Angiotensin II As a Modulator of Baroreceptor Reflexes in the Brainstem of Conscious Rats

Lisete C. Michelini and Leni G.H. Bonagamba

The effect of microinjection into the nucleus tractus solitarii (NTS) of angiotensin II (Ang II) on baroreceptor control of heart rate (HR) in conscious, freely moving rats was evaluated with a new method of long-term cannulation of the dorsal brainstem areas. Reflex changes in HR were produced by intravenous bolus injections of either phenylephrine or sodium nitroprusside (0.2–25.6 μg/kg) both after saline and after unilateral microinjection of Ang II into the NTS (24 ng, 0.2 μl) and compared with those produced after administration of Ang II into the fourth ventricle (24 ng, 0.2 μl) or intravenously (1–2 ng/kg/min). Baseline levels of mean arterial pressure (MAP) and HR were not affected by the route of Ang II application but reflex bradycardia during MAP increase was significantly attenuated after injections of Ang II into the NTS. Both the slope and the intercept of the regression line function between ΔHR and ΔMAP were reduced by 43% from the control value of −1.55±0.13 beats/min/mm Hg (p<0.01) and −14±5 beats/min (p<0.05), respectively. Similar reductions were observed after Ang II administration into the fourth ventricle or intravenously, although microinjections into the cerebellum produced no effect. Endogenous blockade of Ang II by saralasin (22 ng) in the NTS facilitated the bradycardic response (−2.29±0.91 beats/min/mm Hg). Nitroprusside-induced tachycardia was not altered by Saralasin microinjection into the NTS or by Ang II application to the NTS, fourth ventricle, or intravenously. The data suggest a coherent physiological action for peripheral Ang II, central Ang II administration, or both in the attenuation of baroreceptor reflex control of HR to increases but not decreases in MAP and indicate the NTS as a site of action for the effects of both endogenously or exogenously administered Ang II of either central or peripheral origin. (Hypertension 1990;15(suppl I):I-45–I-50)

Several lines of evidence suggest that angiotensin II (Ang II) participates in cardiovascular regulation not only through a direct effect on vascular smooth muscle but also by action on the central nervous system (for review see References 1 and 2). A number of Ang II–sensitive brain sites have been identified both within and outside the blood–brain barrier. Ang II receptors have also been identified at many sites in the forebrain and lower brainstem where dense concentrations of specific high-affinity binding sites have been described in the nucleus tractus solitarii (NTS) and dorsal motor nucleus of the vagus nerve (DMNX), with a lower concentration in the area postrema. Immunohistochemical and biochemical techniques have revealed that the NTS–DMNX complex is rich in Ang II–like immunoreactive cell bodies and nerve terminals and that Ang II is present in measurable concentrations in the cerebrospinal fluid. Such observations, together with the potential role of Ang II in central cardiovascular control and the prominent position of the NTS within the central network of autonomic control, suggest the NTS as a possible site at which Ang II might modulate the regulation of arterial pressure. Several studies of anesthetized animals have shown that Ang II injection into the NTS causes dose-dependent effects on mean arterial pressure (MAP) and heart rate (HR) and impairs baroreceptor reflex control of HR, whereas its endogenous blockade improves sensitivity.

In the present study, we evaluate the physiological significance of Ang II in the NTS and its blockade on the baroreceptor reflex control of HR in unrestrained rats capable of expressing different...
behavioral patterns by using a technique for the long-term cannulation and microinjection of drugs into the brainstem recently developed by us.\(^\text{13}\) We also tested the effect of Ang II as a neurohormone modulating the baroreceptor reflex control of HR in a group of rats chronically implanted with cannulae in the fourth ventricle (4V). Because impairment of reflex bradycardia by intravenous Ang II has been confirmed in several species,\(^\text{14-16}\) we compared the effect of central versus peripheral administration of Ang II on the baroreceptor reflex control of the heart in the same rats.

