Baroreceptor Reflex Modulation by Angiotensin II at the Nucleus Tractus Solitarii

MARIA J. CAMPAGNOLE-SANTOS, DEBRA I. DIZ, AND CARLOS M. FERRARIO

SUMMARY This study characterized the effect of nucleus tractus solitarii (NTS) microinjection of the angiotensin II (Ang II) antagonist [Sar',Thr 8]Ang II on the baroreceptor control of heart rate in anesthetized rats. Reflex changes in heart rate were elicited by bolus intravenous injections of either phenylephrine or sodium nitroprusside before and after bilateral microinjection of [Sar',Thr 8]Ang II (100 pmol) or vehicle into the NTS. The slope of the relationship between the change in pulse interval and the change in mean arterial pressure was used as an index of baroreceptor reflex sensitivity. Bradycardia elicited by phenylephrine-induced increases in pressure was significantly greater after NTS injection of [Sar',Thr 8]Ang II. The slope of the pulse interval–arterial pressure relationship was 0.60 ± 0.09 ms/mm Hg after injection, as compared with 0.42 ± 0.07 ms/mm Hg before. In contrast, the baroreceptor reflex sensitivity index generated by decreases in pressure with nitroprusside was similar before and after injection. Vehicle injections did not alter the baroreceptor reflex index. Collectively, the data suggest that inhibition of endogenous Ang II in the NTS facilitates the baroreceptor reflex sensitivity to increases, but not decreases, in pressure. This new finding reveals the NTS as one site of action for the tonic effects of endogenous Ang II.

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KEY WORDS • angiotensin II antagonist • baroreceptor reflex sensitivity • nucleus tractus solitarii • baroreceptor reflexes • heart rate • brainstem • blood pressure • hypertension

MANY studies indicate that angiotensin II (Ang II) modulates baroreceptor reflex control of heart rate (HR) in part by altering the central integration of sensory information.1 Ang II–sensitive areas have been identified in the brain, both within and outside of the blood-brain barrier.1–4 In the lower brainstem, dense concentrations of specific high-affinity Ang II binding sites exist in the nucleus tractus solitarii (NTS) and dorsal motor nucleus of the vagus nerve (NX), with lower concentrations in the area postrema.5,6–9 We recently showed that Ang II receptors in both rats and dogs are associated with vagal afferent fibers in the NTS and vagal motor neurons.7 Besides containing a high density of Ang II receptors, the NTS is rich in Ang II–like immunoreactive nerve terminals and cell bodies,1 suggesting that locally synthesized Ang II may modulate baroreceptor reflex function. Indeed, direct NTS microinjection of Ang II causes dose-dependent effects on mean arterial pressure (MAP) and HR,8,10 while Ang II infusions in the NTS attenuate the baroreceptor reflex control of HR.11 With this in mind, we evaluated the effect of NTS microinjection of the Ang II antagonist [Sar',Thr 8]Ang II on baroreceptor control of HR in rats.

Materials and Methods

Experiments were performed in 18 male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 253 ± 2 g. After anesthesia with chloralose and urethane (50 mg/kg and 500 mg/kg i.p., respectively), catheters were inserted into a femoral artery and vein. Arterial pressure was continuously measured with a solid state strain gauge transducer (Model MP-15D, Micron Instruments, Los Angeles, CA, USA). HR was measured on a beat-by-beat basis by a cardiotorachometer (Model 2000, Gould, Cleveland, OH, USA) triggered by the arterial pressure wave. All variables were displayed on a Gould polygraph (Series 2400).

Rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) with the head flexed downward at 45 degrees. The dorsal surface of the medulla was exposed by incising the atlanto-occipital membrane. Bilateral microinjections of either 100 ng (100 pmol) of the Ang II antagonist [Sar',Thr 8]Ang II (M. Khosla, Cleveland, OH, USA) or artificial cerebrospinal fluid (aCSF) in a volume of 100 nl was made...
with a triple-barreled glass micropipette (30–50 μm outer diameter), as described previously. In preliminary studies in four rats, this dose of the antagonist blocked the blood pressure response to an NTS injection of Ang II (500 fmol) for up to 30 minutes. [Sar1, Thr8]Ang II was chosen over the antagonist saralasin because it has fewer agonistic effects and is a relatively long-acting blocker.11 Injections were given over 1 minute by air pressure generated by hand-held syringe while the pipette was positioned in the NTS (0.5 mm rostral and 0.5 mm lateral to the obex, 0.3 mm below the dorsal surface).

