Role of central catecholaminergic pathways in the actions of endogenous ANG II on sympathetic reflexes

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Gaudet, Elisabeth A., Shirley J. Godwin, and Geoffrey A. Head. Role of central catecholaminergic pathways in the actions of endogenous ANG II on sympathetic reflexes. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1174–R1184, 1998.—In the present study, we examined the effect of blockade of the brain stem renin-angiotensin system on renal sympathetic baroreflexes and chemoreflexes in conscious rabbits and examined the role of central catecholaminergic pathways in these responses. Eleven rabbits underwent preliminary surgical instrumentation and pretreatment with central 6-hydroxydopamine (6-OHDA, 500 µg/kg) or ascorbic acid 6 wk before the commencement of the experiments. Baroreflex curves were determined under conditions of normoxia and hypoxia (10% O2 + 3% CO2) before and after central administration of either Ringer solution, the ANG II receptor antagonist losartan (10 µg), or the angiotensin-converting enzyme inhibitor enalaprilat (500 ng) on separate days. Losartan increased the upper plateau and the range of the mean arterial pressure (MAP)-renal sympathetic nerve activity (RSNA) curve (79 and 78%, respectively) in intact rabbits, whereas this effect was not observed in 6-OHDA-pretreated rabbits. Hypoxia elicited an increase in resting RSNA (111% in intact rabbits and 74% in 6-OHDA-injected rabbits) and elevated the upper plateau of the RSNA-MAP curve in both groups (89% in intact rabbits and 114% in 6-OHDA-injected rabbits). During hypoxia, losartan and enalaprilat increased the RSNA upper plateau in intact rabbits but had no effect in 6-OHDA-pretreated rabbits. No effects on the MAP-heart rate baroreflex curves were observed. Thus the effect of losartan to increase RSNA, particularly during hypoxia and baroreceptor unloading, being abolished by central noradrenergic depletion suggests that the endogenous ANG II which normally causes an inhibition of renal sympathetic motoneurons is dependent on the integrity of central catecholaminergic pathways.

baroreceptor reflex; hypoxia; chemoreceptor reflex; losartan; angiotensin type 1 receptors; enalaprilat; blood pressure; fourth ventricular administration

THE RENIN-ANGIOTENSIN SYSTEM in the central nervous system (CNS) has long been known to influence the sympathetic nervous control of blood pressure as well as its arterial baroreflex regulation (52). Much of the interest has focused on those CNS sites accessed by circulating ANG II, such as the forebrain and hindbrain circumventricular organs, and the influence of ANG II on the autonomic nervous system (1, 12, 27, 46, 47). However, there are also a number of regions possessing high concentrations of ANG II receptors that lie behind the blood-brain barrier, and these are sites where ANG II modulates the sympathetic nervous system. In the medulla, these include the nucleus of the solitary tract (NTS) (14, 15), the rostral ventrolateral medulla (RVLM), which is known to be one of the main pressor and sympathoexcitatory areas (20), and also the caudal ventrolateral medulla (CVLM), which is a major depressor center mediating baroreceptor reflex responses (16). ANG II applied locally is generally excitatory (17) and produces sympathetically mediated pressor responses when applied to the RVLM of a number of species (2, 4, 49, 53, 54, 56) and sympathoinhibition when applied to the CVLM (50, 56).

Despite the disparate effects of activating ANG II in the various medullary brain regions, relatively consistent pressor effects are observed when ANG II is given into the cerebrospinal fluid of the brain stem, suggesting that mainly the sympathoexcitatory ANG II receptors are activated via this route (32, 35). Studies from our laboratory have shown that injections of low doses of ANG II into the fourth ventricle (4V) of conscious rabbits produced dose-dependent increases in sympathetic activity and arterial pressure (33, 35). We also observed that removal of baroreceptor afferent input by sinoaortic denervation increased the sensitivity to ANG II, suggesting that the ANG II receptors stimulated by 4V administration may be physiologically important, particularly when baroreflex mechanisms are suppressed (24). Furthermore, we found that this facilitation by baroreceptor denervation was absent in animals previously treated with 6-hydroxydopamine (6-OHDA) to deplete spinal noradrenergic pathways (25). Administration of an ANG II antagonist into the RVLM region markedly reduced the pressor responses to 4V ANG II, suggesting that this area is the major site of the pressor effect or that perhaps it lies downstream to the major site (36). Furthermore, local microinfusion of ANG II into the RVLM facilitates the renal sympathetic baroreflex (53) in a similar fashion to that produced by 4V administration, as shown by Dorward and Rudd (22). Thus, because inhibition of the renal sympathetic baroreflex can be observed after ANG II microinjection into the CVLM (54), either the effects of the pressor RVLM ANG II receptors predominate or ANG given into the 4V does not penetrate the parenchyma to the CVLM.

