX rats. Typical histological sections from sham rats and APX rats are shown in Fig. 1, A and B, respectively. The AP was completely lesioned, and the ablation of AP caused little damage to the adjacent NTS in all APX rats. The SD of MAP was not significantly different (P = 0.86) among groups [APX (n = 4), 4.66 ± 1.06; SFR (n = 6), 5.02 ± 0.38; SAL (n = 6), 5.16 ± 0.55 mmHg], further indicating that the AP ablation did not significantly damage baroreflex components of NTS.

Data Analysis and Statistics

A logistic relation, slightly modified from Kent et al. (26), was used to analyze baroreflex curves: \( Y = d + (a - d) / [1 + \exp(b(x - c))] \), where \( X \) represents MAP, \( Y \) LSNA or HR, a maximum of LSNA or HR, b slope coefficient, c MAP at the midpoint of the range of LSNA or HR, and d minimum of LSNA or HR. In each animal, raw MAP and LSNA (or HR) data were fit to the logistic function to generate parameters a, b, c, and d using graphics software (SigmaPlot, Jandel Scientific, Corte Madera, CA). Constraints of maximum and minimum of LSNA or HR were set for the fitting process. The maximal or minimal values of LSNA and HR were determined in each experiment when the high or low plateau of LSNA and HR were reached while MAP was still being decreased or increased by NP or PE infusion, respectively. The range of the baroreflex curve, \( e \), was defined as (a – d), and the maximal gain of the baroreflex curve was calculated using the formula \(-be4\). Means ± SE of individual fitted curve parameters were calculated, and statistical analysis was performed to determine within and between group differences in these parameters (Tables 1 and 2). The averaged a, b, c, and d were then used to generate averaged baroreflex curves (Figs. 3 and 5).

In addition, LSNA (or HR) from all rats in each group was pooled by calculating means ± SE of all data points collected within 5-mmHg MAP increments. Multiple points at the same MAP in each animal were averaged before pooling. The means of pooled data were plotted with SE of LSNA or HR and then fitted to the logistic function (Figs. 4 and 6).

All data are presented as means ± SE. For time course and baroreflex function studies, data were analyzed using two-factor ANOVA, repeated one way (time or drug) (49). For comparison of MAP lability, pressor responses to intravenous bolus of Ang II and changes in MAP at 50 or 100% of control LSNA (MAP50 or MAP100, respectively) after losartan, one-factor ANOVA was used. The Newman-Keuls post hoc test was used for multiple comparisons after ANOVA (49). All analyses were performed using GB-STAT software (Dynamic Microsystems, Silver Spring, MD). A significance level of \( P < 0.05 \) was accepted.

RESULTS

Three groups of rats, APX (n = 10), SFR (n = 9), and SAL (n = 9) were used in this study. In some rats, LSNA was lost either before or during the experiment, but HR was recorded or HR was not detected by the tachograph due to an inadequate trigger but LSNA was recorded. Therefore the final number of rats included for data analysis and presentation in the figures and tables may be less than those totals.

Time Course of Changes in LSNA and HR after Losartan

As shown in Fig. 2, basal LSNA (expressed as %max) was significantly higher (P < 0.05) in APX rats (56 ± 5% max) than SFR and SAL rats (42 ± 2 and 46 ± 4% max, respectively), but basal HR was not significantly different among groups (APX, 399 ± 13; SFR, 386 ± 10; SAL, 405 ± 11 beats/min). Basal MAP in SAL rats (100 ± 3 mmHg) was significantly lower (P < 0.01) than that in APX rats (107 ± 3 mmHg) and SFR rats (108 ± 2 mmHg).

After losartan administration, MAP was maintained by methoxamine infusion at prelosartan levels in all groups (Fig. 2). Losartan decreased LSNA, and the decreases were not different among groups (Fig. 2). At 40 min after losartan administration, LSNA had fallen (P < 0.01) to 36 ± 4, 25 ± 4, and 29 ± 4% max in APX, SFR, and SAL rats, respectively.

Losartan also similarly decreased HR in all groups (Fig. 2). At 40 min after losartan administration, HR was significantly suppressed to 355 ± 13 beats/min in APX rats and 353 ± 8 beats/min in SFR rats (P < 0.05). Although the HR suppression in SAL rats was not significant, HR at 40 min postlosartan (365 ± 8 beats/min) was not significantly different from the corresponding HR values in APX or SFR rats.