**Methods**

For the long-term cannulation of dorsal brainstem areas, male Wistar rats (220–260 g) were anesthetized with Nembutal (Abbott Laboratories, Chicago, Illinois) (40 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf, Tujunga, California) with the head in a horizontal position, as previously described.\(^\text{13}\) Briefly, after exposure of the skull, a small window was opened caudally to the lambda allowing the introduction of a unilateral stainless steel guide cannula (17 mm length, 0.6 mm o.d.) at an angle of 24°. The stereotaxic coordinates were as follows: 1 mm caudal to interaural line, 0.4–0.6 mm lateral (right or left) to the midline, and 8.9 mm (NTS group), 8.7 mm (4V group), or 8.4 mm (cerebellum group) below the skull surface such that the cannula tip lay in the ventral cerebellum (lobule 9 or 10). The cannula was closed by an occluder and fixed with fast polymerizing methacylate. The rats were given 60,000 units penicillin (Pentobiótico Veterinário, Fontoura Wyeth, São Paulo, Brazil) and were allowed to recover for 6–9 days. One day before the experiment, catheters (polyethylene tubing, Clay Adams, Parsippany, New Jersey) were inserted under ether anesthesia into a femoral artery and vein and tunneled subcutaneously to the back of the neck. In the rats submitted to intravenous infusion studies, one jugular vein was also cannulated.

Both arterial pressure and HR were recorded continuously (Statham P23DB transducer, Hato Rey, Puerto Rico) in freely moving rats. Microinjections of small volumes (0.2 µl) of vehicle and peptide were made in conscious unrestrained rats by introduction of a 33-gauge needle (18-mm length) connected by PE-10 tubing to a microliter syringe (701-N, Hamilton, Reno, Nevada) into the guide cannula. Microinjections lasted 15–20 seconds, and the needle was then removed and replaced by the occluder. To stimulate baroreceptors, repeated bolus injections (0.1 ml) of graded doses of phenylephrine (0.2–12.8 µg/kg) and sodium nitroprusside (0.4–25.6 µg/kg) were made into a femoral vein, and control and peak MAP and HR changes for each response were measured. Instantaneous HR was determined by the number of pulses of arterial pressure in 1 second. Phenylephrine and nitroprusside injections were given in a random order, and subsequent injections were not made until the recorded parameters had returned to preinjection levels. The intravenous injection of vehicle (saline) alone did not change the recorded parameters.

An initial period (30–45 minutes) of continuous recording was allowed for stabilization of the arterial pressure and HR of the conscious rat. Saline was then microinjected into the NTS (or 4V or cerebellum), and the baroreceptor reflex control of HR was evaluated from minute 5 to minute 20 (saline test). Twenty to 30 minutes later, 24 ng Ang II (ACM Paiva, EPM, São Paulo, Brazil) was injected at the same site, and the baroreceptor reflex response to phenylephrine and nitroprusside was again tested. The effectiveness of intracerebral (NTS, 4V, and cerebellum) Ang II administration was confirmed by 1) the same magnitude of MAP and HR responses after phenylephrine and nitroprusside injection in the experiments in which the order of injections was reversed to control the decay effect of intracerebral Ang II, 2) administration of phenylephrine and nitroprusside injections over a shorter time interval after intracerebral administration (only 2–3 doses of each were used), while the Ang II effect was still present, and 3) the marked MAP (+35±5 mm Hg) and HR (−61±7 beats/min) responses obtained when the same dose of Ang II was injected intravenously. In preliminary experiments (n=3), two successive (50–60 minutes apart) microinjections of 0.2 µl of saline into the NTS did not change the baroreceptor control of HR (HR=3.92–1.46x vs. −6.49–1.50x during phenylephrine–induced increases in MAP, and HR=6.18–3.27x vs. 3.54–3.21x during nitroprusside–induced decreases in MAP) for the first versus the second saline microinjection, respectively. Twenty-four hours later, some of these rats were submitted to the baroreceptor reflex test during NTS blockade with 22 ng Saralasin (Sar) (ACM Paiva, EPM, São Paulo, Brazil), whereas others were tested during intracerebral infusion of nonpressor doses of Ang II (1–2 ng/kg/min, 0.02 µl/min). The phenylephrine and nitroprusside tests were compared during intracerebral treatments with saline or Sar and during intravenous infusion of either saline or Ang II. The order of agonist and antagonist or intravenous and intracerebral administration was reversed in some animals. At the end of every experiment, 0.2 µl Evans blue dye was microinjected into the brainstem. The rat was then perfused transcardiatically with saline followed by 10% buffered formalin. The brain was removed and frozen and the exact location of the injection site and its extension assessed by histological examination of 50 µm serial coronal cryostat sections.

