Baroreceptor reflex modulation by circulating angiotensin II is mediated by AT1 receptors in the nucleus tractus solitarius

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IT HAS LONG BEEN KNOWN that an increase in the concentration of circulating ANG II can alter the characteristics of the baroreceptor reflex. Ismay et al. (17) showed that, in conscious sheep, the pressor response evoked by a bolus intravenous injection of ANG II is accompanied by little or no change in heart rate (HR), in contrast to the marked reflex bradycardia evoked by an equipressor bolus injection of phenylephrine. ANG II has no effect on the baroreceptors, however (26); therefore, the modulatory effect of circulating ANG II on the baroreceptor reflex is likely to be due to an action on the central mechanisms subserving the reflex. Very similar observations have since been made in several different species, including humans (40). As reviewed by Reid (40, 41), studies in the rabbit indicate that these effects reflect the fact that circulating ANG II resets the baroreceptor cardiac vagal reflex, such that the relationship between arterial pressure and reflex changes in HR are reset to higher levels of arterial pressure and HR, but without reducing the gain of this reflex. In contrast to the cardiac baroreflex, circulating ANG II does not greatly affect the baroreflex control of renal sympathetic nerve activity (RSNA), except under conditions where there are more extreme changes in arterial pressure (21, 43, 44).

ANG II does not cross the blood-brain barrier; therefore, it is generally considered that the effects of circulating ANG II on the baroreflex are due to an action on receptors [particularly ANG II type 1 (AT1)] on neurons in circumventricular organs, which are outside the blood-brain barrier (41). In animals with chronic lesions of the area postrema, circulating ANG II no longer affects the cardiac baroreflex, leading to the conclusion that its effects on the baroreflex are dependent on AT1 receptors within the area postrema (29, 44).

Similar to the area postrema, the nucleus tractus solitarius (NTS) contains a high density of AT1 receptors (14, 16, 34). Furthermore, microinjection of ANG II directly into the NTS can also powerfully attenuate the cardiac baroreflex, even in animals in which the area postrema has been removed (5, 35, 37). In this case, the effects of ANG II appear to be mediated by nitric oxide generated by activation of endothelial nitric oxide synthase (35, 36). Thus, according to the “vascular-neuronal signaling” hypothesis proposed by Paton et al. (35, 38), activation of AT1 receptors on blood vessels within the NTS can trigger the sequence of events leading to inhibition of the cardiac baroreflex. Consistent with this hypothesis, it has been shown that activation of endothelial nitric oxide synthase in blood vessels in the brain triggers the release of nitric oxide, causing changes in neuronal activity (1, 12). Furthermore, AT1 receptors are located on blood vessels as well as neurons in the NTS (16, 38). As suggested by Paton et al. (35), it is therefore possible that circulating ANG II may act directly on AT1 receptors on endothelial cells in the NTS, leading to the release of nitric oxide and modulation of the baroreflex. In addition, it is also conceivable that AT1 receptors on neurons in the NTS might mediate the modulatory effects on the baroreflex via inputs from ANG II-sensitive neurons in the area postrema, in the same way that excitative inputs from the subfornical organ to neurons in the hypothalamic paraventricular nucleus are mediated, at least in part, by AT1 receptors in the hypothalamic paraventricular nucleus (10, 24).

There do not appear to be any previous studies that have examined the role of AT1 receptors in the NTS in the modulation of the baroreflex by circulating ANG II. Furthermore, although previous studies have examined the effects of chronic lesions of the area postrema on the modulation of the baroreflex by circulating ANG II (29, 44), they do not appear to have
been any previous studies on the effects of acute blockade of AT1 receptors in the area postrema on this modulation. The main aim of the present study, therefore, was to test whether AT1 receptors in the NTS have a significant role in the modulation of the baroreflex by circulating ANG II, as predicted by the hypothesis of Paton et al. (35). We have also tested the effect of specific blockade of AT1 receptors in the area postrema on this modulation. For this purpose, we have performed a comprehensive analysis of the effects of circulating ANG II on the baroreceptor reflex in the rat. These studies have been performed in other species (e.g., rabbit, sheep, and dog) (17, 22, 26, 27, 29, 31, 42–44), as a first step, we confirmed that the baroreceptor reflex is modulated by circulating ANG II in a similar fashion in the rat as in these other species.

MATERIALS AND METHODS

General procedures. All experiments were carried out on male Sprague-Dawley rats (380–550 g body wt; Laboratory Animal Services, University of Sydney, NSW, Australia). All experimental procedures were approved by the Animal Ethics Committee of the University of Sydney. The rats had free access to a normal rat pellet diet containing 0.36% sodium chloride (61 meq/kg sodium) and tap water. Anesthesia was initially induced by inhalation of isoflurane (2–3% in O2-enriched air). Catheters were placed in a femoral artery, femoral vein, and jugular vein. Isoflurane was withdrawn while being replaced (over a period of ~10 min) with urethane (1.3 g/kg iv, with supplemental doses of 0.1 g/kg iv if required). The adequacy of anesthesia was verified by the absence of the corneal reflex and a withdrawal response to nociceptive stimulation of a hindpaw. A tracheotomy was performed, and the animals were artificially ventilated at a level that maintained end-tidal CO2 at 3.5–4.5%. Body temperature was monitored with a rectal probe and maintained at 37–38°C with a heating pad. Mean arterial pressure (MAP), HR, and RSNA were recorded according to procedures described previously (15).