Perhaps the key question concerning these two opposing endogenous ANG II systems in the brain stem is their respective physiological roles, which are not at all well defined. It has been suggested that endogenous ANG in the RVLM and the CVLM is tonically active (56). Recent studies from our laboratory have shown that the angiotensin AT1-receptor antagonist losartan produced an increase in renal sympathetic nerve activity (RSNA) and renal sympathetic reflexes (8). We pro-
posed that in conscious normotensive rabbits, the sympathoinhibitory activity of ANG II outweighed the sympathoexcitatory activity. In contrast, using an angiotensin-converting enzyme inhibitor, Dorward and Rudd (22) found little effect on sympathetic reflexes. The reason for these differences is not clear, but the result of blocking central ANG II may depend on the balance of the sympathoinhibitory and sympathoexcitatory effects. Alternatively, the effect of losartan may not relate to its effects on blocking AT1 receptors, as suggested by Averill and colleagues (5). We therefore reexamined the effects of blocking the renin-angiotensin system on sympathetic baroreflex using the ANG II receptor antagonist losartan and compared its effects to the angiotensin-converting enzyme inhibitor enalaprilat. Given that the effects of losartan on sympathetic baroreflex were particularly marked during hypoxia plus CO2 (8), we examined baroreflex function during both normoxic and hypoxic conditions.

The ventrolateral medulla, which contains a high density of AT1-receptor binding sites (48), also corresponds to a region containing norepinephrine-synthesizing cells (10). Hirooka et al. (37) recently showed that 4V infusion of ANG II in conscious rabbits increased the number of Fos-positive neurons in the RVLM, CVLM, and A5 region. Furthermore, ~60% of the Fos-positive neurons were also immunoreactive for tyrosine hydroxylase, a marker of catecholamine cells. Because the actions of applied ANG II involved central noradrenergic pathways, the second aim of the study was to investigate whether any of the functional effects of endogenous ANG II were dependent on central catecholaminergic neurons by using the noradrenergic neurotoxin 6-OHDA.

METHODS

Animal preparation. Experiments were conducted in 11 rabbits of either sex bred and housed at the Baker Medical Research Institute whose weight ranged from 2.6 to 2.9 kg. The colony is derived from an original multicolored English strain with the "Dutch Belted" strain, introduced in 1994. All procedures were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) and were approved by the Animal Experimentation Committee of the Alfred Hospital-Baker Medical Research Institute. Rabbits were housed under controlled temperature, humidity, and dark-light cycle and were fed a controlled diet of pellets (0.5% sodium chloride) and vegetables but with water ad libitum.

At least 6 wk before experiments, rabbits were implanted with a vinyl 4V catheter (SV10, ID 0.28 mm and OD 0.61 mm; Dural Plastics and Engineering, Auburn, New South Wales, Australia) through the atlantooccipital membrane as described previously (34). The rabbits were intubated after induction of anesthesia with an intravenous administration of propofol (10 mg/kg Diprivan; ICI, Melbourne, Australia) and then placed on halothane (Fluothane; ICI) open-circuit anesthesia with use of a vaporizer (Goldman). The animal was placed prone in a sling, and its head was flexed to 45° using a modified stereotaxic head holder (David Kopf Instruments, Tujunga, CA) (6). The neck muscles were parted in the midline, and the atlantooccipital membrane was exposed. With a 25-gauge, 16-mm needle, a hole was made in the midline at a 45° angle through the rostral part of the membrane. The catheter was then inserted into the hole, with the 8-mm tip placed rostrally inside the 4V and the end buried under the skin in the neck for later retrieval. After at least 1 wk recovery, an inflatable Silastic balloon was placed around the intrathoracic vena cava (41). In brief, the rabbits were anesthetized as for the surgery described above and placed on a respirator, an incision was made through the sixth right intercostal space, and, after deflation of the lungs, a Silastic perivascular cuff was positioned around the inferior vena cava just above the diaphragm. The end of the balloon tube was brought out through the rib incision and positioned under the skin at the back of the neck. The incision was closed, and a further 3-wk recovery period was allowed.

One month before the experiments, rabbits were pretreated with either 6-OHDA (500 µg/kg, n = 5; Sigma, St. Louis, MO) freshly dissolved in 100 µl 0.9% NaCl containing 1 mg/ml ascorbic acid (Sigma) or given only ascorbic acid vehicle (n = 6) into the 4V. The dose of 6-OHDA was chosen in accordance with results from Korner et al. (40) to minimize nonspecific side effects. The animals remained in good condition after the injection, as indicated by their daily body weights.

Several days before the first experiment, a bipolar renal nerve electrode for recording RSNA was implanted according to the method of Dorward and colleagues (21) while rabbits were under halothane anesthesia after induction with propofol (10 mg/kg Diprivan, ICI). The left kidney was exposed by retroperitoneal approach, and the renal nerve was identified using a dissecting microscope and was placed inside a caged pair of electrodes. The nerve and recording electrode assembly was insulated from the surrounding tissue by a silicone gel (Elastosil RT 604; Wacker-Chemie, Munich, Germany).