All data are reported as the mean±SEM. For each rat, the relation between changes in HR and MAP was estimated by using linear regression analysis. The regression coefficient (slope) was taken as an index of baroreceptor reflex sensitivity. Significant differences between groups and treatments
TABLE 1. Baseline Values of Mean Arterial Pressure and Heart Rate and Regression Parameters in Response to Phenylephrine-Induced Increases and Nitroprusside-Induced Decreases in Mean Arterial Pressure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>PE-induced increases in MAP</th>
<th>NP-induced decreases in MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Slope (Δbeats/min/mm Hg)</td>
<td>Intercept (Δbeats/min)</td>
</tr>
<tr>
<td>NTS (n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>114±2</td>
<td>363±5</td>
<td>-1.55±0.13</td>
<td>-1.12±3.20</td>
</tr>
<tr>
<td>Ang II</td>
<td>117±3</td>
<td>362±6</td>
<td>-0.89±0.21*</td>
<td>-15.07±5.15†</td>
</tr>
<tr>
<td>4V (n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>115±3</td>
<td>372±10</td>
<td>-1.68±0.17</td>
<td>-4.38±3.16</td>
</tr>
<tr>
<td>Ang II</td>
<td>116±4</td>
<td>383±10</td>
<td>-0.83±0.21†</td>
<td>-19.44±5.13†</td>
</tr>
<tr>
<td>CER (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>125±4</td>
<td>390±13</td>
<td>-1.80±0.15</td>
<td>-1.94±3.81</td>
</tr>
<tr>
<td>Ang II</td>
<td>130±6</td>
<td>380±14</td>
<td>-2.05±0.72</td>
<td>7.39±13.75</td>
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<tr>
<td>IV (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>111±2</td>
<td>382±11</td>
<td>-2.08±0.41</td>
<td>4.97±4.26</td>
</tr>
<tr>
<td>Ang II</td>
<td>114±3</td>
<td>382±13</td>
<td>-1.15±0.54†</td>
<td>-11.56±13.42</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; HR, heart rate; PE, phenylephrine; NP, nitroprusside; NTS, nucleus tractus solitarii; Ang II, angiotensin II; 4V, fourth ventricle; CER, cerebellum; IV, intravenous.

* p<0.01, † p<0.05; as compared with appropriate saline treatment. There were no statistically significant differences in baseline values for saline between groups (analysis of variance).

Results

Basal MAP and HR values in freely moving cannulated rats were similar for all groups studied before and during saline treatment (Table 1). Intracerebral administration of 24 ng Ang II into the NTS, 4V, or cerebellum did not change baseline levels of MAP and HR, although significant transient MAP increases were observed 1.1±0.1 and 1.6±0.4 minutes after Ang II microinjection into the NTS and 4V, respectively (NTS=+3±0.4 mm Hg, and 4V=+6±2 mm Hg). No change in MAP or HR was observed during the intravenous administration of Ang II (Table 1).

Injection of graded doses of phenylephrine caused similar dose-related increases in MAP (on average, +9±4 up to +37±12 mm Hg for 0.2–12.8 μg/kg) both after saline and after Ang II treatment by NTS, 4V, cerebellum, or intravenously. The HR response to stimulation of the baroreceptor reflex is shown in Figure 1. Rats treated with Ang II in the NTS or 4V exhibited significantly smaller reflex changes in HR in response to phenylephrine-induced increases in MAP than after the respective saline treatment. The sensitivity of the baroreceptor reflex, taken as the mean slope of the individual regression lines for each animal was significantly and similarly less after Ang II microinjection into the NTS (reduction of 43%) or 4V (reduction of 50%) (Table 1). The intercepts of both regression lines were significantly reduced by -14±5 and -15±5 beats/min after exogenous administration of Ang II into the NTS or 4V, respectively. Curiously, when a nonpressor dose of Ang II was infused intravenously in the same animals, a similar alteration in baroreceptor reflex control of HR was observed (Table 1), that is, the slope of the ΔHR×ΔMAP regression was significantly reduced.
by 45% with an equivalent (not significant) reduction in the intercept of $-17\pm11$ beats/min. In contrast, baroreceptor reflex function of the heart to increases in MAP was the same before and after Ang II treatment in the cerebellum group.