The rat was fixed in a stereotaxic apparatus with the head flexed forward at a 45° angle. The dorsal surface of the medulla was exposed, and candesartan solution (100 pmol in 50 nl) was microinjected into sites within the medulla via a single-barreled micropipette held in a micromanipulator at a 13° angle (tip rostral). The vehicle solution was artificial cerebrospinal fluid (aCSF) adjusted to pH 7.4. Injections were made by pressure, and the volume injected was measured by displacement of the meniscus in the pipette with respect to a horizontal grid viewed through an operating microscope. The solution was microinjected over ~30 s. A fluorescent marker (rhodamine-labeled microspheres, Lumafluor) was added to the injectate. The calamus scriptorius was used as the rostrocaudal reference point for microinjections. For injections into the area postrema, the coordinates of the tip of the micropipette were in the midline, 0.5 mm rostral to calamus scriptorius, and 0.2 mm below the dorsal surface. For injections into the NTS, the coordinates were 0.4 mm rostral and 0.6 mm lateral to calamus scriptorius and 0.35 mm below the dorsal surface of the medulla. These coordinates were chosen because they correspond to the dorsomedial NTS, which contains a high density of ANG II receptors (16), and is the main site of termination of fibers arising from neurons in the area postrema (6, 45).

At the end of the experiment, the rat was killed with an overdose of pentobarbital sodium. The brain was removed and placed in 4% paraformaldehyde in 0.1 M PBS (pH 7.4) at 4°C for ≥24 h. After this period of fixation, the brain was placed in 30% sucrose in PBS for another 24 h at 4°C. Two series of 48-μm-thick coronal sections of brain stem tissue were cut on a CO2 freezing microtome. One series was used to view the fluorescent-labeled injection sites, and the other was stained with neutral red to identify the boundaries of the brain nuclei.

Baroreflex function tests. Two methods were used to determine the effect of circulating ANG II on the baroreflex control of HR and RSNA.

First, MAP was produced by intravenous infusion of phenylephrine (0.125 mg/ml) or ANG II (25 μg/ml), and the accompanying reflex changes in HR and RSNA were measured. The rate of infusion of the drugs was varied as necessary (from 40 to 80 μl/min) over a period of 1–2 min, so that MAP increased in a ramplike fashion, reaching a maximal increase of ~50 mmHg by the end of the infusion period and then decreasing more rapidly back to the baseline level.

Second, increases and decreases in MAP were evoked by intravenous infusions of phenylephrine and sodium nitroprusside, respectively, as described in detail previously (33). This allowed variation of the MAP over the full operating range of the baroreflex while reflex changes in HR and RSNA were measured. Then ANG II was infused continuously at a rate (400 ng/kg “min”1 iv) that resulted in an increase in MAP to a new stable baseline ~25 mmHg above the original baseline. After a 15-min waiting period following commencement of the ANG II infusion, the baroreflex test was again performed by infusion of phenylephrine and sodium nitroprusside.

Experimental procedures. Five different series of experiments were performed, and the procedures for these are summarized schematically in Fig. 1. In the first series of experiments, phenylephrine was infused intravenously to produce a ramplike increase in MAP, while HR and RSNA were recorded as described above. A 10- to 15-min waiting period allowed MAP, HR, and RSNA to return to their resting levels; then the test was repeated, except ANG II, instead of phenylephrine, was infused. Candesartan was then microinjected into the area postrema, and after a further 2- to 5-min waiting period, ANG II was again infused to produce a ramplike pressor response of similar magnitude. In control experiments, the procedure was the same, except the vehicle solution, instead of candesartan, was injected. In the second series of experiments, the procedure was the same as that described for the first series, except candesartan or vehicle solution was injected into the NTS, instead of the area postrema.

In the third and fourth series of experiments, the procedure was similar to that described for the first and second series, except the phenylephrine-nitroprusside baroreflex test was performed. A control baroreflex test was carried out, and then, after the variables had returned to their baseline levels (~15 min), a continuous infusion of ANG II was commenced and continued for the remainder of the experiment. A second baroreflex test was performed 15 min after the start of the ANG II continuous infusion. Then, when the cardiovascular variables had stabilized again (~15 min), candesartan was microinjected into the area postrema or NTS, and, after a 2- to 5-min waiting period, the baroreflex test was repeated. In this series, the same procedure was used for control experiments, except the vehicle solution, instead of candesartan, was injected.

In a fifth series of experiments, the procedure was the same as that described for the fourth series, except, after the initial control baroreflex test, the area postrema was removed via vacuum aspiration, and then all the other procedures were performed (Fig. 1).

Data analysis. For experiments in which ramplike increases in MAP were induced by phenylephrine or ANG II infusion, the changes in HR and RSNA (compared with the preinfusion baseline level) were calculated and plotted against each progressive 5-mmHg increase in MAP. For the purposes of statistical analysis, the changes in HR and RSNA in each experiment are expressed as normalized units, where the decreases in HR and RSNA that occurred when the MAP was increased by 50 mmHg by infusion of phenylephrine in that experiment were defined as ~100 units.

For the experiments in which phenylephrine and sodium nitroprusside induced increases and decreases in MAP, logistic sigmoidal
curves of best fit for the MAP-HR and MAP-RSNA relationships were determined according to the procedure previously described (32, 33) using Prism (version 4.0) software (Graphpad). The correlation coefficient ($R^2$) was calculated as a measure of the goodness of fit of each sigmoidal curve to the raw data points. In a few experiments, the program was not able to produce sigmoidal curves that were an adequate fit ($R^2 > 0.9$) for the MAP-HR and MAP-RSNA relationships; in these cases, only one curve was computed.

