Actions of Angiotensin Peptides After Partial Denervation of the Solitary Tract Nucleus

Maria J. Campagnole-Santos, Debra I. Diz, and Carlos M. Ferrario

We determined the excitatory effects of direct nucleus tractus solitarii injection of angiotensin peptides after the sinoaortic nerves were cut unilaterally in rats under halothane anesthesia. Twenty-four hours later, recordings of mean arterial pressure and heart rate were obtained during injections of 2.5 ng angiotensin II or angiotensin-(1-7) in chloralose-urethane-anesthetized rats. Both peptides caused reductions in pressure and heart rate after nucleus tractus solitarii injections. In unilateral sinoaortic denervated rats, the hypotension and bradycardia produced with angiotensin II injections in either the ipsilateral (denervated) or contralateral (nondenervated) nucleus tractus solitarii were comparable. Angiotensin-(1-7), however, produced a larger decrease in pressure on the denervated side when compared with the nondenervated side. There were no differences in baseline pressure or heart rate between control rats and those with unilateral sinoaortic denervations. The effects of both angiotensin II and angiotensin-(1-7) were blocked by previous administration of the angiotensin II antagonist [Sar¹, Thr⁸]angiotensin II into the nucleus tractus solitarii. Assessment of angiotensin II binding sites in the solitary-vagal complex 24 hours after denervation showed a 13% reduction in angiotensin receptors. These findings confirm that both angiotensin II and angiotensin-(1-7) express biological activity through receptor-mediated actions in the dorsal medulla oblongata. That the effects produced by angiotensin II do not require the integrity of baroreceptor input further suggests that the receptors responsible for the acute cardiovascular actions of this peptide reside on postsynaptic elements in the vagal-solitary complex. (Hypertension 1990;15[suppl I]:I-34–I-39)

It is now recognized that angiotensin II (Ang II) acts to oppose the activity of the baroreceptors. Recent studies by us¹-⁴ revealed that a main component of this action resides in the vagal-solitarii complex of the medulla oblongata. For example, specific high affinity Ang II receptors present within this brain region are dependent on vagal afferent fibers that synapse in the nucleus tractus solitarii (NTS).³ Furthermore, baroreceptor reflex control of heart rate (HR) is augmented by NTS injection of the Ang II antagonist [Sar¹, Thr⁸]Ang II.⁵ Direct injections of Ang II into the NTS, however, trigger a hemodynamic response similar to that produced by acute stimulation of the baroreceptor nerves.²,⁵-⁷ Further, we have recently noted that acute NTS injections of the amino terminal heptapeptide angiotensin-(1-7) [Ang-(1-7)], a fragment that is endogenous to the medulla oblongata,⁸ cause similar reductions in both blood pressure and HR and has direct neuronal actions.⁵,⁶ Therefore, we ascertained whether the integrity of baroreceptor input is required for the acute NTS actions of angiotensin peptides by sectioning the carotid sinus and aortic depressor nerves (SAD) unilaterally and comparing the acute responses to the two peptides in both the denervated and nondenervated NTS.

Methods

Surgical Procedures and Protocols

Thirty male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Indiana) weighing 220-280 g were used in this study. All rats were housed in a temperature- and humidity-controlled environment on a 12-hour light/dark cycle (6 AM to 6 PM). Twelve rats underwent unilateral (right or left) sectioning of the SAD under halothane anesthesia, as described elsewhere.¹⁰ Twenty-four hours later, under chloralose-urethane anesthesia (35 mg/kg
and 750 mg/kg i.p., respectively), catheters were inserted into a femoral artery and vein. Arterial pressure was monitored by a solid-state strain gauge transducer (model MP-15D, Micron Instrument, Los Angeles, California), and HR was determined with a cardiotachometer (model 2000, Gould, Cleveland, Ohio). All variables were recorded continuously on a direct-writing Gould polygraph (Series 2400).

Rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, California) with the head flexed downward at 45°. The dorsal surface of the medulla oblongata was exposed by incising the atlanto-occipital membrane. Microinjections of 2.5 ng Ang II and Ang-(1-7) (100 nl vol) into the NTS (0.5 mm rostral and 0.5 mm lateral to the obex, and 0.3 mm below the dorsal surface) were made with a multibarreled glass micropipette (30–50 μm o.d.), as described previously. Ang II and Ang-(1-7), synthesized in our laboratories by M. C. Khosla, were dissolved in artificial cerebrospinal fluid (aCSF). Rats received injections of both Ang II and Ang-(1-7) into both the ipsilateral and contralateral (with respect to the denervation procedure) NTS. The order of the injections of the two peptides, as well as the side of the medulla first tested, were assigned randomly. A minimum of 30 minutes was allowed between injections. Rats with arterial baroreceptors intact (n=10) received unilateral injections of both Ang II and Ang-(1-7) in random order and served as a control group. In subgroups of the control rats, NTS injections of Ang II (n=5) or Ang-(1-7) (n=5) were repeated after NTS administration of 100 ng of the Ang II antagonist [Sar¹,Thr⁸]Ang II.