Experimental preparation. On the day of the experiment, the rabbit was placed in a standard single-rabbit holding box (15 cm high and wide and 35 cm long) with wire top and raised wire grid floor. The rabbit contained in the holding box was placed in a larger sealed Perspex box (30-cm width, 50-cm length, 25-cm height, and 40-liter volume) which contained an inlet and outlet valve for continuous flow of various gas mixtures (9). The end of the 4V catheter, the perivascular balloon catheter, and the renal nerve electrode were retrieved from under the skin while rabbits were under local anesthesia with 1% prilocaine (Citanest, Astra Pharmaceuticals). The central ear artery was cannulated transcervically with a 22-gauge, 25-mm Teflon catheter (Jelo, Critikon) under local anesthesia. The catheter was then connected to a Statham P23Dc pressure transducer (Gould, Glen Burnie, MD) for continuous measurements of mean arterial pressure (MAP) and heart rate (HR), and the animal was allowed a 1-h recovery period before commencement of the experiment. The raw RSNA signal was amplified 10,000–100,000 times, filtered between 50 and 5,000 Hz, and integrated using a resetting integrator with a time constant of 2 s. The baseline or zero position of the RSNA recording system was set to the average value of the recording during "silent periods" between bursts of RSNA. Arterial pressure and RSNA were digitized at 200 Hz using a data acquisition card (PC plus; National Instruments, Austin, TX) and a computer program written in Labview graphical programming language (National Instruments). The program calculated MAP instantaneously from the detected systolic and diastolic pressures as well as heart period (interval between beats) and a corresponding instantaneous HR. All parameters were saved onto disk as 2-s averages for later offline analysis. Cardiovascular parameters were also recorded on a Grass polygraph (model 7D; Grass Instruments, Quincy, MA) during the
experiment, in which case the arterial pressure was dampened to derive the MAP and also used to trigger a heart rate meter (model 173B, Baker Institute). The respiration rate was estimated throughout the experiment by visual count of the breathing movements.

Experimental protocol. The effects of losartan, enalaprilat, or Ringer solution on baroreceptor and chemoreceptor stimulation were examined in three experiments conducted 2 days apart. Hemodynamic parameters (MAP, HR, and RSNA) were recorded for 15 min while the rabbit was breathing room air. An arterial blood sample (0.5 ml) was taken and analyzed to determine the partial pressures of O₂ and CO₂ in arterial blood (Pao₂ and Paco₂, respectively). The box containing the rabbit was sealed and perfused with a mixture of 10% O₂ and 3% CO₂ to maintain arterial CO₂ at normal levels. The same recordings were performed, and a similar blood sample was withdrawn, under hypoxia plus CO₂. The hypoxia was stopped, and the rabbit was allowed to breathe room air. After the hemodynamic parameters had returned to baseline levels, the same protocol (normoxia then hypoxia + CO₂) was repeated after the administration of either 4V osartan (10 µg; DuPont, Wilmington, DE), enalaprilat (500 ng MK-422; Merck Sharpe & Dohme, Rahway, NJ), or Ringer solution (Baxter, Old Toongabbie, New South Wales, Australia) randomly assigned to one of the experimental days. The dose of losartan used in this study was shown to completely inhibit the pressor response over a 3-h period (8), and the dose of enalaprilat was able to block conversion of endogenous ANG II to ANG II for at least 60 min (22). To check for efficacy of blockade, we examined the pressor response to an acute injection of 4V ANG II (25 pmol human 11e form; Peninsula Laboratories, Belmont, CA) and ANG I (0.5 mg) at the end of the experiment.

All drugs were dissolved in Ringer solution immediately before use and injected in a volume of 25 µl using 50-µl glass syringes. Each injection was followed by 25 µl using 50-µl freedom, the MAP and also used to trigger a heart rate meter (model 173B, Baker Institute). The respiration rate was estimated throughout the experiment by visual count of the breathing movements.

Effect of Ringer solution, losartan, and enalaprilat on MAP-RSNA and MAP-HR baroreflex curves. In 6-OHDA-pretreated rabbits, the resting MAP and HR were unchanged by 4V injection of Ringer solution, losartan, or enalaprilat (Table 1). Although Ringer solution and enalaprilat did not affect resting RSNA, there was an 83% increase after losartan that was of borderline significance (P = 0.09). Losartan increased the upper plateau of the RSNA-MAP curve by 79% and the range by 78% (P < 0.05, Table 1 and Fig. 1) without changing the LP. Thus a decrease in MAP resulted in a much greater increase in RSNA after losartan compared with control. Losartan did not significantly alter the slope (gain) of the curve because a decrease in rate of curvature toward the UP offset the marked increase in the UP. By contrast, there were minimal effects of Ringer solution and enalaprilat on RSNA baroreflex curves (Table 1 and Fig. 1). In 6-OHDA-pretreated rabbits, as observed in the intact group of rabbits, the resting MAP, HR, and RSNA were unaltered by the 4V injection of either Ringer solution, losartan, or enalapril-
However, the changes induced by losartan on RSNA baroreflex curves observed in the intact group did not appear in these animals (Table 2 and Fig. 2). The MAP-HR baroreflex curves were not affected by any central treatment in either group of rabbits (Figs. 1 and 2).