The sigmoidal curves are described by the following equation: $y = A_1/[1 + \exp(A_2(x - A_3))] + A_4$, where $y$ is HR or RSNA, $x$ is MAP, $A_1$ is the $y$ range (value of $y$ at the top plateau $-$ value of $y$ at the bottom plateau), $A_2$ is the gain coefficient, $A_3$ is the value of MAP at the midpoint (which is also the point of maximum gain), and $A_4$ is the value of $y$ at the bottom plateau (20). The threshold (THR) and saturation (SAT) values for MAP are defined as the values of $x$ at which $y$ is 5% (of the $y$ range) below and above the top plateau and bottom plateau, respectively, and are given by the following equations: $\text{THR} = A_3 - 2.944/A_2$ and $\text{SAT} = A_3 + 2.944/A_2$ (32). The maximum gain of the baroreceptor reflex function is defined as the maximum slope of the sigmoidal curve and is equal to $-A_1 A_2/4$ (20, 32).

For the purposes of statistical comparison of these curves obtained under different experimental conditions, MAP, HR, and RSNA were also expressed in normalized units, which in this case were defined as follows. In each experiment, the sigmoidal curves of best fit relating HR and RSNA to MAP under control baseline conditions were determined. Then the value of HR or RSNA at the midpoint was defined as 100 normalized units, and the values corresponding to the top and bottom plateaus were defined as 150 and 50 normalized units, respectively. Similarly, for MAP, the value at the midpoint of the curve was taken as 100 units, while the values corresponding to THR and SAT were defined as 50 and 150 normalized units, respectively. For all subsequent baroreflex tests in the same experiment, the calculated sigmoidal curves were plotted using these normalized units.

For experiments in which ramped increases in MAP were produced by infusions of phenylephrine or ANG II, the changes (compared with the preinfusion baseline level) in HR and RSNA associated with each 5-mmHg increase in MAP were determined, up to a maximum increase of 50 mmHg. Two-factor ANOVA was used to compare the changes in HR or RSNA associated with different phenylephrine- or ANG II-induced increases in MAP where the sources of variation were the increase in MAP and the experimental condition (i.e., phenylephrine infusion, ANG II infusion, or ANG II infusion + microinjection of candesartan into the area postrema or NTS). Separate ANOVAs were used to compare responses to 1) ANG II infusion vs. phenylephrine infusion, 2) ANG II infusion before vs. after microinjection of candesartan into the area postrema or NTS, and 3) ANG II infusion after microinjection of candesartan into the area postrema or NTS vs. phenylephrine infusion. For the experiments in which the MAP-HR or MAP-RSNA relationships were determined using sigmoidal equations of best fit, the parameters of these equations under different experimental conditions were compared using a t-test. $P < 0.05$ was regarded as statistically significant. Values are means ± SE.

**RESULTS**

Baseline levels of MAP and HR before any experimental procedures were similar in all groups of rats (Table 1). After lesions of the area postrema in one group of rats, however, baseline MAP and HR were significantly decreased (Table 1).

**Effects of ramped increases in MAP induced by phenylephrine or ANG II.** In the first group of experiments, ramped increases in MAP were produced by intravenous infusion of phenylephrine or ANG II over a period of 1–2 min. In confirmation of previous observations in other species (11, 17, 21, 22, 29, 31, 40, 50), the pressor response induced by phenylephrine was accompanied by a large decrease in HR, whereas that induced by ANG II was accompanied by much smaller changes in HR (Fig. 2). In contrast, the pressor response induced by phenylephrine or ANG II was in both cases accompanied by large decreases in RSNA, although the decreases in RSNA associated with ANG II-induced >30-mmHg increases in MAP were significantly less than those associated with phenylephrine-induced increases of the same magnitudes (Fig. 2B). For more moderate increases (up to 25 mmHg) in MAP

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline resting MAP and HR</th>
<th>ANG II infusion after microinjection of candesartan into the area postrema or NTS</th>
<th>Phenylephrine infusion vs. ANG II infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (bolus injections)</td>
<td>23</td>
<td>93 ± 2</td>
<td>365 ± 7</td>
</tr>
<tr>
<td>Group 2 (continuous ANG II infusion)</td>
<td>21</td>
<td>90 ± 3</td>
<td>345 ± 9</td>
</tr>
<tr>
<td>Group 3</td>
<td>Before AP lesion</td>
<td>14</td>
<td>91 ± 2</td>
</tr>
<tr>
<td>After AP lesion</td>
<td>14</td>
<td>77 ± 3</td>
<td>303 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; AP, area postrema. *$P < 0.05$ vs. before AP lesion.
induced by phenylephrine or ANG II, however, there was no significant difference in the reflex changes in RSNA ($P > 0.2$).