Baroreceptor reflex control of HR was determined both before and after NTS microinjections in each rat by eliciting reflex HR changes in response to transient drug-induced variations in MAP. Kornner et al.12 demonstrated that such determinations are more sensitive to alterations in parasympathetic than sympathetic drug-induced variations in MAP. Korner et al.12 determined increases in HR related to increases in MAP (range, 10–40 mm Hg) were then apposed to x-ray film (SB-5, Kodak, Rochester, NY, USA) for 2 to 4 weeks, and the dimensions of the images were microscopically quantitated.

### Results

Basal values of MAP and HR were similar before NTS microinjections of either aCSF or the Ang II antagonist (Table 1). A small but significant increase in HR (−13 ± 5 beats/min; p < 0.05) sustained throughout the study, occurred without changes in the resting MAP in response to [Sar1, Thr8]Ang II injection in the NTS. No changes in MAP or HR occurred after injection of aCSF.

Injections of phenylephrine caused similar dose-related increases in MAP (range, 10–40 mm Hg) before and after NTS microinjection of either aCSF or [Sar1, Thr8]Ang II (Figure 1A). After NTS injection of the Ang II antagonist, bradycardia produced by phenylephrine was significantly greater than that obtained before microinjection. This effect was also discernible in the pulse interval values of [Sar1, Thr8]Ang II–injected rats compared with those of aCSF-injected rats (see Figure 1B). The sensitivity of the baroreceptor reflex control of HR, taken as the mean of the slopes of individual regression lines for each animal, was significantly greater (about 50%) after [Sar1, Thr8]Ang II microinjection (see Table 1 and Figure 2A). When the data were expressed as the ΔHR/ΔMAP ratio, the values before aCSF and before [Sar1, Thr8]Ang II were similar

### Table 1. Baroreceptor Control of Heart Rate

<table>
<thead>
<tr>
<th>NTS injection</th>
<th>MAP (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Slopes (msec/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF</td>
<td></td>
<td></td>
<td>Phenylinephrine</td>
</tr>
<tr>
<td>Before</td>
<td>108 ± 4</td>
<td>326 ± 19</td>
<td>0.50 ± 0.09</td>
</tr>
<tr>
<td>After</td>
<td>114 ± 7</td>
<td>319 ± 11</td>
<td>0.46 ± 0.09</td>
</tr>
<tr>
<td>Before</td>
<td>106 ± 3</td>
<td>360 ± 10</td>
<td>0.42 ± 0.07</td>
</tr>
<tr>
<td>After</td>
<td>109 ± 4</td>
<td>347 ± 10*</td>
<td>0.60 ± 0.09*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. NTS = nucleus tractus solitarii; aCSF = artificial cerebrospinal fluid.

*Significant differences between before and after values (p < 0.05). There were no statistically significant differences in baseline (before) values between the aCSF- and the [Sar1, Thr8]Ang II-treated groups.

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Figure 1. Change in MAP (A) or pulse interval (B) produced by injection of graded doses of phenylephrine (expressed as μg/rat) after microinjection of [Sar¹, Thr⁸]Ang II (solid circle and line; n = 11) or artificial cerebrospinal fluid (aCSF; open circle, hatched line; n = 7) into the NTS. A range of doses of phenylephrine was used to achieve similar increases in MAP in each rat.

Figure 2. Average reflex changes in heart rate (expressed as change in pulse interval) in response to increases or decreases in MAP produced by intravenous injection of phenylephrine or sodium nitroprusside, respectively. Panel A: Before (open circles, hatched lines) and after (solid circles and lines) NTS injection of [Sar¹, Thr⁸]Ang II. Panel B: Before (open circles, hatched lines) and after (solid circles and lines) NTS injection of artificial cerebrospinal fluid (aCSF). Significant differences in reflex control of heart rate were found by analyzing the slopes of individual regression lines (p<0.05; see Table 1).