Effect of hypoxia on blood gases, respiration, resting hemodynamics, and MAP-RSNA curves. The effects of hypoxia (+ CO₂) on PaO₂, PaCO₂, respiration rate, and resting HR are shown in Fig. 3. Over the 3 experimental days, a 20-min period of hypoxia induced a fall in PaO₂ from 109 ± 4 to 41 ± 1 mmHg (P < 0.05) in intact rabbits and from 116 ± 3 to 47 ± 3 mmHg (P < 0.05) in 6-OHDA-pretreated rabbits. Hypoxia did not alter PaCO₂ in either group (Fig. 3), presumably due to the addition of CO₂ in the mixture, which counteracted the increase in respiration rate (from 72 ± 2 to 96 ± 2 breaths/min in intact rabbits and from 78 ± 7 to 114 ±

![Image of graphs](http://ajpregu.physiology.org/)

**Fig. 1.** Average relationship between mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) (A, B, and C) or heart rate (HR) (D, E, and F) in 6 intact rabbits during normoxia before treatment (○, dotted lines) and after Ringer solution (■, solid line in A and D), losartan (●, solid line in B and E), or enalaprilat (●, solid line in C and F) administered into fourth ventricle. ○ and ■ Resting values. Error bars are average SE calculated from ANOVA, indicating variation between animals. bpm, Beats/min. *P < 0.05 for comparison between control and treatment from ANOVA.
9 breaths/min in 6-OHDA-injected rabbits; \( P < 0.05 \) for both). Hypoxia did not modify MAP (−1.0 ± 2 and −3.8 ± 2 mmHg in intact and 6-OHDA animals, respectively) but significantly decreased the HR from 261 ± 2 to 233 ± 4 beats/min \( (P < 0.05) \) in intact rabbits and from 263 ± 4 to 217 ± 10 beats/min \( (P < 0.05) \) in the 6-OHDA group.

The most pronounced effect of hypoxia was on resting RSNA, which increased by 111% \( (25 ± 4 to 52 ± 4 \text{ NU}, \quad P < 0.05) \) in the intact group and by 74% \( (34 ± 6 to 59 ± 8 \text{ NU}, \quad P < 0.05) \) in the 6-OHDA group. The RSNA baroreflex curve was markedly altered by hypoxia, with the upper RSNA plateau (reached during hypotension) increasing by 89 and 114% in both the intact and 6-OHDA groups, respectively, with little change to the lower RSNA plateau (reached during hypertension) (Fig. 4). This meant that in both groups hypoxia approximately doubled the RSNA range as well as the

### Table 2. Basal values and baroreflex parameters describing MAP-RSNA curves before and after 4V administration of Ringer solution, losartan, and enalaprilat in 6-OHDA-treated rabbits during normoxic conditions

<table>
<thead>
<tr>
<th></th>
<th>Before Ringer Solution</th>
<th>After Ringer Solution</th>
<th>Before Losartan</th>
<th>After Losartan</th>
<th>Before Enalaprilat</th>
<th>After Enalaprilat</th>
<th>Within-Animal SE</th>
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<td><strong>Basal parameters</strong></td>
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<tr>
<td>Resting MAP, mmHg</td>
<td>80 ± 2</td>
<td>81 ± 3</td>
<td>85 ± 3</td>
<td>82 ± 2</td>
<td>80 ± 2</td>
<td>78 ± 5</td>
<td>2.5</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>258 ± 11</td>
<td>265 ± 7</td>
<td>259 ± 11</td>
<td>285 ± 8</td>
<td>272 ± 9</td>
<td>294 ± 14</td>
<td>11.2</td>
</tr>
<tr>
<td>Resting RSNA, NU</td>
<td>33 ± 7</td>
<td>29 ± 3</td>
<td>45 ± 19</td>
<td>45 ± 25</td>
<td>24 ± 9</td>
<td>23 ± 11</td>
<td>15.7</td>
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<tr>
<td><strong>Baroreflex parameters</strong></td>
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</tr>
<tr>
<td>Lower plateau, NU</td>
<td>20 ± 4</td>
<td>12 ± 5</td>
<td>24 ± 12</td>
<td>3 ± 8</td>
<td>4 ± 4</td>
<td>7 ± 8</td>
<td>8.4</td>
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<tr>
<td>Upper plateau, NU</td>
<td>100</td>
<td>110 ± 10</td>
<td>100</td>
<td>96 ± 10</td>
<td>100</td>
<td>91 ± 17</td>
<td>27.5</td>
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<td>Range, NU</td>
<td>80 ± 4</td>
<td>97 ± 5</td>
<td>76 ± 12</td>
<td>93 ± 5</td>
<td>96 ± 4</td>
<td>84 ± 12</td>
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<tr>
<td>BP50, mmHg</td>
<td>71 ± 3</td>
<td>67 ± 2</td>
<td>85 ± 5</td>
<td>75 ± 2*</td>
<td>69 ± 4</td>
<td>70 ± 4</td>
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<td>Lower curvature (P3), 1/100 mmHg</td>
<td>−39 ± 8</td>
<td>−17 ± 7</td>
<td>−52 ± 30</td>
<td>−41 ± 16</td>
<td>−27 ± 4</td>
<td>−21 ± 5</td>
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<td>Upper curvature (P5), 1/100 mmHg</td>
<td>−29 ± 13</td>
<td>−19 ± 5</td>
<td>−28 ± 19</td>
<td>−23 ± 5</td>
<td>−15 ± 4</td>
<td>−13 ± 1</td>
<td>9</td>
</tr>
<tr>
<td>Average gain, NU/mmHg</td>
<td>2.9 ± 0.4</td>
<td>3.7 ± 1.2</td>
<td>6.4 ± 3.6</td>
<td>6.6 ± 2.4</td>
<td>4.3 ± 0.7</td>
<td>2.9 ± 0.4</td>
<td>2.3</td>
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</table>