Microinjection of candesartan into the area postrema had no significant effect on baseline MAP or RSNA (changes of $-2 \pm 2$ mmHg and $3 \pm 3\%$ baseline, respectively) but did result in a small but significant decrease in baseline HR of $14 \pm 5$ beats/min ($P < 0.05$). After microinjection of candesartan into the area postrema, however, the increase in MAP induced by intravenous infusion of ANG II was accompanied by a large decrease in HR, in contrast to the much smaller and irregular HR response to ANG II infusion before candesartan microinjection (Fig. 3). At the same time, the reflex decreases in HR were not completely restored to the control responses: they were significantly ($\sim 40\%$) less ($P < 0.001$) than those associated with equivalent increases in MAP induced by phenylephrine in the same experiments (Fig. 3B). The decreases in RSNA accompanying the ANG II-induced pressor response, however, were not significantly different before and after candesartan microinjection (Fig. 3B). Consistent with the results presented in Fig. 2B, the decreases in RSNA associated with ANG II-induced $>30$-mmHg increases in MAP before or after candesartan microinjection into the area postrema were significantly less than those associated with phenylephrine-induced increases of the same magnitudes in the same experiments (Fig. 3B). In control experiments, the changes in HR and RSNA accompanying the ANG II-induced pressor response were not affected by microinjection of aCSF (Fig. 3B).

In contrast to its effects in the area postrema, microinjection of candesartan into the NTS in seven experiments resulted in a significant decrease in baseline MAP of $14 \pm 5$ mmHg ($P < 0.05$) and a significant decrease in baseline HR of $43 \pm 7$ beats/min ($P < 0.01$) but with no significant change in RSNA ($-7 \pm 4\%$ baseline). Similar to the effects of candesartan in the area postrema, however, after bilateral microinjections of candesartan into the NTS, the increase in MAP induced by intravenous infusion of ANG II was accompanied by a large decrease in HR, in contrast to the much smaller and irregular HR response before candesartan microinjection (Fig. 4). In this case, however, there was no significant difference ($P > 0.8$) between the reflex decreases in HR induced by ANG II after candesartan in the NTS and those associated with equivalent increases in MAP induced by phenylephrine in the same experiments (Fig. 4B). In addition, the decreases in RSNA accompanying the ANG II-induced pressor response were not significantly different before and after candesartan microinjection into the NTS (Fig. 4B). Consistent with the results presented in Fig. 2B, the decreases in RSNA associated with ANG II-induced $>30$-mmHg increases in MAP before or after candesartan microinjection into the NTS were significantly less than those associated with phenylephrine-induced increases of the same magnitudes in the same experiments (Fig. 4B). In two control experiments, the changes in HR and RSNA accompanying the ANG II-induced pressor response were not affected by microinjection of aCSF solution (data not shown).

Effects of continuous infusion of ANG II on the baroreflex. Traces from one experiment in which ANG II was infused continuously are shown in Fig. 5, and parameters of the sigmoidal curves for the MAP-HR and MAP-RSNA relationships under the different experimental conditions are shown in Tables 2 and 3. In all experiments, ANG II infusion resulted in an increase in baseline MAP of $25 \pm 2$ mmHg and a decrease in RSNA of $37 \pm 6\%$ below the original baseline level but no significant change in baseline HR. During ANG II infusion, the sigmoidal curve of best fit describing the relationship between sodium nitroprusside- and phenylephrine-induced changes in MAP and the reflex changes in HR was shifted upward, such that the upper plateau and, to a lesser extent, the lower plateau were significantly increased compared with the control MAP-HR curve when ANG II was not infused (Fig. 6A). The range of the HR response (difference between upper and lower plateaus) was also significantly increased ($P < 0.001$), as was the maximum gain (Fig. 6A). In contrast, ANG II infusion had no significant effect on any of the parameters of the MAP-RSNA curve, except for a slight increase in the midpoint value (Fig. 6A).

After microinjection of candesartan into the area postrema during continuous ANG II infusion in 13 experiments, there

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**Fig. 2.** A: changes in arterial pressure induced by bolus intravenous injections of phenylephrine or ANG II and reflex changes in HR and RSNA in 1 experiment. B: grouped results showing relationship between changes in MAP induced by bolus injections of phenylephrine or ANG II and reflex changes in HR or RSNA. Changes in HR and RSNA are expressed in normalized units (nu), where $-100$ nu is defined as reflex change in these variables evoked by a $50$-mmHg increase in MAP induced by infusion of phenylephrine. Values are means $\pm$ SE. $P$ values are determined from ANOVA comparisons of responses to changes in MAP induced by ANG II vs. those induced by phenylephrine over the range of changes in MAP from 5–50 mmHg.
were moderate decreases in MAP and HR (8 ± 2 mmHg and 25 ± 5 beats/min, respectively, \( P < 0.05 \) in both cases), but there was no significant change in RSNA (−8 ± 6% baseline). Similarly, after microinjection of candesartan into the NTS during continuous ANG II infusion in eight experiments, there were also moderate decreases in MAP and HR (8 ± 2 mmHg and 18 ± 6 beats/min, respectively, \( P < 0.05 \) in both cases), but there was no significant change in RSNA (17 ± 8% baseline).

After microinjection of candesartan into the area postrema, the baroreflex MAP-HR curve was shifted back to the control curve before the start of ANG II infusion (i.e., a significant reduction in the upper and lower plateaus), but the range of the HR response was not altered (\( P > 0.2 \); Fig. 6B). The maximum gain of the reflex was significantly decreased (Fig. 6B). After microinjection of candesartan into the NTS, the baroreflex MAP-HR curve was also shifted back to the control curve before the start of the ANG II infusion (Fig. 6C), and, in this case, the range of the HR response was significantly decreased (\( P < 0.001 \)). The gain of the reflex was also decreased, but the decrease was not statistically significant (\( P = 0.084 \)). The decreases in the upper plateau and range of the MAP-HR curve after candesartan injection into the NTS (Fig. 6C) were significantly greater (\( P < 0.05 \) and \( P < 0.01 \), respectively) than the decreases in these parameters after candesartan injection into the area postrema (Fig. 6B).

