Nucleus of the solitary tract lesions enhance drinking, but not vasopressin release, induced by angiotensin

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SchreihofeR, Ann M., Edward M. Stricker, and Alan F. Sved. Nucleus of the solitary tract lesions enhance drinking, but not vasopressin release, induced by angiotensin. Am J Physiol Regulatory Integrative Comp Physiol 279: R239–R247, 2000.—Rats with chronic nucleus of the solitary tract lesions (NTS-X) drink water and release vasopressin (VP) in response to reduced blood volume despite an absence of neural signals from cardiac and arterial baroreceptors. The present study determined whether rats with NTS-X have a greater sensitivity to circulating ANG II, which may contribute to the drinking and VP responses to hypovolemia. In conscious control rats and rats with NTS-X, ANG II was infused intravenously for 1 h at 10, 100, or 250 ng·kg⁻¹·min⁻¹. At the two higher doses, ANG II stimulated more water intake with a shorter latency to drink in rats with NTS-X than in control rats. In contrast, infusion of ANG II produced comparable increases in plasma VP in the two groups. At the two higher doses, ANG II produced an enhanced increase in arterial pressure (AP) in rats with NTS-X, and the bradycardia seen in control rats was reversed to a tachycardia. Infusion of hypertonic saline, which did not alter AP or heart rate, produced comparable drinking and VP release in the two groups. These results demonstrate that chronic NTS-X increases the dipsogenic response of rats to systemic ANG II but has no effect on ANG II-induced VP release or the osmotic stimulation of these responses.

INCREASED CIRCULATING LEVELS of ANG II promote neurohypophysial vasopressin (VP) release and water intake (12, 37). However, it has been proposed that the true efficacy of endogenous ANG II for stimulating these effects has been underestimated, because the increase in arterial pressure (AP) produced by intravenous infusion of exogenous ANG II simultaneously inhibits VP release and drinking (9, 20, 23, 37). Indeed, the inhibitory effect of acute increases in AP on the firing rate of hypothalamic VP neurons in anesthetized rats has been well established (e.g., Refs. 15, 28). Furthermore, intravenously infused ANG II in relatively low doses stimulates the firing of VP neurons in a dose-related manner in anesthetized rats, but at higher doses, which also increase AP, the firing of VP neurons is inhibited by ANG II (24). In agreement, denervation of arterial baroreceptor inputs in rats allows normally inhibitory high doses of ANG II to stimulate the firing of VP neurons (23). On the other hand, in conscious dogs ANG II-induced VP release is not enhanced by chronic cardiac and arterial baroreceptor denervation (3), as would be expected if an increase in AP inhibited VP release. At present, it is difficult to reconcile these seemingly conflicting observations.

The examination of the effect of AP on ANG II-induced water intake also has produced some seemingly inconsistent results. When the pressor effect of ANG II is potentiated by administration of the ganglionic antagonist hexamethonium, ANG II-induced water intake is inhibited in dogs (21). Conversely, in rats the drinking response to ANG II is enhanced when the pressor effect of intravenously infused ANG II is counteracted by concomitant infusion of diazoxide, isoproterenol, or minoxidil (10, 31). Furthermore, ANG II-induced water intake is enhanced in chronic cardiac- and arterial baroreceptor-denervated dogs (20). These data are consistent with the notion that ANG II-induced increases in AP inhibit ANG II-induced drinking via a signal detected by baroreceptor afferents. However, selective removal of arterial baroreceptor inputs by sinoaortic denervation in rats does not appear to enhance the drinking response to the systemic administration of ANG II (18, 30).

The present study examines whether ANG II-induced VP release and water intake in rats are enhanced by chronic bilateral lesions of nucleus of the solitary tract (NTS), which eliminates inputs from both arterial baroreceptors and cardiac receptors (32, 33). If increased AP blunts these ANG II-induced responses by acting on arterial baroreceptors and/or cardiac receptors, then rats with chronic NTS lesions (NTS-X) should drink more water and have a greater increase in plasma VP levels in response to intravenous infusion of ANG II. To examine potential effects of NTS-X on drinking and VP secretion unrelated to changes in AP, these responses were induced by an osmotic stimulus that did not alter AP.

MATERIALS AND METHODS

Animals. Studies were performed using adult male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA). Rats were housed singly in hanging wire-mesh cages in

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a temperature-controlled room (22–23°C). Lights were on between 0800 and 2000. Food (Purina 5001 Rodent Chow) was available ad libitum except during experiments. Tap water was available except as noted. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh, and studies were conducted in accordance with the guidelines established by the American Physiological Society and Public Health Service.