Values are means ± SE. 6-OHDA, 6-hydroxydopamine. *\( P < 0.05 \) for differences between pre- and posttreatment values from ANOVA.
baroreflex gain (slope). The similarity in both intact and 6-OHDA group RSNA baroreflexes under normoxia and hypoxia were not simply due to the RSNA normalization procedure. The resting levels of RSNA in absolute microvolts were similar in vehicle and 6-OHDA-treated rabbits (4 ± 1 and 5 ± 2 µV, respectively), as was the UP of the RSNA baroreflex (12 ± 2 and 21 ± 9 µV, respectively).

Effect of hypoxia on MAP-HR baroreflex curves. The MAP-HR baroreflex curves in vehicle- and 6-OHDA-treated animals were very similar (Fig. 4). The HR range was 214 ± 6 and 225 ± 8 beats/min in both groups, respectively, whereas the average gain was also similar, being 4.2 ± 0.5 and 4.0 ± 0.5 beats·min⁻¹·mmHg⁻¹, respectively. The parameters of the HR baroreflex curve were not significantly altered during hypoxia on both experimental days in intact rabbits (Fig. 4C). However, in 6-OHDA-pretreated rabbits, hypoxia produced a leftward shift in the HR baroreflex curve characterized by a decrease in the BP50 (from 97 ± 8 to 79 ± 10 mmHg, P < 0.05, average for the 3 experimental days; Fig. 4D).

Effect of Ringer solution, losartan, and enalaprilat on the MAP-RSNA baroreflex curves under hypoxia. Neither Ringer solution, losartan, nor enalaprilat injection into the 4V altered the resting MAP or HR during hypoxia + CO₂ (Table 3). Losartan increased the UP of the RSNA baroreflex curve by 40% (P < 0.05) and the range by 39% (P < 0.05) (Table 3 and Fig. 5) but had no other effects on the baroreflex curve parameters. Enalaprilat produced an upward shift in the RSNA baroreflex curve, with the UP elevated by 74% (P < 0.05), the LP elevated by 206%, and the range increased by 61% (P < 0.05) (Table 3 and Fig. 5). The only other baroreflex parameter affected by enalaprilat was the BP50, which was reduced with enalaprilat (Table 3). After Ringer solution administration, there was a tendency for an increase in the UP and range of the RSNA baroreflex curve, but these effects did not reach statistical significance.

The resting MAP, HR, and RSNA during hypoxia + CO₂ were also unaltered by the 4V injection of either Ringer solution, losartan, or enalaprilat in 6-OHDA-pretreated rabbits. However, the changes induced by losartan and enalaprilat on RSNA baroreflex curves observed in the intact group were not observed in these animals (Table 4 and Fig. 6).

Effect of 6-OHDA pretreatment on brain norepinephrine levels. In rabbits given 6-OHDA, norepinephrine concentrations after 1 mo were reduced to 46% in the medulla oblongata (vehicle, 416 ± 59 ng/g; 6-OHDA, 176 ± 7 ng/g; P < 0.05) and to 22% in the spinal cord (vehicle, 68 ± 6 ng/g; 6-OHDA, 15 ± 5 ng/g; P < 0.05) of the values measured in vehicle-injected rabbits.
DISCUSSION

In the present study, we confirmed a previous finding in conscious rabbits (8) that losartan has a pronounced excitatory influence on central pathways influencing RSNA, resulting in an increase in the UP and range of the RSNA baroreflex curve. More importantly, we observed a qualitatively similar effect with the angiotensin-converting enzyme inhibitor enalaprilat, but only during hypoxic conditions when there was a larger degree of sympathetic excitation. The other major finding was that the effects of losartan and enalaprilat were abolished in 6-OHDA-pretreated rabbits, suggesting that the sympathoinhibitory actions of endogenous ANG II are dependent on central catecholaminergic pathways.