The MAP-RSNA curve was not significantly altered after candesartan microinjection into the area postrema (Fig. 6B), but the upper plateau of the MAP-RSNA curve was significantly shifted downward after candesartan microinjection into the NTS (Fig. 6C) to below the level measured under control conditions (Fig. 6A). Microinjection of aCSF solution into the area postrema or NTS had no significant effect on any of the parameters of the MAP-HR or MAP-RSNA curves (Tables 2 and 3).

**Effects of continuous infusion of ANG II on the baroreflex after removal of the area postrema.** As noted previously, the baseline levels of MAP and HR were significantly reduced after removal of the area postrema (Table 1). As shown in Tables 2 and 3, however, the parameters of the MAP-HR and MAP-RSNA baroreflex curves were not significantly altered after removal of the area postrema, except for the upper and lower plateaus of the MAP-HR curve, which were significantly decreased (\( P < 0.001 \)) by 103 ± 12 and 55 ± 8 beats/min, respectively. Even after removal of the area postrema, ANG II infusion resulted in a significant upward shift in the upper plateau of the MAP-HR relationship (Fig. 7A), which was restored after microinjection of candesartan into the NTS (Fig. 7B).
The magnitude of this upward shift (25 ± 8 beats/min), however, was significantly less (P < 0.01) than that evoked by ANG II infusion in intact rats (57 ± 8 beats/min). ANG II infusion did not result in a significant change in the maximum gain or the lower plateau of the MAP-HR relationship in area postrema-lesioned rats (Fig. 7A), in contrast to the significant increases in these parameters in intact rats (Fig. 6A). Similarly, subsequent microinjection of candesartan into the NTS of area postrema-lesioned rats reduced the upper plateaus of the MAP-HR relationship by 39 ± 8 beats/min, which was significantly less (P < 0.01) than the decrease in this parameter (82 ± 10 beats/min) after microinjection of candesartan into the NTS of intact rats.

As was observed in rats in which the area postrema was intact, ANG II infusion resulted in a small but significant increase in the midpoint of the MAP-RSNA relationship but did not significantly change the upper or lower plateau of this relationship (Fig. 7A). Subsequent microinjection of candesartan into the NTS of area postrema-lesioned rats, however, decreased the upper plateau of the MAP-RSNA relationship to a level below the original control level (Fig. 7B), as observed in intact rats (Fig. 6). The magnitude of the decrease in the upper plateau of this curve after candesartan microinjection into the NTS was not significantly different (P > 0.05) between area postrema-lesioned and intact rats.

The centers of the injection sites in the area postrema and NTS for all experiments are shown in Fig. 8.

DISCUSSION

It is well established that an increase in the level of circulating ANG II can powerfully modulate the cardiac component of the baroreceptor reflex. This was first shown by Ismay et al. (17) and Lumbers and co-workers (26, 27), who demonstrated in sheep and dogs that the pressor response evoked by a bolus injection of ANG II was accompanied by little or no reflex change in HR, in contrast to the powerful reflex bradycardia...
that accompanies a pressor response of similar magnitude evoked by a bolus injection of phenylephrine. Later studies demonstrated the same phenomenon in several other species, including rabbit (21, 29, 31), baboon (11), and mouse (50).

In the present study, we first confirmed that, in the rat, as in other species, an ANG II-induced pressor response was accompanied by little or no reflex bradycardia. We also showed that this reflex bradycardia was largely restored after microinjection of candesartan into the area postrema, consistent with previous studies showing that chronic lesions of the area postrema also prevented the ANG II-induced modulation of the cardiac baroreflex (29, 44, 50). The major new finding of this study, however, is that microinjection of the AT1 receptor antagonist candesartan into the medial NTS prevented the modulation of the cardiac baroreflex that results from an increase in the level of circulating ANG II. This was shown to be the case whether this modulatory effect was assessed by comparing the changes in HR associated with an ANG II-induced pressor response with that associated with a phenylephrine-induced pressor response or by determining the sigmoidal curves that best describe the relationship between induced changes in MAP and reflex changes in HR in the presence or absence of a continuous infusion of ANG II.

Because lesion of the area postrema (29, 44, 50) or blockade of AT1 receptors in the area postrema (present study) reduces the modulatory effect of ANG II, the following question arises: Is the effect of candesartan microinjection into the NTS due to diffusion of the drug to the area postrema, rather than an action on AT1 receptors in the NTS? This seems unlikely, because the modulatory effect of intravenous ANG II on the cardiac baroreflex was still present after acute ablation of the area postrema, and this effect was reversed after subsequent bilateral microinjections of candesartan into the NTS. This is further supported by the fact the cardiac baroreflex response to ANG II-induced increases in MAP was more effectively restored by microinjections of candesartan into the NTS than into the area postrema.