Figure 3. Composite of 18 pipette tip placements (shaded region) in the NTS as determined by deposition of Alcian blue dye in each experiment. Maps and coordinates (in mm, right margin) are from the atlas of Pellegrino et al.11 AP = area postrema; FG = fasciculus gracilis; NTS = nucleus tractus solitarii; NX = dorsal motor nucleus of the vagus; NXII = hypoglossal nucleus; TS = tractus solitarius.
Discussion

The data presented in this study show that bilateral injections of an Ang II antagonist confined to the NTS selectively facilitate the baroreceptor reflex control of HR in response to increases in MAP in anesthetized rats. These observations are consistent with the localization of Ang II specific binding sites in the NTS and the demonstration that direct microinjection of Ang II into this nucleus produces dose-related effects on MAP. More important, the results suggest a functional role for endogenous Ang II in the central regulation of the HR component of the baroreceptor reflex. Since there were no changes in the level of MAP after \(\text{[Sar}^1,\text{Thr}^8\text{]}\) Ang II injection into the NTS, the differences in reflex sensitivity cannot be attributed to changes in baseline MAP. Because basal HR decreased after Ang II antagonist injections, the data also suggest a small but tonic effect of the peptide on cardiac rate in anesthetized animals. This latter finding contrasts with the observations of Rettig et al. who showed that similar doses of the Ang II antagonist saralasin produced increases in HR. Although reasons for this discrepancy are not apparent, differences may be related to the partial agonist actions of saralasin, the bilateral application of the antagonist in our study, or a combination of both.

It should be emphasized that our experiments were performed in rats under anesthesia, a state known to exhibit high levels of plasma renin. Several studies have shown that anesthesia elevates the threshold for baroreceptor activation of vagal efferents and may even eliminate the response. Thus, one possibility in our experiments is that anesthesia increased the amount of either blood or brain concentrations of Ang II. Therefore, even though the anesthetic effect did not modify the basal level of MAP or HR from normal conscious values, it may have unmasked an inhibitory effect of Ang II on baroreceptor reflex sensitivity. Ang II exerts an inhibitory influence on baroreceptor reflex control of HR after either intravenous or intracerebroventricular administration, in many species. The responses appear to be accompanied by withdrawal of parasympathetic tone to the heart and excitation of sympathetic vascular tone. The depressor responses after stimulation of the carotid sinus nerve are significantly smaller after intraventricular infusion of Ang II. The demonstration of Ang II receptors and Ang II-like immunoreactivity in the dorsal medulla suggested this region as a site of action for this peptide. Indeed, Casto and Phillips recently reported that injection of Ang II into the NTS attenuates the baroreceptor reflex sensitivity. That endogenous Ang II influences the baroreceptor reflex can be inferred from studies by Berecek et al., in which selective increases in the baroreceptor reflex gain in response to increases in MAP were observed after intracerebroventricular infusion of captopril (a converting enzyme inhibitor) in both spontaneously hypertensive and Wistar-Kyoto rats. Our study extends these findings by providing a specific brain site for the actions of endogenous Ang II on baroreceptor reflex control of HR in normotensive rats. The selective effect of the Ang II antagonist to facilitate the baroreceptor reflex only for increases in MAP may be related to a specific attenuation by Ang II of baroreceptor reflex–evoked activity in cardiac vagal efferent nerves. Ang II has been reported to selectively inhibit parasympathetic tone to the heart after peripheral administration. In addition, Guo and Abboud showed selective impairment of the baroreceptor reflex in response to increases in MAP during peripheral infusion of Ang II without changes in reflex control of HR in response to decreases in MAP. The present data confirm a selective action of Ang II to impair the sensitivity of the vagal component of baroreceptor control of HR to increases in MAP. Furthermore, the new findings suggest the NTS as one brain site for this selectivity of action. An alternative explanation that cannot be ruled out, however, is that multiple sites within the central nervous system are involved in regulating baroreceptor reflex control of parasympathetic outflow to the heart, as suggested by Reis and Cuenod. It is possible that these sites can be reached or affected differently by Ang II given by intracerebroventricular or intravenous routes.

Taken together, the results of this study indicate that endogenous, as well as exogenous, Ang II may act on specific receptors in the NTS to modulate the central integration of the baroreceptor inputs. Whether the source of the endogenous Ang II is central, peripheral, or both remains to be determined.

References

EFFECT OF ANG II ANTAGONIST ON BARORECEPTOR REFLEX/Campagnole-Santos et al.


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