Baroreflex Control of LSNA

Effect of AP lesion. The AP lesion did not alter the sensitivity or gain of baroreflex control of LSNA, but...
decreased maximal reflex LSNA. Before losartan administration, maximal gain of the LSNA-MAP curve in APX rats was not different from that in SFR or SAL rats either when nerve activity was expressed as percentage of control or percentage of maximum (Figs. 3 and 4, Table 1). MAP50 was also not different between groups (Table 1). When LSNA was expressed as percentage of control, maximal LSNA in APX rats was significantly lower ($P < 0.01$) than that in SFR and SAL rats, as was the range (Fig. 3, Table 1). The lesser maximal LSNA and range in APX rats was not revealed when LSNA was expressed as percentage of maximum (Fig. 4, Table 1) because of the normalization.

Effect of losartan. Losartan shifted baroreflex control of LSNA to lower MAP levels similarly in all groups of rats without altering baroreflex sensitivity. Losartan did not affect maximal gain in any group, regardless of whether nerve activity was expressed as percentage of maximum or percentage of control (Figs. 3 and 4, Table 1). MAP50 was similarly decreased ($P < 0.01$) by losartan in all three groups, when LSNA was expressed as either percentage of control (Fig. 3, Table 1) or percentage of maximum (Fig. 4, Table 1). Another index of the position of the LSNA-MAP curve, MAP100 (Fig. 3), was also similarly decreased ($P < 0.01$) by losartan in all groups (APX: 106 ± 2 to 88 ± 2; SFR: 108 ± 2 to 89 ± 5; SAL: 103 ± 3 to 84 ± 5 mmHg). Minimal LSNA was similarly suppressed ($P < 0.05$) in all groups when nerve activity was expressed as percentage of control, but no significant suppression in SFR rats was detected when LSNA was expressed as percentage of maximum (Table 1). Finally, losartan suppressed ($P < 0.01$) maximal LSNA in SFR and SAL rats but not APX rats (Figs. 3 and 4, Table 1).
In summary, the data suggest that in sodium-deprived rats, lesion of the AP suppresses maximal reflex LSNA and prevents the action of losartan to decrease maximal LSNA at low MAP levels. However, the lesion does not alter the ability of losartan to decrease LSNA at normal or elevated MAP.

**Baroreflex Control of HR**

**Effect of the AP lesion.** The AP lesion generally did not affect baroreflex control of HR (Figs. 5 and 6, Table 2). Before losartan administration, MAP 50, maximal and minimal HR in APX rats were not significantly different from those in SFR or SAL rats, indicating that AP lesion did not shift the HR-MAP curve (Table 2). However, although maximal gain in APX rats was not significantly different from that in SFR rats, it was larger (P < 0.05) than that in SFR rats (Table 2).

**Effect of losartan.** Maximal gain was significantly decreased (P < 0.05) by losartan in APX rats but not SFR or SAL rats (Table 2). Losartan shifted the HR-MAP curve to a lower MAP level in all groups (Figs. 5 and 6, Table 2). Losartan also significantly decreased MAP 50 in APX rats and SFR but not in SAL rats (Table 2); however, the decrease in MAP 50 in APX rats (∼13 ± 4 mmHg) was similar to that in APX (∼11 ± 4 mmHg) and SFR rats (∼12 ± 4 mmHg), indicating that the HR-MAP curve was similarly shifted to a lower MAP level in all groups. Minimal HR was not altered in any group, but maximal HR was similarly suppressed in all groups (P < 0.05; Table 2).

In summary, the data suggest that, in sodium-deprived rats, the AP lesion does not alter baroreflex control of HR, nor does it prevent losartan’s action to suppress HR.

**Pressor Response to ANG II**

Before losartan administration, the pressor response to an ANG II bolus (100 ng/kg iv) in APX rats (24 ± 3 mmHg, n = 6) was significantly smaller (P < 0.05) than in SFR (44 ± 4 mmHg, n = 9) but not SAL rats (37 ± 4 mmHg, n = 8). At the end of each experiment, the same dose of ANG II did not change MAP in any rat, indicating complete blockade of AT 1 receptors.

**DISCUSSION**

The present study is the first to demonstrate that ablation of the AP does not have major impact on the suppression of LSNA and HR produced by acute intravenous losartan at most MAP levels in conscious, sodium-deprived rats. The present study also demonstrates that ablation of the AP does not have major impact on the suppression of LSNA and HR produced by acute intravenous losartan at most MAP levels in conscious, sodium-deprived rats.