The rats used in our experiments were fed a normal-sodium diet. A tonic activation of the endogenous renin-angiotensin system has been shown in rats fed a normal- or low-sodium diet (7, 8), so the effects of AT1 receptor blockade in the NTS could be partly due to blockade of the effects of endogenous ANG II. It was shown previously that microinjection of candesartan into the NTS of anesthetized rats in which ANG II was not infused also modulates the baroreflex (30). In that case, however, the range and gain of the cardiac baroreflex were significantly increased by candesartan (30); in our experiments, candesartan in the NTS of ANG II-infused rats had opposite effects on these parameters. It therefore seems very unlikely that reversal of the modulatory effects of increased levels of circulating ANG II by candesartan is simply a consequence of blocking the effects of endogenous tonically released ANG II within the NTS independently of the increased level of circulating ANG II.

The experiments in the present study were performed in anesthetized rats to ensure precise placement of small microinjections of candesartan in the medial NTS or area postrema. At the same time, anesthesia may have influenced the modulatory effects of ANG II on the baroreflex. Nevertheless, as mentioned above, our observation in the anesthetized rat that the ANG II-induced pressor response, in contrast to the phenylephrine-induced pressor response, is associated with little or no reflex bradycardia is consistent with similar observations in conscious animals of several different species (11, 17, 21, 22, 29, 31).

Table 2. Parameters describing baroreflex control of HR

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Midpoint, mmHg</th>
<th>Lower Plateau, beats/min</th>
<th>Upper Plateau, beats/min</th>
<th>Max Gain, beats·min⁻¹·mmHg⁻¹</th>
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<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>102±3</td>
<td>282±6</td>
<td>395±10</td>
<td>-1.77±0.18</td>
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<td>ANG II infusion</td>
<td>20</td>
<td>108±3</td>
<td>298±8</td>
<td>343±11</td>
<td>-1.32±0.43</td>
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<tr>
<td>ANG II infusion + CAND in AP</td>
<td>13</td>
<td>108±5</td>
<td>275±8</td>
<td>392±14</td>
<td>-2.44±0.45</td>
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<td>ANG II infusion + aCSF in AP</td>
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<td>100±7</td>
<td>272±14</td>
<td>425±30</td>
<td>-4.96±1.37</td>
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<tr>
<td>ANG II infusion + CAND in NTS</td>
<td>7</td>
<td>112±3</td>
<td>284±14</td>
<td>357±17</td>
<td>-1.91±0.27</td>
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<tr>
<td>ANG II infusion + aCSF in NTS</td>
<td>4</td>
<td>114±5</td>
<td>329±8</td>
<td>446±21</td>
<td>-3.54±0.39</td>
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<tr>
<td>AP-lesioned control</td>
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<td>233±13</td>
<td>324±10</td>
<td>-2.12±0.32</td>
</tr>
<tr>
<td>AP-lesion + ANG II infusion</td>
<td>10</td>
<td>114±4</td>
<td>232±15</td>
<td>349±10</td>
<td>-2.48±0.31</td>
</tr>
<tr>
<td>AP-lesion + ANG II infusion + CAND in NTS</td>
<td>10</td>
<td>127±4</td>
<td>227±14</td>
<td>310±11</td>
<td>-2.15±0.33</td>
</tr>
</tbody>
</table>

Values are means±SE. Maximum gain (Max Gain) is gain of sigmoidal curve of best fit at MAP corresponding to midpoint value. CAND, candesartan; aCSF, artificial cerebrospinal fluid (vehicle); NTS, nucleus tractus solitarius.

Table 3. Parameters describing baroreflex control of RSNA

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Midpoint, mmHg</th>
<th>Lower Plateau, %baseline</th>
<th>Upper Plateau, %baseline</th>
<th>Max Gain, %baseline:mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>106±2</td>
<td>18±2</td>
<td>126±5</td>
<td>-2.63±0.15</td>
</tr>
<tr>
<td>ANG II infusion</td>
<td>15</td>
<td>110±3</td>
<td>22±2</td>
<td>123±5</td>
<td>-2.59±0.21</td>
</tr>
<tr>
<td>ANG II infusion + CAND in AP</td>
<td>7</td>
<td>103±7</td>
<td>24±2</td>
<td>101±7</td>
<td>-2.48±0.33</td>
</tr>
<tr>
<td>ANG II infusion + aCSF in AP</td>
<td>4</td>
<td>100±3</td>
<td>28±4</td>
<td>144±17</td>
<td>-3.22±0.53</td>
</tr>
<tr>
<td>ANG II infusion + CAND in NTS</td>
<td>8</td>
<td>115±2</td>
<td>25±5</td>
<td>105±9</td>
<td>-3.12±0.34</td>
</tr>
<tr>
<td>ANG II infusion + aCSF in NTS</td>
<td>4</td>
<td>120±4</td>
<td>50±15</td>
<td>141±12</td>
<td>-2.42±0.51</td>
</tr>
<tr>
<td>AP-lesioned control</td>
<td>9</td>
<td>99±3</td>
<td>22±5</td>
<td>115±3</td>
<td>-2.76±0.60</td>
</tr>
<tr>
<td>AP-lesion + ANG II infusion</td>
<td>9</td>
<td>105±4</td>
<td>24±5</td>
<td>102±10</td>
<td>-1.80±0.27</td>
</tr>
<tr>
<td>AP-lesion + ANG II infusion + CAND in NTS</td>
<td>9</td>
<td>111±3</td>
<td>26±5</td>
<td>86±6</td>
<td>-1.72±0.26</td>
</tr>
</tbody>
</table>