Electrolytic NTS lesions. NTS-X were produced as described previously (33). Rats were anesthetized with halothane and injected with an autonomic ganglionic antagonist (chlorisondamine, 2 mg/kg sc; Ciba-Geigy) and a VP1 receptor antagonist ([1-(β-mercaptop-β,β-cyclo pentamethylene-propionyl)1,2-O- methyl]-tyrosine[Arg6-VP, 10 μg/kg sc; Bachem]. The rats were placed in a Kopf stereotaxic instrument with the incisor bar positioned 11 mm below the interaural line. The dorsal surface of the medulla was exposed, and bilateral electrolytic lesions of the NTS were produced by passing anodal current (1 mA, 15 s) through a Teflon-coated tungsten electrode (375-μm tip exposed) placed 0.5 mm rostral to calamus scriptorius, 0.6 mm lateral to the midline, and 0.6 mm below the dorsal surface of the brain stem. After NTS-X were produced bilaterally, the wound was closed and the administration of halothane was terminated. Rats were treated with an antibiotic (Combimic, 0.1 ml im) and isotonic saline (20 ml sc) and returned to their home cages. Control rats did not receive surgery. The decision to use unoperated control rats rather than surgical control rats was based on the need to use weight-matched animals for studies measuring fluid intake. Because rats with NTS-X gain weight more slowly than control rats after placement of the lesions, it is difficult to produce surgical controls with both comparable weights and recovery periods.

Physiological assessment of NTS lesions. Ten to fourteen days after production of NTS-X, rats were anesthetized with halothane. A catheter was inserted into the right femoral artery for recording AP and heart rate (HR) and for withdrawing blood samples. Another catheter was placed into the right femoral vein for administration of drugs and infusion of ANG II. The free ends of the catheters were tunneled subcutaneously to exit between the scapulae. Each rat was fitted with a jacket connected to a tether and swivel system as described previously (33), placed in a cylindrical Plexiglas cage, and allowed to regain consciousness.

Testing of baroreceptor reflexes occurred 1–2 h later while the animals were conscious and unrestrained. Arterial baroreceptor reflexes were assessed by measuring HR changes induced by changes in AP. All rats with NTS-X showed an absence of reflexive HR changes to phenylephrine-induced increases in AP and to nitroprusside-induced decreases in AP (33). Testing reflex-evoked changes in HR to both increases and decreases in AP are required to functionally assess the quality of the denervation (35), because they assess different components of the baroreceptor reflex. Specifically, the bradycardia evoked by increased AP is mediated by the parasympathetic nervous system, whereas the tachycardia evoked by decreased AP is mediated by the sympathetic nervous system; it is unclear at present which of these responses might better reflect baroreceptor reflex control of drinking or VP secretion. In addition, all rats with NTS-X showed an absence of hypotension and bradycardia to the 5-hydroxytryptamine3 agonist phenyl biguanide (33).

One day after implantation of catheters, groups of control rats and rats with NTS-X were used in one of the following experiments.

ANG II-induced changes in AP, HR, and VP levels. A baseline blood sample (1.2 ml) was withdrawn from the arterial line, and the volume was not replaced. The arterial line was then connected to a Statham pressure transducer (Grass Instruments, Quincy, MA) and a polygraph chart recorder (Grass Instruments, model 7) for baseline measurement of AP and HR. On the basis of the animal’s body weight from the previous day, solutions of ANG II (Sigma Chemicals, St. Louis, MO) were prepared for one of three doses (10, 100, or 250 ng/kg) in isotonic saline and drawn into a syringe. The syringe was connected to the venous line, and the previously determined dead space of the catheter was filled with the ANG II solution. The syringe was placed in a constant infusion pump (Harvard Apparatus, Holliston, MA) that was set to infuse at a rate of 25 μl/min for 1 h. Blood samples (1.2 ml) were taken 15 and 45 min after the start of the infusion. Except during blood sampling, AP and HR were measured continuously for the 1-h infusion period.

All blood samples were immediately centrifuged (10,000 g, 2 min), and the plasma was removed for later analysis of VP concentration and osmolality. VP was extracted from the plasma with the use of microcolumns of Amberlite CG-50 resin and analyzed by radioimmunoassay as described previously (40). Plasma osmolality was measured by osmometry (μOsmette, Precision Instruments, Natick, MA).