Histological Identification of Injection Sites

At the completion of each experiment, the location of the pipette tip was marked by injection of 50 nl of 2% Alcian Blue dye through one of the barrels of the pipette. The deposition of the dye within the structures of the medulla was examined in 20–50 μm serial stained with Neutral red.

In Vitro Receptor Autoradiography

Ang II receptor binding was analyzed in three other rats. Two rats underwent unilateral sinoaortic denervation, and 24 hours later, the brain was perfused and the brainstem removed. One rat with intact baroreceptors served as a control. Forty-four μm sections were incubated with 0.3 nM [125I]Ang II for in vitro receptor autoradiography as described previously. Binding was quantified by computerized densitometry using brain paste standards exposed with the tissue sections. Measurements of binding density were made in the caudal (caudal to the caudal tip of the area postrema) and intermediate (at the level of the area postrema) solitary vagal complex. Between 10 and 21 determinations were made on each side of the brain in each rat. Data are expressed as the ratio of the side ipsilateral to the denervation procedure versus the contralateral nondenervated side (SAD rats), or right versus left for the control rat.

Statistical Analyses

For each peptide, comparisons between the control group and each side in the denervated group were made by unpaired Student's t test. Comparisons between two observations (i.e., before vs. after; or ipsilateral vs. contralateral) in the same rat were assessed by Student's paired t test. Differences between the two peptides with respect to responses on the contralateral and ipsilateral side of the medulla were assessed by repeated-measures analyses of variance followed by Tukey's multiple comparisons tests. The criterion for statistical significance was set at a p value less than 0.05. Numerical values are given as mean±SEM.

Results

Baseline Data

The baseline mean arterial pressure (MAP) and HR of rats with unilateral SAD under chloralose-urethane anesthesia averaged 96±3 mm Hg and 346±17 beats/min, respectively. These values are not different from those observed in control rats (93±2 mm Hg and 363±6 beats/min, respectively).

Effects of Unilateral Sinoaortic Denervation on Nucleus Tractus Solitarii Responses to Angiotensin II or Angiotensin-(1-7)

Ang II (2.5 ng) microinjections into the NTS produced significant decreases in both MAP and HR in intact rats (Figure 1), consistent with our previous findings. Twenty-four hours after unilateral SAD, Ang II produced similar reductions in MAP and HR in both the ipsilateral and contralateral NTS. Additionally, responses were essentially unchanged from those of control rats.

The MAP and HR effects produced by Ang-(1-7) microinjected into the NTS of control rats were also similar to those reported previously. After unilateral SAD, Ang-(1-7) produced a 50% larger reduction in MAP in the ipsilateral NTS when compared with responses in the contralateral NTS (p<0.05; paired t test). HR effects, however, were similar between the two sides. Although there was a tendency for the depressor response on the denervated side to be greater than that in the control rats (see Table 1), MAP and HR responses to Ang-(1-7) on either side were not different from those observed in control rats.

Effects of [Sar¹,Thr⁸]Angiotensin II on the Responses to Angiotensin II or Angiotensin-(1-7)

To verify that the responses to the two peptides could be blocked by a receptor antagonist, the Ang II antagonist [Sar¹,Thr⁸]Ang II was injected into the NTS of intact rats 5 minutes before injections of either Ang II or Ang-(1-7). The antagonist effectively blocked the cardiovascular responses to injections of
**Discussion**

The present findings are the first demonstration that the acute cardiovascular actions of Ang II are not dependent on baroreceptor primary afferent input and suggest a postsynaptic mechanism for these effects. Because interruption of baroreceptor reflex input potentiated the acute depressor responses produced by Ang-(1-7), the data further suggest that the two peptides act on different postsynaptic elements.

The original observation that Ang II binding sites are reduced or abolished in the dorsal medulla 10-14 days after nodose ganglionectomy was made at time points when anatomic studies indicated degeneration of afferent fibers within the NTS. 

Subsequently, others reported that 7-10 days after bilateral SAD, Ang II binding sites were reduced 10-70%. 