### Table 1. Effects of losartan on baroreflex control of LSNA in APX, SFR, and SAL rats

<table>
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<th>SFR (n = 7)</th>
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<th>SAL (n = 8)</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>Maximum</td>
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<td>231 ± 21</td>
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<td>Slope coeff</td>
<td>0.092 ± 0.010</td>
<td>0.110 ± 0.013</td>
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<td>0.098 ± 0.013</td>
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<tr>
<td>MAP 50</td>
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<td>86 ± 5a</td>
<td>97 ± 3</td>
<td>85 ± 3a</td>
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</tr>
<tr>
<td>Minimum</td>
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<td>24 ± 7b</td>
<td>36 ± 4</td>
<td>15 ± 3a</td>
<td>42 ± 5</td>
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<tr>
<td>Range</td>
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<td>195 ± 18</td>
<td>182 ± 25</td>
<td>150 ± 11c</td>
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<td>Maximal gain</td>
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<td>−4.96 ± 0.89</td>
<td>−4.39 ± 0.69</td>
<td>−3.20 ± 0.20</td>
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**LSNA, %con**

**LSNA, %max**

### Table 2. Effects of losartan on baroreflex control of heart rate in APX, SFR, and SAL rats

<table>
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<tr>
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<th>APX (n = 7)</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Maximum</td>
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<td>479 ± 14a</td>
<td>524 ± 13</td>
<td>489 ± 17a</td>
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<td>Slope coeff</td>
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<td>0.090 ± 0.021</td>
<td>0.061 ± 0.007</td>
<td>0.090 ± 0.013†</td>
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<tr>
<td>MAP 50</td>
<td>109 ± 5</td>
<td>98 ± 3a</td>
<td>110 ± 4</td>
<td>97 ± 3</td>
<td>111 ± 3</td>
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<tr>
<td>Minimum</td>
<td>273 ± 12</td>
<td>259 ± 11</td>
<td>288 ± 13</td>
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<td>Range</td>
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<td>236 ± 14</td>
<td>200 ± 17</td>
<td>268 ± 19</td>
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<td>Maximal gain</td>
<td>−3.71 ± 0.36</td>
<td>−3.28 ± 0.43</td>
<td>−5.11 ± 0.98</td>
<td>−3.05 ± 0.40</td>
<td>−5.76 ± 0.58‡</td>
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**Values are means ± SE of baroreflex logistic curve parameters. LSNA, lumbar sympathetic nerve activity; APX, area postrema lesioned; SFR, sham with food restriction; SAL, sham without food restriction; Pre and Post, pre- and postlosartan, respectively. MAP 50, mean arterial pressure at 50% of range of LSNA. Data were analyzed with ANOVA and Newman-Keuls test. Post- vs. pre-losartan in each group: *P < 0.01; †P < 0.05. APX vs. SFR and SAL during pre-losartan period: ‡P < 0.01; ††P < 0.05. APX vs. SFR and SAL during post-losartan period: P < 0.05.
sodium-deprived rats. An important new finding is that many actions of losartan are similar in APX and sham rats; i.e., losartan similarly decreases LSNA and HR at basal MAP, suppresses baroreflex-mediated changes in LSNA at all but low MAP levels, and suppresses baroreflex-mediated changes in HR. Nevertheless, a key difference is that maximal reflex LSNA (%con) is lower in APX rats before losartan, and losartan does not reduce maximal LSNA further in the lesioned animals. Collectively, these results indicate that in contrast to our hypothesis, the AP is not necessary for endogenous ANG II to chronically support LSNA in sodium-deprived rats.
deprived rats, except at low MAP levels that correspond to maximal LSNA. The AP is also not necessary for endogenous ANG II to maintain HR.

Basal MAP and HR in APX rats were not significantly different from those in SFR rats, suggesting that the AP is not critical in the maintenance of basal MAP or HR during sodium deprivation. The results are similar to those of previous studies, suggesting that the AP plays little or no role in the maintenance of MAP in sodium-replete rats (2, 4, 15, 16, 20, 27, 33, 54), dogs (25, 37, 40), and rabbits (34) or HR in rats (10, 15, 33), dogs (25, 37), and rabbits (34). However, hypotension (13, 43, 44) and bradycardia (4, 27, 43, 44) after AP lesion has also been reported in animals with normal sodium intake. One explanation for the differences in basal MAP and HR could be the length of time allowed for recovery after AP lesion. Feeding behavior is depressed a few weeks after AP lesions (22, 23, 27), and this could affect cardiovascular regulation. For example, Skoog et al. (43, 44) reported basal MAP and HR were lower in rats 1 wk after AP ablation compared with sham-operated rats. In the present and previous studies (2, 10, 15, 33, 54), APX animals were allowed more time to regain steady-state feeding and growth rates (4, 22). On the other hand, it could be argued that the lack of APX-induced hypotension and bradycardia in the present study was because rats were studied 1–2 days after the stress of surgery for nerve electrode implantation. The stress could eliminate group differences in MAP or HR. However, sodium-replete APX rats studied well after the AP lesion and surgery for catheter implantation are also found to have normal arterial pressure (4).