To determine whether the withdrawal of blood affected the ANG II-induced changes in AP and HR, the next day a second infusion of ANG II was given to each rat, but blood samples were not taken. Each animal received one of three doses of ANG II but not the same dose they had received previously. Measurement of AP and HR during the 1-h infusion of ANG II confirmed that blood sampling had not altered these responses.

ANG II-induced water intake and urine Na+ excretion. Each rat was removed from its tethering jacket, and the arterial line was plugged as it exited between the scapulae. The venous line was attached to a syringe filled with ANG II but not the same dose they had received previously. To determine whether the withdrawal of blood affected the ANG II-induced changes in AP and HR, the next day a second infusion of ANG II was given to each rat, but blood samples were not taken. Each animal received one of three doses of ANG II but not the same dose they had received previously. Measurement of AP and HR during the 1-h infusion of ANG II confirmed that blood sampling had not altered these responses.

Hypertonic saline-induced VP release. A baseline blood sample (2 ml) was drawn from the arterial line, and the volume was not replaced. A syringe containing 1 M NaCl was connected to the venous line, the previously determined dead space was filled with the hypertonic saline solution, and the syringe was placed in a constant infusion pump. Blood samples (2 ml) were taken 2, 4, 6, and 8 h after the start of the infusion (1 ml/h). Through the arterial line, AP and HR were continuously measured, except during the periods of blood withdrawal.

All blood samples were immediately centrifuged (10,000 g, 2 min), and the plasma was removed for analysis of VP concentration, osmolality, and Na+ concentration.
Hypertonic saline-induced water intake and urine Na⁺ excretion. On the day before the experiment, a baseline blood sample (1 ml) was drawn for analysis of plasma Na⁺ concentration. The blood cells were resuspended in isotonic saline and returned to the animal through the venous line. The rat was removed from its jacket to plug its catheters and then returned to its home cage. The next day rats were placed in the special cages described previously. The venous line was connected to a syringe filled with 1 M NaCl, the previously calculated dead space of the line was filled with the saline solution, and a 25-ml burette containing water was placed on the front of the cage. The rats were allowed to acclimate for 1 h before infusion of hypertonic saline began. Hypertonic saline was infused at 1 ml/h, beginning at 0900. At every hour for 8 h, water intake was measured and the urine vial was replaced with an empty vial. The urine volume was recorded, and the Na⁺ concentration was measured. After 8 h, the infusion was terminated and a blood sample was taken from the arterial line (1 ml) for analysis of plasma Na⁺ concentration.

Statistical analysis. Data are expressed as means ± SE. The effects of chronic NTS-X, infusion of ANG II, and infusion of hypertonic saline were determined with the use of the appropriate ANOVA (Statview 512, Abacus Systems) in each case. The VP values were log transformed before analysis. When a significant P value was obtained with the ANOVA, differences at each time or dose were analyzed by post hoc Tukey's honestly significant difference tests.

RESULTS

Angiotensin-induced changes in VP, AP, and HR. Baseline plasma VP levels in control rats and rats with NTS-X were similar (Fig. 1), as previously noted (33). The infusion of ANG II caused a dose-dependent increase in plasma VP levels in control rats and rats with NTS-X, with no significant differences between groups (Fig. 1). The smallest dose of ANG II tested, 10 ng·kg⁻¹·min⁻¹, was below threshold for eliciting an increase in plasma VP levels in either control rats or rats with NTS-X (Fig. 1).

Plasma osmolality did not differ significantly between control rats and rats with NTS-X (Table 1), either before or after the infusion of any of the doses of ANG II.

Baseline mean AP and HR in control rats and rats with NTS-X were comparable (Fig. 2), although lability of AP was markedly exaggerated in rats with NTS-X (not shown), as previously observed (33, 41). In control rats, intravenous infusion of 100 or 250 ng·kg⁻¹·min⁻¹ of ANG II produced rapid and sustained increases in mean AP (Fig. 2A), whereas the 10-ng·kg⁻¹·min⁻¹ dose of ANG II did not significantly alter mean AP (Fig. 2A). In the control rats, the ANG II-induced increases in mean AP were accompanied by significant decreases in HR (Fig. 2B), presumably mediated by baroreceptor reflexes. In rats with NTS-X, the pressor responses evoked by the two higher doses of ANG II, 100 and 250 ng·kg⁻¹·min⁻¹, were significantly greater than those seen in control rats (Fig. 2A), and the pressor responses were accompanied by increases in HR (Fig. 2B). As seen in the control rats, infusion of 10 ng·kg⁻¹·min⁻¹ of ANG II did not alter HR in rats with NTS-X (Fig. 2B).

