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 [Sar', 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)

I

From the Department of Brain and Vascular Research, Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio.

Supported by grants HL-6835 and HL-38535 from the National Heart, Lung, and Blood Institute, National Institutes of Health, the American Heart Association, Northeastern Ohio Affiliate, the Reinberger Foundation, and the Storer Foundation. M.J.C.-S. is a recipient of a fellowship from the American Heart Association, Northeastern Ohio Affiliate. D.I.D. is a recipient of an Established Investigatorship of the American Heart Association.

Address for correspondence: Debra I. Diz, PhD, Department of Brain and Vascular Research, Cleveland Clinic Foundation/FF2, One Clinic Center, Cleveland, OH 44195–5070.

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 [Sar', 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)

duced 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,6 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 240).

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.1-2-5 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. Fourteen μm sections were incubated with 0.3 nM [125I]Ang II for in vitro receptor autoradiography as described previously.3,11 Binding was quantified by computerized densitometry using brain paste standards exposed with the tissue sections.11 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 (angiotensin-peptide and baroreceptor reflexes, 1-35) 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.5 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.5 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
either peptide (Table 1). In contrast, injections of a CSF vehicle 5 minutes before Ang II had no effect on the MAP or HR responses (-18±11 mm Hg and -20±14 beats/min before vs. -16±8 mm Hg and -13±4 beats/min after, respectively; n=2).

Effects of Sinoaortic Denervation on Angiotensin II Binding Sites in the Dorsal Medulla

To document the effect of SAD on Ang II binding sites in the solitary-vagal complex in the region where injections were made (Figure 2A), in vitro autoradiographic analysis of [125I]Ang II binding density was determined over a 1.5-mm rostro-caudal segment of the dorsal medulla. Figures 2B and 2C illustrate the binding densities in the dorsal medulla of a rat 24 hours after section of the sinoaortic nerves on the right side. There was only a modest difference in binding in the intermediate solitary-vagal region when ipsilateral and contralateral sides were compared (ipsilateral-contralateral ratio, 0.87±0.04; n=2; 0.1>p>0.05). In the more caudal part of the solitary-vagal complex (caudal to the area postrema), there was essentially no difference in binding when the two sides of the brain were compared (ipsilateral vs. contralateral ratio, 0.96±0.06). In a control rat, the ratio of the right versus the left side averaged 0.98 over the entire area.

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.12,13 Subsequently, others reported that 7–10 days after bilateral SAD, Ang II binding sites were reduced 10–70%.14,15 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.12,13 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.2 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>ΔMAP (mm Hg)</th>
<th>ΔHR (beats/min)</th>
<th>ΔMAP (mm Hg)</th>
<th>ΔHR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before antagonist</td>
<td>-10±2</td>
<td>-6±1</td>
<td>-21±3</td>
<td>-21±5</td>
</tr>
<tr>
<td>After antagonist</td>
<td>-3±3*</td>
<td>-2±2*</td>
<td>-4±2*</td>
<td>-1±1*</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 [Sar^1, Thr^2]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

**FIGURE 2.** Panel A: Diagram of dorsal medulla 0.5 mm rostral to obex indicating injection tip placements as determined by deposition of Alcian blue dye. Scale bar, 0.5 mm. Injections were all located within 200-μm rostrocaudal segment of medial nucleus tractus solitarii. ◦ represent location of one pipette tip at that site. ● indicate more than one pipette tip placement at that site. Maps and coordinates are from atlas of Paxinos and Watson (see Reference 24). AP, area postrema; cc, central canal; nTS, nucleus tractus solitarii; TS, tractus solitarius; dmNCX, dorsal motor nucleus of vagus. Gr, gracilis nucleus; nXII, hypoglossal nucleus. Panel B: Autoradiographic image of total L[125I]angiotensin II (Ang II) binding in 14-μm section of rat medulla, approximately 0.5 mm rostral to obex, 24 hours after right sinoaortic denervation. This level corresponds to level shown in Panel A for pipette tip placements. Note that only modest reduction (13%) in Ang II binding sites occurs at this level. AP, area postrema; nTS, nucleus tractus solitarii, dmNCX, dorsal motor nucleus of the vagus. Scale bar, 0.5 mm. Panel C: Lower magnification of autoradiographic image in Panel B showing entire dorsal medullary section. Scale bar, 0.5 mm.
Ang II in some but not all regions of the canine brain ventricles. Additionally, the potentiation of Ang II in this nucleus. This significant observation suggests a selective effect of SAD on a subclass of receptors that have a high affinity for Ang-(1-7) and Ang II were unknown. The present observations provide the first evidence that the hypothensive and bradycardic actions of Ang-(1-7) are effectively blocked by NTS injections of [Sar^1,Thr^3]Ang II, while confirming previous findings by others that Ang II antagonists block the effects of Ang II in receptor binding studies. The receptor characteristics responsible for the equipotent actions of Ang-(1-7) and Ang II were unknown. 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 hypothensive and bradycardic actions of Ang-(1-7) are effectively blocked by NTS injections of [Sar^1,Thr^3]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, [Sar^1,Thr^3]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 vasopressin release and produce cardiovascular effects in the dorsal medulla, Ang-(1-7) is devoid of dipsogenic actions and does not 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 two peptides, possibly situated on different parts of the neuronal circuits comprising the baroreceptor reflex arc. Indeed, we have recent evidence to suggest that Ang-(1-7) is capable of displacing [125I]Ang II binding with an affinity similar to that of Ang II in some but not all regions of the canine dorsal medulla. The physiological significance of this finding is yet to be determined. The evidence, however, supports the contention that different components of the NTS exhibit different angiotensin receptor binding characteristics. The present findings reveal that multiple angiotensin peptides express cardiovascular activity through receptor-mediated events in the dorsal medulla oblongata. That these effects do not require the complete integrity of baroreceptor input further suggests that the receptors responsible for the acute cardiovascular actions of angiotensin peptides reside on postsynaptic elements within the dorsal medulla.

Acknowledgments

We thank Geri A. Locker, BS, and Mark Schluchter, PhD, of the Department of Biostatistics, The Research Institute of the Cleveland Clinic Foundation, for their assistance in the analysis of the data. The expert technical assistance of Susan M. Bosch is gratefully acknowledged.

References

14. Healy DP, Rettig R, Nguyen T, Printz MP: Autoradiographic evidence that angiotensin II receptors are associated
with vagal afferents and efferents within the solitary-vagal area of the rat brainstem. *J Hypertens* 1986;4(suppl 6):S462–S464


23. Diz DI, Ferrario CM: Angiotensin (Ang) receptors in the rostral dorsomedial medulla recognize Ang-(1-7) with an affinity similar to that of Ang II. *Hypertension* 1989;14:343


**KEY WORDS** • angiotensin II • angiotensin-(1-7) • blood pressure • heart rate • baroreceptors • angiotensin antagonist • rat studies
Actions of angiotensin peptides after partial denervation of the solitary tract nucleus.
M J Campagnole-Santos, D I Diz and C M Ferrario

Hypertension. 1990;15:I34
doi: 10.1161/01.HYP.15.2_Suppl.I34

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/2_Suppl/I34

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/