The qualitatively similar effects of losartan and enalaprilat suggest that the sympathoexcitation observed is most likely due to inhibition of AT$_1$ receptors in the medulla and hence inhibition of the brain stem renin-angiotensin system, rather than to a nonspecific effect, as suggested by Averill and colleagues (5). They suggested that losartan, when injected into the RVLM, produced a pressor response due to potassium ions, because it is formulated as a potassium salt. Although potassium might have actions when it is injected locally into the RVLM, it is very unlikely to produce long-lasting sympathoexcitatory effects when injected into cerebrospinal fluid at such low concentrations. We had previously observed that the effect of the antagonist losartan was paradoxically similar to the effects of 4V administration of ANG II (8). When ANG II is injected into the 4V of conscious rabbits, a marked sympathoexcitation is observed (24, 33, 35) that is mediated by activation of AT$_1$ receptors (8). It has been previously suggested by Sasaki and Dampney (56) that there are both sympathoinhibitory and sympathoexcitatory AT$_1$ receptors in the medulla in different nuclei. Our present data in nonanesthetized rabbits with losartan and

Table 3. Basal values and baroreflex parameters describing MAP-RSNA curves before and after 4V administration of Ringer solution, losartan, and enalaprilat in intact rabbits during hypoxic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ringer Solution</th>
<th>After Ringer Solution</th>
<th>Before Losartan</th>
<th>After Losartan</th>
<th>Before Enalaprilat</th>
<th>After Enalaprilat</th>
<th>Within-Animal SE</th>
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<tr>
<td>Resting MAP, mmHg</td>
<td>84 ± 2</td>
<td>80 ± 5</td>
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<td>79 ± 4</td>
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<td>Resting HR, beats/min</td>
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<td>Resting RSNA, NU</td>
<td>60 ± 4</td>
<td>62 ± 12</td>
<td>51 ± 13</td>
<td>60 ± 12</td>
<td>46 ± 3</td>
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<tr>
<td>Lower plateau, NU</td>
<td>21 ± 10</td>
<td>15 ± 10</td>
<td>13 ± 5</td>
<td>18 ± 14</td>
<td>16 ± 5</td>
<td>49 ± 14*</td>
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<td>Upper plateau, NU</td>
<td>151 ± 20</td>
<td>185 ± 33</td>
<td>216 ± 22</td>
<td>303 ± 47*</td>
<td>199 ± 26</td>
<td>345 ± 49*</td>
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<tr>
<td>Range, NU</td>
<td>130 ± 27</td>
<td>169 ± 31</td>
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<td>284 ± 45*</td>
<td>184 ± 29</td>
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<td>BP50, mmHg</td>
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<td>69 ± 3</td>
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<td>Lower curvature (P3), 1/100 mmHg</td>
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<td>Upper curvature (P5), 1/100 mmHg</td>
<td>−14 ± 4</td>
<td>−16 ± 4</td>
<td>−20 ± 4</td>
<td>−14 ± 3</td>
<td>−14 ± 2</td>
<td>−9 ± 1</td>
<td>3</td>
</tr>
<tr>
<td>Average gain, NU/mmHg</td>
<td>4.9 ± 1.3</td>
<td>4.9 ± 0.9</td>
<td>6.5 ± 0.7</td>
<td>6.8 ± 1.0</td>
<td>5.1 ± 0.8</td>
<td>2.9 ± 1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 for differences between pre- and posttreatment values from ANOVA.

Fig. 5. Average relationship between MAP and RSNA in 6 intact rabbits during hypoxia before treatment (○, dotted lines) and after Ringer solution (■, solid line in A), losartan (●, solid line in B), or enalaprilat (●, solid line in C) administered into fourth ventricle. ○ and ■, Resting values. *P < 0.05 for comparison between control and treatment from ANOVA.
enalaprilat suggest that the sympathoinhibitory activity of endogenous ANG II reduces the effect of chemoreceptor activation and baroreceptor unloading and that this is greater than the contribution from any sympathoexcitatory AT₁ receptors. From mapping studies, the latter are likely to be within the RVLM (56). Very recently, Fontes and colleagues (28) reported a pressor effect with losartan and L-158809 (a non-potassium salt AT₁-receptor antagonist) as well as with an AT₂-receptor antagonist injected into the RVLM of conscious rats. They also observed that a nonselective peptide antagonist and a selective ANG 1–7 antagonist decreased blood pressure. No explanation of these results was forthcoming for the pressor effects of the AT₁-receptor and AT₂-receptor antagonists. They did not consider that the lipophilic nature of losartan compared with the peptide antagonists may enable it to access the AT₁-receptor sympathoinhibitory sites.

In the present study, losartan and enalaprilat did not change blood pressure, indicating there was not generalized sympathetic activation but rather an increase in sympathetic vasomotor outflow to specific target organs such as the kidney. The UP of the RSNA baroreflex curve gives an estimate of the excitatory capacity of the motoneuron pool when tonic baroreceptor inhibition is reduced to low levels. The increase in this parameter, produced by losartan and enalaprilat, indicates that both of these compounds resulted in an excitatory influence on renal sympathetic motoneurons. Losartan increased resting renal nerve activity similar to what we had observed previously (8), although in the present study this was of borderline significance. However, the effect of losartan to increase the UP of the RSNA baroreflex curve both during normoxic and hypoxic conditions was clear. Enalaprilat only increased this parameter during hypoxia. Thus it appears that a much larger sympathetic activation was required before the effect of enalaprilat could be seen. This explains why Dorward and Rudd (22) did not observe any effect of 4V enalaprilat on renal sympathetic baroreflexes.