Consistent with findings in previous studies (9, 50, 51), losartan decreased LSNA and HR over the entire range of MAP in AP intact rats, indicating that endogenous ANG II chronically maintains sympathetic outflow during sodium deprivation. It is important to emphasize that the ability of losartan to decrease nerve activity is due to blockade of ANG II receptors rather than other nonspecific effects. For example, the decrease is not a consequence of time, since nerve activity does not decrease in low-salt rats injected with saline vehicle or in high-salt rats given the same dose of losartan (50). It is also unlikely that the suppression of LSNA is due to an effect of methoxamine to directly decrease nerve activity by an action in the brain or at the baroreceptors. Previous studies have demonstrated that the sympathoinhibitory effect of intravenous phenylephrine administration is eliminated after combined sinoaortic denervation and vagotomy or maintenance of carotid sinus pressure in closed-loop experiments (5,

Fig. 5. Baroreflex control of HR in APX, SFR, and SAL rats. Curves were obtained before and after intravenous losartan injection. Sigmoidal baroreflex curves were generated from averaged logistic parameters as described in text.

Fig. 6. Baroreflex control of HR in SFR (A) and SAL (B) and APX (C) rats. Curves were obtained before (○) and after (●) intravenous losartan injection. Data were pooled as described in text.
Moreover, sustained methoxamine infusion does not decrease renal nerve activity when arterial pressure is returned to basal by inflation of a vena cava cuff (41). These results suggest that the sympathoinhibitory effects of intravenous α-adrenergic agonists are mediated via increased pressure activating baroreceptors. An important feature of the present experiments is that methoxamine was used to maintain arterial pressure, not increase it. Finally, it is unlikely that methoxamine is acting directly at arterial baroreceptors to decrease LSNA, since, at concentrations achieved in the present study, α-adrenergic agonists decrease aortic baroreceptor activity in vitro (which, if anything, should increase efferent sympathetic outflow) via constriction of the vessel in which the baroreceptor ends are embedded (35, 53).

AP lesions did not alter the magnitude or time course of the suppression of LSNA and HR by losartan when MAP was clamped at prelosartan levels (Fig. 2), nor did the lesion affect the suppression of baroreflex-mediated LSNA and HR at most levels of MAP. Interestingly, the lesion did prevent the ability of losartan to decrease baroreflex-mediated maximal LSNA. These data indicate that, in sodium-deprived rats, the AP is not necessary for the action of endogenous ANG II to maintain lumbar sympathetic outflow at high and normal MAP levels but is required for maximal increases in LSNA during hypotension.

These results may seem surprising given reports that AP lesions reduce MAP in rats with hypertension induced by elevated circulating ANG II levels or insertion of the mouse mRen-2Rd gene into the rat genome (1, 14, 15). Moreover, the hypotensive effect of chronic losartan infusion is reduced in rats with AP lesions (4). However, the antihypertensive effect of the AP lesion may not be due to the loss of a sympathoexcitatory action of circulating ANG II. Indeed, the AP lesion has also been shown to eliminate DOCA-salt hypertension in rats (16), which is a low-renin model of hypertension. Alternatively, the AP may act as a mediator of sympathoexcitatory effects of circulating ANG II in these models of hypertension, but not during sodium deprivation. In support of this idea is a study showing that ANG II binding sites in the rat AP decrease during sodium deprivation (52).

Maximal LSNA, expressed as percentage of control, was lower in APX rats compared with sham rats (Fig. 3, Table 1). This lower maximal LSNA may be due to an elevated absolute basal LSNA or a decreased absolute maximal LSNA or both, since LSNA was normalized to basal LSNA. However, it seems unlikely that absolute basal sympathetic outflow would be increased after the AP lesion, because basal HR was not increased in APX rats in both the present investigation and studies by others (2, 4, 15, 16, 27, 33, 44, 54). Therefore a decreased absolute maximal LSNA with a smaller or no decrease in absolute basal LSNA in APX rats is probable. Moreover, because losartan abolished the difference in maximal LSNA between APX and sham rats (Fig. 3, Table 1), it appears that the smaller maximal LSNA in APX rats is due to the loss of a chronic effect of ANG II to maintain LSNA at low arterial pressure levels. In agreement with this conclusion is the study of Skoog et al. (43), in which hemorrhage reduced MAP to lower levels in APX rats than in sham rats. In addition, c-fos expression (a marker of activated neurons in the brain) in the rat AP, induced by hypotension (−50 mmHg) (3, 18, 45), is blunted by intravenous administration of the ANG II antagonist, [Sar1, Ile8]ANG II (3). Therefore the present data suggest that circulating ANG II may act at the AP to maintain maximal sympathetic outflow during hypotension in rats.