There is equivocal information concerning whether fiber degeneration occurs in the NTS after these maneuvers because neuronal cell bodies in the nodose ganglion are still intact. Because sinoaortic afferents account for a small portion of the total vagal or glossopharyngeal sensory afferent input to the NTS, the data suggest that baroreceptor integrity is required for the maintenance of Ang II binding sites within this brain area. In the present study, we documented that binding was 13% less ipsilaterally, 24 hours after unilateral SAD. At this time, actual degeneration of fibers is unlikely and the reduction in binding may represent interruption of axonal traffic from the periphery, or metabolic changes occurring in the nucleus after transection of afferent nerve input. Because the cardiovascular actions of Ang II were unaltered by the loss of tonic baroreceptor impulses, and a substantial amount of binding was still present within components of the dorsal medulla after SAD, the data suggest that binding sites on postsynaptic elements within the NTS are responsible for these actions. Indeed, earlier studies by us had clearly shown that the acute depressor actions of Ang II in the medulla were mediated mostly by activation of cardiac vagal outflow. 

Alternately, although there

**Table 1.** Effects of the Antagonist [Sar¹, Thr⁴] Angiotensin II on Responses to Nucleus Tractus Solitarii Injections of Angiotensin Peptides

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Angiotensin-(1-7)</th>
<th>Angiotensin II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔMAP (mm Hg)</td>
<td>ΔHR (beats/min)</td>
</tr>
<tr>
<td>Before antagonist</td>
<td>-10±2</td>
<td>-6±1</td>
</tr>
<tr>
<td>After antagonist</td>
<td>-3±3*</td>
<td>-2±2*</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=5 rats for each peptide. Mean arterial pressure (MAP) and heart rate (HR) responses to antagonist alone averaged 4±2 mm Hg and 2±2 beats/min in 10 rats, respectively.

*Significant difference when before and after antagonist values are compared (p<0.05).
is a predominant ipsilateral projection of baroreceptor input, we cannot rule out the possibility that after unilateral denervation, contralateral projections from the remaining intact baroreceptors are sufficient to maintain the response.

That Ang II reduces the gain of the HR component of the baroreceptor reflex is well established. The exact sites for this action, however, have not been determined. Our recent work confirms that the dorsal medulla oblongata is one site for this action because bilateral NTS injections of the Ang II antagonist [Sar¹,Thr⁵]Ang II facilitate the baroreceptor reflex control of HR. Others have shown that Ang II infusions attenuate the reflex. Further, inhibition of endogenous Ang II in the NTS facilitated baroreceptor reflex sensitivity to increases but not decreases in MAP. The additional observation that Ang II binding sites are associated with vagal afferent fibers in the NTS provided the first evidence that one mechanism by which Ang II can modify the baroreceptor reflex is by presynaptic mechanisms involving vagal afferent fibers in the...
Ang II in some but not all regions of the canine brain ventricles. Additionally, the potentiation of cardiovascular effects in the dorsal medulla, Ang-(1-7). We favor this latter hypothesis because it further suggests that Ang-(1-7) acts through an Ang II receptor. Alternately, [Sar1, Thr2]Ang II may bind to a subclass of receptors that have a high affinity for Ang-(1-7). We favor this latter hypothesis because the actions of the two peptides are not always identical. For example, although both Ang II and Ang-(1-7) cause vasopressor release and produce pressor responses after injections into the brain ventricles. Additionally, the potentiation of the response to Ang-(1-7) after denervation may also reflect the presence of different receptors for the peptide on presynaptic vagal afferent fibers, the acute actions of Ang II may be caused by stimulation of postsynaptic elements within the dorsal medulla. In fact, we have previously shown that acute administration of Ang II elicits hypotension and bradycardia from dorsal medullary sites; these effects are mediated by activation of cardiac vagal efferent tone.

Equally important, we found that unilateral SAD potentiates the magnitude of the depressor response produced by Ang-(1-7) on the ipsilateral side without altering the HR component. This surprising observation suggests a selective effect of SAD on a population of angiotensin receptors that regulate tonic sympathetic outflow. Previous studies from this laboratory provide strong evidence for the generation and endogenous presence of Ang-(1-7) in brain. Because Ang-(1-7) was reported to be approximately 100-300-fold less potent than Ang II in receptor binding studies, the receptor characteristics responsible for the equipotent actions of Ang-(1-7) and Ang II were unknown. The present observations provide the first evidence that the hypotensive and bradycardic actions of Ang-(1-7) are effectively blocked by NTS injections of [Sar1, Thr2]Ang II, while confirming previous findings by others that Ang II antagonists block the effects of Ang II in this nucleus. This significant observation further suggests that Ang-(1-7) acts through an Ang II receptor. Alternately, [Sar1, Thr2]Ang II may bind to a subclass of receptors that have a high affinity for Ang-(1-7). We favor this latter hypothesis because the actions of the two peptides are not always identical. For example, although both Ang II and Ang-(1-7) cause vasopressor release and produce pressor responses after injections into the brain ventricles. Additionally, the potentiation of the response to Ang-(1-7) after denervation may also reflect the presence of different receptors for the peptide on presynaptic vagal afferent fibers, the acute actions of Ang II may be caused by stimulation of postsynaptic elements within the dorsal medulla.

Acknowledgments

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