Table 4. Resting values and baroreflex parameters describing MAP-RSNA curves before and after 4V administration of Ringer solution, losartan, and enalaprilat in 6-OHDA-treated rabbits during hypoxic conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Ringer Solution</th>
<th>After Ringer Solution</th>
<th>Before Losartan</th>
<th>After Losartan</th>
<th>Before Enalaprilat</th>
<th>After Enalaprilat</th>
<th>Within-Animal SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
<td>78 ± 3</td>
<td>75 ± 3</td>
<td>81 ± 2</td>
<td>80 ± 2</td>
<td>75 ± 3</td>
<td>74 ± 4</td>
<td>2.5</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>217 ± 21</td>
<td>225 ± 22</td>
<td>200 ± 21</td>
<td>235 ± 17*</td>
<td>234 ± 19</td>
<td>242 ± 15</td>
<td>11.2</td>
</tr>
<tr>
<td>Resting RSNA, NU</td>
<td>44 ± 9</td>
<td>63 ± 11</td>
<td>60 ± 26</td>
<td>85 ± 40</td>
<td>73 ± 28</td>
<td>88 ± 37</td>
<td>15.7</td>
</tr>
<tr>
<td>Baroreflex parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower plateau, NU</td>
<td>20 ± 14</td>
<td>20 ± 6</td>
<td>24 ± 11</td>
<td>3 ± 12</td>
<td>5 ± 5</td>
<td>15 ± 12</td>
<td>8.4</td>
</tr>
<tr>
<td>Upper plateau, NU</td>
<td>196 ± 61</td>
<td>220 ± 53</td>
<td>206 ± 38</td>
<td>232 ± 20</td>
<td>240 ± 34</td>
<td>225 ± 35</td>
<td>27.5</td>
</tr>
<tr>
<td>Range, NU</td>
<td>172 ± 52</td>
<td>200 ± 51</td>
<td>182 ± 29</td>
<td>229 ± 24</td>
<td>235 ± 38</td>
<td>211 ± 41</td>
<td>24.2</td>
</tr>
<tr>
<td>BP50, mmHg</td>
<td>72 ± 4</td>
<td>67 ± 1</td>
<td>76 ± 3</td>
<td>75 ± 2</td>
<td>64 ± 1</td>
<td>69 ± 3</td>
<td>3.3</td>
</tr>
<tr>
<td>Lower curvature (P3), 1/100 mmHg</td>
<td>-22 ± 5</td>
<td>-29 ± 2</td>
<td>-16 ± 6</td>
<td>-18 ± 3</td>
<td>-12 ± 7</td>
<td>-16 ± 6</td>
<td>11</td>
</tr>
<tr>
<td>Upper curvature (P5), 1/100 mmHg</td>
<td>-26 ± 7</td>
<td>-14 ± 1</td>
<td>-29 ± 16</td>
<td>-23 ± 1</td>
<td>-15 ± 1</td>
<td>-26 ± 7</td>
<td>9</td>
</tr>
<tr>
<td>Average gain, NU/mmHg</td>
<td>10.3 ± 4.0</td>
<td>9.8 ± 3.0</td>
<td>10.0 ± 4.8</td>
<td>10.7 ± 3.2</td>
<td>6.4 ± 1.3</td>
<td>8.6 ± 1.4</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 for differences between pre- and posttreatment values from ANOVA.

Fig. 6. Average relationship between MAP and RSNA in 6-OHDA-pretreated rabbits during hypoxia before treatment (○, dotted lines) and after Ringer solution (■, solid line in A), losartan ( ■, solid line in B), or enalaprilat ( ■, solid line in C) administered into fourth ventricle. ○ and ■, Resting values.
flexes and concluded that there was little tonic endogenous ANG II activity, because they only examined normoxic conditions. They also found no effect of a peptide ANG II receptor antagonist. The reason for the difference between the effect of losartan and enalaprilat is not clear but may result from the difference in mechanism of the two drugs or possibly their physicochemical properties. Angiotensin-converting enzyme, also known as kininase II, is able to cleave substrates other than ANG I, including bradykinin, neurotensin, and substance P, all of which are known to influence the cardiovascular system (26). An alteration in the concentrations of these peptides could explain the lack of excitatory action of enalaprilat, and an extra sympathoinhibition induced by the use of hypoxia is needed to see the effect of enalaprilat on the UP of the RSNA baroreflex curve. Alternatively, losartan may simply provide more effective blockade of the system, compared with inhibiting kininase II, by being a lipophilic ANG II receptor antagonist.