The sensitivity of baroreceptor reflex control of HR to nitroprusside-induced decreases in MAP was not affected by Ang II regardless of whether the peptide was administered centrally (NTS or 4V) or infused intravenously. The dose-related depressor responses ($-5\pm4$ to $-28\pm10$ mm Hg for 0.4–25.6 µg/kg) produced by intravenous nitroprusside injections and the tachycardia were similar after saline and Ang II treatment, that is, the slopes and intercepts did not change significantly in the NTS, 4V, and intravenous groups (Table 1 and Figure 1).

However, Ang II administration into the ventral cerebellum reduced the sensitivity of the tachycardic response to decreases in MAP by 44%.

In four rats of the NTS group, endogenous blockade of Ang II by Sar did not alter the baseline levels of MAP and HR (120±7 mm Hg; 398±15 beats/min) but did cause changes opposite those observed after Ang II administration (Figure 2), that is, the bradycardia was greatly enhanced, with a 50% increase in the slope of the AHR versus AMAP regression line from a control value of $-1.53\pm0.25$ beats/min/mm Hg. Again, no change in reflex tachycardia was observed.

Histological verification of the injection sites (Figure 3) revealed that the Evans blue dye–stained area included the commissural and medial NTS, the center of the injections were located from 0.7 mm caudal to 0.5 mm rostral to the obex. The dye showed a preferential spread (average, 0.6±0.03 mm) in the rostrocaudal direction, apparently after the structure of the NTS. In six of the 13 rats in the NTS group part of DMNX was also stained.

Discussion

With use of the technique recently developed by us for long-term cannulation of dorsal brainstem areas, we proved the physiological role of Ang II in the NTS in attenuating the baroreceptor reflex control of HR in response to increases in MAP in...
conscious, freely moving rats. The data presented here are consistent with the localization of Ang II–specific binding sites in the NTS,7,8 with the demonstration of Ang II–like immunoreactive fibers in this nucleus,3,9 and with the previous demonstration in anesthetized animals that direct microinjection of Ang II into the NTS produced dose-related effects on MAP4–6 and the impairment of reflex decreases in HR during transient hypertension.11 A functional role for endogenous Ang II in the central regulation of the HR component of the baroreceptor reflex, as suggested previously,12 has been confirmed in conscious unrestrained rats by endogenous NTS blockade of Ang II. More importantly, the similarity of the reflex HR responses after NTS-, 4V-, or intravenously administered Ang II, indicates a coherent action of circulating and of brain-synthetized Ang II on the modulation of reflex bradycardia in the NTS.

That Ang II exerts an inhibitory influence on baroreceptor reflex control of HR has been described in dogs,16 rabbits,14 sheep,15 and rats,6,11,12,17 after either intravenous,14–16 intracerebroventricular,17 or NTS administration.6,11,12 Because there were no sustained changes in MAP and HR levels after Ang II treatment in any group of conscious rats, the differences in reflex sensitivity observed cannot be attributed to changes in baseline MAP. Lack of alteration in both basal MAP11,12 and basal HR12 also has been described in anesthetized rats after NTS administration of Ang II; other researchers have reported variable changes in MAP,4–6 which are related to the doses applied (from hypotension with small doses to hypertension with large doses) and are accompanied by reductions, increases, or no HR changes. Contrasting with the unchanged baseline MAP and HR observed in the 4V group, increases in MAP after intracerebroventricular Ang II administration were also reported.1,3,17 Although reasons for these discrepancies are not apparent, they might concern the different doses of Ang II used, the lack of anesthesia (a state known to cause high plasma renin levels and to elevate the threshold for baroreceptor activation12) in our study, or a combination of both factors.

The ability of centrally applied Ang II to attenuate reflex bradycardia is not surprising in light of its property to directly increase sympathetic tone through central mechanisms.1–3 The impairment of the HR component of the baroreceptor reflex appears to be accompanied by less inhibition of sympathetic vascular tone11,14 and withdrawal of parasympathetic tone to the heart,12,15,16 It is likely that a decrease in vagus tone was the main mechanism to impair the bradycardia of conscious rats after Ang II administration into the NTS, 4V, or even intravenously, because HR changes were produced in these experiments by transient drug-induced variations in MAP, which were more sensitive to alterations in parasympathetic outflow12; maximal HR changes are observed 1–2 seconds after maximal MAP changes when only the parasympathetic component is fully manifested.18 Because in half of the rats in the NTS group, part of the DMNX was also stained, a possible direct action of Ang II on this nucleus, impairing parasympathetic outflow, must be considered.