Values are means±SE. RSNA, renal sympathetic nerve activity.
29, 31, 40, 50). Similarly, in agreement with previous studies in the conscious rabbit (31, 43), we found that the reflex inhibition of RSNA in response to increases in MAP was unaffected by background continuous infusion of ANG II. Furthermore, our results are also consistent with previous studies in the conscious rabbit in which a background infusion of ANG II resets the MAP-HR relationship, such that HR is increased at a given level of MAP (21, 29, 31, 42, 43, 48). At the same time, in contrast to the rabbit (43, 48), the midpoint of the MAP-HR sigmoidal curve was not increased significantly in the presence of a background infusion of ANG II. Conversely, we found that the maximum gain of the MAP-HR relationship was significantly increased when ANG II was infused continuously, whereas in the rabbit it was not changed significantly (43, 48). Furthermore, we did not find, as reported in the conscious rabbit (43), a reduction in the maximum reflex increase in RSNA in response to baroreceptor unloading in the presence of ANG II. These differences may reflect species differences or might be due to the effects of anesthesia.

Postulated mechanisms. Our results confirm previous observations that neurons in the area postrema contribute to modulation of the baroreflex by circulating ANG II (29, 50) and extend them by demonstrating that the effects are mediated specifically by AT1 receptors in the area postrema. Consistent with this, we also found that, after acute removal of the area postrema, the magnitude of the modulatory effect of circulating ANG II on the cardiac baroreflex was reduced compared with that observed in rats with an intact area postrema. It is unlikely that this was a result of an impaired baroreflex as a consequence of damage to the underlying NTS, because the maximum gain of the cardiac baroreflex was not significantly affected by removal of the area postrema.

Application of ANG II to the area postrema affects the firing rate of a significant proportion (~40%) of neurons in the dorsomedial NTS, and the large majority of these are inhibited (4). The dorsomedial NTS is also the site of termination of a prominent direct projection from the area postrema, whereas surrounding subregions of the NTS receive only sparse projections from the area postrema (6, 45). It therefore seems likely that the modulatory effects of circulating ANG II on the baroreflex that are dependent on AT1 receptors in the area postrema are mediated via a direct projection from the area postrema to the dorsomedial NTS. It is important to note that the dorsomedial NTS also contains a high density of ANG II receptors (14) and corresponds to the NTS region into which candesartan was microinjected in the present study. Thus our finding that candesartan microinjections into the medial NTS or the area postrema abolished or greatly reduced the modulatory effects of circulating ANG II on the baroreflex could be explained by the hypothesis that circulating ANG II activates AT1 receptors on neurons in the area postrema, which then, in turn, project to and modulate the firing rate of neurons in the medial NTS, also via an AT1 receptor-dependent mechanism. There is considerable evidence that an analogous mechanism...
operates in the hypothalamus, where circulating ANG II first activates (via AT₁ receptors) neurons in the subfornical organ, which then project to and excite neurons in the paraventricular nucleus, also via AT₁ receptors (10, 24).

In addition to the above-postulated mechanism, our results also indicate that part of the modulatory effects of circulating ANG II on the baroreflex is independent of the area postrema, as shown by the fact that, even after removal of the area postrema, ANG II infusion caused a shift in the MAP-HR baroreflex, which was then reversed by candesartan in the medial NTS. In this case, circulating ANG II could act on ANG II-sensitive neurons in other circumventricular organs, such as the subfornical organ in the hypothalamus, which could influence the baroreflex via connections with the NTS (9).

Gross et al. (13) demonstrated that a subregion of the NTS, in the dorsomedial caudal part of the nucleus 0.6–0.9 mm caudal to obex, contains a high density of capillaries with endothelial cells that have morphological characteristics indicative of capillaries that are highly permeable to plasma solutes. They suggested that hormones such as ANG II may access neurons within this specialized dorsomedial NTS subregion (13). Also, the location of this subregion appears to be close to the site in the present study at which candesartan microinjection was found to be highly effective in reversing the modulatory effect of ANG II on the cardiac baroreflex. Thus it is conceivable that circulating ANG II may directly act on neurons within this NTS subregion, leading to the modulation of the baroreflex.

Finally, another possible mechanism as proposed by Paton and co-workers (35) is that ANG II modulates the baroreflex via activation of AT₁ receptors on blood vessels (the vascular-neuronal signaling mechanism). According to this hypothesis, activation of AT₁ receptors on vascular endothelial cells within the NTS activates endothelial nitric oxide synthase, leading to generation of nitric oxide, which then causes modulation of the cardiac baroreflex. Thus circulating ANG II could affect neurons subserving the baroreflex via this mechanism, which is independent of the area postrema or any other circumventricular organ.

Circulating ANG II and ANG II in the NTS. Microinjection of ANG II directly into the NTS of the rat greatly decreases the gain of the cardiac baroreflex, even in animals in which the area postrema has been removed (5, 35, 37). Furthermore, blockade of AT₁ receptors in the NTS has been shown to increase the gain of the cardiac baroreflex (30), indicating that endogenous ANG II in the NTS also has an inhibitory effect on the cardiac baroreflex. These findings are in contrast to our observation that the gain of the cardiac baroreflex was increased in the presence of a continuous intravenous infusion of ANG II. Thus, even though, as suggested above, circulating ANG II and ANG II within the NTS may act on AT₁ receptors on vascular endothelial cells, the fact that circulating ANG II and ANG II within the NTS have quite different effects on the baroreflex indicates that they activate, at least in part, different AT₁ receptor-dependent mechanisms within the NTS. It is possible, for example, that direct or indirect inputs to the NTS...
AT1 receptors have been located on diverse structures within the NTS, including cell bodies, dendrites, axon terminals, glia, and endothelial cells on blood vessels (16). Consistent with this, AT1 receptors have been located on diverse structures within the NTS that are not affected by exogenous ANG II. Thus our results support the view that AT1 receptors within the NTS can mediate different effects, depending on the precise location of the receptors (18, 37). Consistent with this, AT1 receptors have been located on diverse structures within the NTS, including cell bodies, dendrites, axon terminals, glia, and endothelial cells on blood vessels (16).