To our knowledge, the role of the AP in baroreflex control of sympathetic nerve activity has not been studied before in conscious rats. In the present study, the maximal gain of baroreflex control of LSNA was not altered by the AP lesion (Figs. 3 and 4, Table 1). Our data suggest that the AP may not be necessary for maintaining the sensitivity of baroreflex control of sympathetic outflow in sodium-deprived rats. However, it may be required for maintaining the sensitivity of baroreflex control of HR in sodium-deprived rats (Fig. 6) and normal sodium intake (34). Considering the evidence that the rat AP has connections with both NTS and other parts of the brain, such as the paraventricular nucleus (28, 38, 42, 48), it is possible that in rats, the AP may participate in other functions influenced by baroreceptor input or circulating ANG II, such as water and sodium intake (23) and vasopressin release (24).

In the present study, we found that blockade of endogenous ANG II with losartan decreased the maximal gain of baroreflex control of HR in APX but not sham rats (Table 2). Our result is consistent with previous findings indicating that ANG II infusion may increase the slope of cardiac baroreflex curves in conscious APX but not AP-intact animals (33, 34). In addition, losartan similarly shifted the cardiac baroreflex curve to the left and similarly suppressed maximal HR without affecting minimal HR in APX and sham rats, suggesting that the AP does not play a major role, if any, in the maintenance of HR in sodium-deprived rats at most arterial pressure levels.

A diminished pressor response to intravenous ANG II bolus was observed in APX rats compared with sham rats before losartan administration in the present study, in agreement with previous studies in dogs (13) and rabbits (34). It is not clear whether the total number of vascular ANG II receptors is downregulated after AP ablation. If this is true, it could, at least in part, explain the lower ANG II pressor response in APX rats. However, there is also a report showing that short-term (5–10 min) intravenous infusion of ANG II increases MAP identically in APX and sham rats (15). The difference may be due to different methods for ANG II administration, e.g., bolus vs. infusion.

Baroreflex control of LSNA and HR is apparently mediated by different neuromodulatory pathways in rats, since the AP lesion differentially affected LSNA and HR in the present study. The AP lesion decreased maximal LSNA but not maximal HR and increased maximal gain of the HR-MAP curve but not that of the LSNA-MAP curve. In addition, losartan affected baroreflex-mediated LSNA
and HR differently in APX rats. For example, losartan did not significantly affect maximal LSNA or maximal gain of LSNA-MAP curve but decreased maximal HR and maximal gain of the HR-MAP curve in APX rats. These differences may be explained in part by the fact that HR is influenced by both sympathetic and parasympathetic innervation of the heart or that sympathetic drive to the lumbar nerve and the heart is differentially regulated or both.

If circulating ANG II does not act at the AP to increase lumbar sympathetic activity and HR in sodium-deprived rats, then where else could it act? Forebrain CVOs could be involved, because these areas, including the organum vasculosum of the lamina terminalis and the subfornical organ, like the AP, have numerous ANG II receptors and connections with other brain regions that participate in the regulation of sympathetic outflow (for review, see Ref. 39). Sympathetic outflow in sodium-deprived rats could also be maintained by local brain angiotensin acting at sites behind the blood-brain barrier, since losartan can pass across the blood-brain barrier to block AT1 receptors (31, 55).

In conclusion, the present results suggest that the AP is not necessary for endogenous ANG II to chronically maintain LSNA at most levels of MAP, nor is it necessary for ANG II to chronically maintain HR in conscious, sodium-deprived rats. However, the AP is required for endogenous ANG II to enhance maximal reflex LSNA during decreases in MAP.

Perspectives

It is not clear from the data in this study, whether absolute sympathetic activity is altered by chronic AP lesion. The direct nerve activity recording technique is only suitable for assessing changes in, but not absolute, nerve activity, because the recorded nerve activity depends on the contact conditions between the nerve branch and the electrode wire. However, indirect evidence supports the view that sympathetic outflow is not necessary for ANG II to chronically maintain HR in conscious, sodium-deprived rats. Nevertheless, despite these well-known drawbacks of the lesion technique, the present study indicates that the AP is not required for chronic increases in ANG II to support LSNA and HR in conscious, sodium-deprived rats.

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