The second major finding from our study was that the losartan- and enalaprilat-induced increase in the UP was not observed in 6-OHDA-treated rabbits, which suggests that central noradrenergic pathways are required for the endogenous ANG II sympathoinhibitory response. The lack of effect of the ANG II antagonists is not likely to be due to a perturbation by the process through which the RSNA reflex has been normalized to arbitrary units, because the absolute levels of microvolts were similar in each group. Furthermore, the baroreceptor-HR reflex was very similar in both intact and catecholamine-depleted rabbits, suggesting that both cardiac and vasomotor baroreflexes in both groups were not altered 4 wk after the 6-OHDA treatment. Thus the effect of 6-OHDA in blocking the losartan- and enalaprilat-induced facilitation of the RSNA baroreflex appears to be a rather specific one. These data suggest that the association of central catecholamine pathways with central ANG II responses that we have observed previously (24, 37) also appears to be the case for endogenous ANG II pathways. For a decade it has been known that there is a close association of central catecholamine-containing pathways and the location of ANG II receptors, as first suggested by Mendelsohn and colleagues (48). The importance of the forebrain noradrenergic pathways in the actions of ANG II has also been suggested by a number of studies involving pressor and drinking responses to lateral ventricul administration of ANG II in rats (7, 19). Depletion of norepinephrine with 6-OHDA did not, however, alter ANG II receptor binding (59). More recently, Qadri et al. (51) showed that ANG II injected intraventricularly selectively increased norepinephrine release from the anterior hypothalamus without changing the release of other catecholamines or metabolites. Thus there does appear to be a widespread association of ANG II and norepinephrine throughout the CNS.

From our current study, we did not determine the site of action of 4V-injected inhibitors of ANG II and hence can only speculate on the location of the relevant “endogenous sympathoinhibitory ANG II.” We do know, however, that there is a 6-OHDA-sensitive, presumably noradrenergic pathway downstream from the ANG II receptors. Perhaps the most likely candidate for the site of action of ANG II is the CVLM, which contains norepinephrine-synthesizing cells of the A1 group (10), ANG II-immunoreactive fibers (43), and angiotensin-converting enzyme (55). It also contains two groups of neurons, those required for an effective baroreceptor reflex and those that serve to limit sympathetic nerve activity and blood pressure independent of the baroreceptor reflex (18). Sasaki and Dampney (56) showed in baroreceptor-denervated rabbits that injection of the ANG II receptor antagonist [Sar^1, Thr^3]ANG II into the CVLM produced sympathoexcitation, whereas a sympathoinhibition was observed when it was administered into the RVLM. These data suggest that endogenous ANG II is present in both the CVLM and RVLM and under physiological conditions is tonically active in both sites. Furthermore, it has been shown that ANG II antagonist injected into the CVLM increased RSNA baroreflex sensitivity and range in rats and rabbits, indicating that ANG II facilitates baroreflex control of RSNA in the CVLM (54, 57). Intraventricular injection of ANG II produces an increase in activity of the A1 noradrenergic cells, as measured by norepinephrine turnover (58) or c-Fos protein product (37), which suggests that the sympathoinhibitory effect of ANG II could be mediated by an increase in the activity of A1 cells. Alternatively, endogenous ANG II may act on the non-noradrenergic cells in the CVLM with the 6-OHDA-sensitive link further downstream.

A possibility for the noradrenergic component in the RSNA reflex may be the A5 noradrenergic cells in the pons, which receive both chemo- and baroreceptor information and have been postulated to be involved in both the baroreflex (38) and the sympathetic response to hypoxia (30, 42). Most A5 cells are inhibited by aortic nerve stimulation or an increase in blood pressure (29, 39) but are excited by arterial hypoxia, an effect eliminated by section of carotid sinus nerves (31). Direct projections from A5 have been found in the NTS, the RVLM, and the intermediolateral column of the spinal cord (13, 44). Recently we have shown that inhibition of the A5 region with muscimol facilitates the RSNA baroreflex in a qualitatively similar fashion to that produced by 4V-administered losartan (45).

Taken altogether, there is good evidence to suggest that perhaps both the CVLM and the A5 regions are involved. However, there is actually no reason why the endogenous ANG II might not also act directly in the A5 region itself, because moderate levels of ANG II binding have been observed in the human A5 region (3) and cells in the A5 region are activated after ANG II infusion into the 4V (37). Furthermore, the spinal projection of the A5 cells (11) may be responsible for the alteration in the sensitivity to 4V-applied ANG II that we observed previously after spinally injected 6-OHDA (24).

The NTS is the site of termination of the primary baroreceptor afferents and also the chemoreceptor afferents and thus is crucial in the central integration of
baroreceptor and chemoreceptor information. The region also has a very high concentration of ANG II receptors (48), contains catecholamine fibers (10), and is a site where injection of ANG II is active in modulating cardiac baroreflexes (14, 15). However, it seems unlikely to be the site relevant to the RSNA effects of 4V losartan because this treatment had no effect on the cardiac baroreflex (8). Thus, although the NTS remains a possibility, it is a less likely choice than the CVLM for the sympathoinhibitory actions of endogenous angiotensin.

In conclusion, the present study found that the effect of losartan to increase RSNA, particularly during hypoxia and baroreceptor unloading, was also observed with enalaprilat, suggesting that endogenous angiotensin normally inhibits renal sympathetic baroreceptor and chemoreceptor reflexes via AT1 receptors. The effect of inhibiting the brain stem angiotensin system reflects the balance of the sympathoexcitatory and sympathoinhibitory actions of AT1 receptors and suggests that the sympathoinhibitory pathways predominate during chemoreceptor activation and during baroreceptor unloading. Our observation that this effect was abolished by central noradrenergic depletion suggests that this effect is dependent on the integrity of central catecholaminergic pathways and may involve a noradrenergic component.

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