Acute and chronic increases in the level of circulating ANG II. Xu and Brooks (49) showed that, in the conscious rat, an increase in the level of endogenous circulating ANG II, as a consequence of a reduced dietary sodium intake, increases the upper plateau and range of the baroreflex control of HR. This effect was prevented by intravenous administration of the AT1 receptor antagonist losartan (49). These findings are consistent with our observation that an acute increase in the level of circulating ANG II, as a consequence of a reduced dietary sodium intake, increases the upper plateau and range of the baroreflex control of HR. Xu and Brooks did not investigate the site of action of losartan in reversing the effects of increased levels of circulating ANG II on the cardiac baroreflex, but they suggested that because losartan can cross the blood-brain barrier (23, 51), the site of action could be AT1 receptors within the brain or circumventricular organs. This suggestion is entirely compatible with our findings that AT1 receptors in the NTS and area postrema play important roles in mediating the modulatory effect of circulating ANG II.

DiBona and co-workers (8) showed that, in rats fed a low- or normal-sodium diet, but not rats fed a high-sodium diet, intravenous injection of losartan modulates the baroreflex control of RSNA by decreasing the midpoint of the MAP-RSNA sigmoidal baroreflex curve without altering other parameters. This suggests that an increased level of endogenous circulating ANG II increases the midpoint of this reflex, which again is consistent with our observation that an acute increase in the level of circulating ANG II modestly, but significantly, increased the midpoint of the MAP-RSNA curve without affecting other parameters. DiBona et al. also found that intracerebroventricular and intravenous injection of losartan had the same effect on the baroreflex, suggesting that central AT1 receptors may mediate this effect. A more recent study from the same group showed that microinjection of candesartan into the rostral ventrolateral medulla (RVLM) reduces the midpoint of the MAP-RSNA curve (7), indicating that the RVLM is an important site of modulation of baroreflex control of RSNA by ANG II. Although this observation is not consistent with our finding on the role of AT1 receptors in the NTS in modulating this reflex, there are important potential differences between the effects of an acute increase in the level of circulating ANG II and a chronic increase that occurs in response to a low-sodium diet: 1) a low-sodium diet in humans increases the concentration of ANG II in the cerebrospinal fluid, as well as in the plasma (19), raising the possibility that this may also occur in rats, and 2) a chronic sustained increase in the level of circulating ANG II could affect the properties of neurons within the baroreflex circuitry by alterations in gene expression. For example, in severe heart failure, there is an increased expression of AT1 receptors within the NTS and RVLM, which is thought to be associated with increased activation of the renin-angiotensin system (52).

Perspectives

Regardless of the precise mechanisms involved, our results clearly demonstrate the importance of AT1 receptors in the NTS in subserving the modulation of the baroreflex by circulating ANG II. An important question that remains, however, is the physiological significance of this modulation. In agreement with previous studies in the rabbit (21, 31, 42, 43, 48), an increase in the level of circulating ANG II in the rat does not reduce the sensitivity of the cardiac baroreflex but does increase HR at any given level of MAP. This may be beneficial in conditions such as dehydration or hemorrhage, which stimulate the renin-angiotensin system, because the increase in HR associated with increased levels of circulating ANG II would tend to compensate for the reduction in cardiac output that occurs in these conditions. Consistent with this, intravenous injection of an AT1 receptor antagonist reduces baroreflex increases in HR in water-deprived animals (2) and also reduces the onset time of the decompensatory phase following severe hemorrhage (28). During pregnancy, a large progressive increase in the level of circulating ANG II is associated with a significant increase in HR and cardiac output (39, 47). Further-
more, in pregnant rabbits, AT₁ receptor blockade decreases the maximum reflex tachycardia in response to induced hypotension (3), indicating that the increase in HR in pregnancy also may be, at least in part, a consequence of the increased levels of ANG II.

Similarly, heart failure is also associated with increased levels of circulating ANG II (52). In this condition, as mentioned above, AT₁ receptors are upregulated in several brain regions, including the NTS (52). In contrast to conditions such as dehydration, where an increased level of ANG II is associated with an increase in the sensitivity of the cardiac baroreflex (2), in severe heart failure the sensitivity of this reflex is reduced (25, 52). In heart failure, however, depression of the baroreflex appears to be in large part due to increased activation of the cardiac sympathetic afferent reflex, which affects the baroreflex via AT₁ receptors in the NTS (46). This further emphasizes the point made previously that the role of AT₁ receptors in the NTS in the modulation of the baroreflex is complex and does not depend only on the level of circulating ANG II. Nevertheless, the present study indicates that AT₁ receptors in the NTS make a crucial contribution to the altered regulation of cardiovascular function under various physiological and pathophysiological conditions in which levels of circulating ANG II are increased.

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GRANTS

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