The similarity of effect caused by central or intravenous Ang II administration, already suggested by several studies,11,12,14–17 is confirmed by the present results. The data for the 4V and NTS groups indicate that Ang II present in the ventricular system exerts its modulatory action on baroreceptor reflex through receptors located in the NTS (or DMNX) and that the cerebrospinal fluid is one possible pathway of Ang II access to the NTS-DMNX complex, strengthening the action of Ang II as a central neurohormone as suggested previously.3 The similar modulation of HR control by centrally and intravenously administered Ang II indicates that circulating Ang II can diffuse in the brain and act on central receptors after gaining access to the cerebral parenchyma through the circumventricular organs. Ferrario et al1 provided evidence that the biological expression of Ang II action results from binding to putative brain receptors accessible by the bloodstream, by the cerebrospinal fluid, or even produced locally by brain elements containing the necessary proteins for formation of the peptide. An additional possibility that cannot be ruled out is that bidirectional translocation of high affinity Ang II–binding sites along the cervical vagus nerve might contribute to the unified action of peripheral-central Ang II because an association between Ang II receptors in the NTS and afferent sensory vagal fibers has been demonstrated.19 The identical action of central and peripheral Ang II is also stressed by our results obtained with nitroprusside-induced decreases in MAP, which showed no changes before or after Sar or Ang II administration into the NTS, 4V, and intravenously in the same conscious rats. Other researchers12,14 have also reported lack of alteration in reflex tachycardia after intravenous Ang II treatment14 or after its central blockade12 in anesthetized animals.

The dose of Ang II administered centrally (24 ng) is far greater than the physiological levels detected in the brainstem (1 pg/g of tissue). However, this dose will not be encountered during the reflex tests due to the breakdown of Ang II (metabolization time in the brainstem is about 8–10 minutes; M. Kohsla, personal communication). Elevated doses were used for technical reasons, that is, 1) infusion into the NTS of small doses over a long time period was impractical because the rats were conscious and freely moving, and 2) all injections were unilateral, the contralateral side remaining intact and able to develop compensatory changes. Thus, the high initial doses would ensure that the effect of Ang II persisted during the transient pressure challenges. The dose used was similar to or less than those used by other workers4–6,11 and close to that seen in
cerebrospinal fluid during the development of renal hypertension.10

To date, Ang II has not been administered in or even blocked in the NTS of conscious unrestrained animals. The results reported here indicate that long-term cannulation of the NTS is a viable, nontraumatic technique for study of the modulation of baroreceptor reflex control of the heart (and even peripheral resistance) in freely moving rats able to express different behavioral patterns. Because the local application of Ang II produces effects similar to those after intracerebroventricular or intravenous administration, its endogenous blockade produces opposite effects, and because cerebellum administration was without effect, the above data clearly show that the NTS is a site of action for the effects of both exogenous and endogenous Ang II (of either central or peripheral origin) on the baroreceptor reflex control of HR. The present observations also suggest that this physiological action of Ang II on the NTS-DMNX complex might integrate a larger central pathway, likewise triggered by Ang II in the area postrema, which in several species has been demonstrated to mediate the central pressor response to circulating Ang II.1 Because the first synapse of the pressor pathway that originates in the area postrema, which in several species has been demonstrated to mediate the central pressor response to circulating Ang II, is located in the medial NTS at exactly the same region where Ang II administration in this study caused reduction of reflex bradycardia, it is possible that the release, by centrally acting Ang II of peripheral pressor mechanisms to such a degree that baroreceptor reflex inhibition of HR is not fully capable of compensation, might represent a specific cardiovascular adaptation to a specific behavioral pattern. These considerations also provide support for a coherent action of central-peripheral Ang II on the modulation of cardiovascular parameters during behavioral adjustment.

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Key Words • angiotensin II • baroreceptor reflexes • blood pressure • heart rate • nucleus tractus solitarii
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