ANG II-induced water intake and urine Na⁺ excretion. The 10-ng·kg⁻¹·min⁻¹ dose of ANG II failed to produce a significant water intake in either control rats or rats with NTS-X (Fig. 3A). At this dose, three of six control rats and four of nine rats with NTS-X drank <1 ml during the 1-h test period. In contrast, both of the higher doses of ANG II elicited drinking in all rats. In both groups, 250 ng·kg⁻¹·min⁻¹ of ANG II elicited

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight, g</th>
<th>Plasma Osmolality, mosmol/kgH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline 15 min 45 min</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>335 ± 9</td>
<td>285 ± 1 286 ± 2 285 ± 1</td>
</tr>
<tr>
<td>NTS-X</td>
<td>15</td>
<td>378 ± 5</td>
<td>283 ± 1 285 ± 1 284 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE from control rats and rats with chronic nucleus of the solitary tract lesions (NTS-X). Plasma vasopressin, mean arterial pressures, and heart rates of these rats are depicted in Figs. 1 and 2. Plasma osmolalities from the 3 doses of ANG II are combined within each group (5 rats/dose). Plasma osmolality was not different between groups and did not change during the infusion of ANG II.
more drinking than 100 ng kg⁻¹ min⁻¹ (Fig. 3A). At each dose, the rats with NTS-X drank significantly more than control rats (Fig. 3A). In addition, the rats with NTS-X began drinking significantly earlier than control rats during the infusion of the two higher doses of ANG II (Fig. 3B).

The urine volumes excreted by control rats and rats with NTS-X were comparable (Table 2), leaving the rats with NTS-X in greater positive water balance at the end of the 1-h test period with the two higher doses of ANG II (Table 2). Total urinary excretion of Na⁺ in response to infusion of ANG II did not differ significantly between groups (Table 2).

Hypertonic saline-induced VP release. Baseline plasma VP levels in control rats and rats with NTS-X again were comparable (Fig. 4). In both groups, infusion of 1 M NaCl (1 ml/h) produced a progressive increase in plasma VP levels throughout the 8-h test period. VP levels were significantly elevated above baseline within 2–4 h in both groups. Moreover, the rate at which plasma VP levels increased throughout the infusion period did not appear to differ between the two groups, nor did the VP levels at 8 h differ between these groups (Fig. 4).

As shown in Table 3, baseline plasma osmolalities and Na⁺ concentrations did not differ between control rats and rats with NTS-X. The hypertonic saline infusion produced a comparable osmotic stimulus in both groups. Beginning with the 2-h measurement, plasma osmolality was significantly increased above baseline levels in both groups. At 8 h, the infusion had produced a 6 ± 1% and a 7 ± 1% increase in plasma osmolality in control rats and rats with NTS-X, respectively. Similarly, the infusion produced a 5 ± 1% increase in

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**Fig. 2.** Effect of intravenous infusion of ANG II on mean AP (A) and HR (bpm, beats/min, B) in control rats (n = 5/dose of ANG II) and rats with NTS-X (n = 5/dose of ANG II). Infusion of ANG II began at time 0. The effect of ANG II on plasma VP levels from these rats are shown in Fig. 1, and plasma osmolalities are in Table 1. *Significant difference from baseline values within group, P < 0.05. †Significant difference from control rats at the analogous time point and ANG II dose.

**Fig. 3.** Effect of ANG II infusion on water intake in control rats and rats with NTS-X. A: water intakes after 1 h of ANG II infusion. B: latency to first drinking bout after start of ANG II infusion. The average value excludes rats that did not drink during the test period. Group sizes, urine volumes, and urine Na⁺ values are in Table 2. *Significant difference from lower dose of ANG II within group, P < 0.05. †Significant difference from control rats at the same dose of ANG II.
plasma Na\(^+\) concentrations in both groups. The infusion of 1 M NaCl and blood sampling did not produce any significant changes in AP or HR in either group. **Hypertonic saline-induced water intake and urine Na\(^+\) excretion.** Infusion of 1 M NaCl produced a steady rate of water intake for 8 h in both control rats and rats with NTS-X (Fig. 5A). The volumes of water consumed did not differ between the two groups at any point during the 8-h period (Fig. 5A). Urine excretion between the two groups was also comparable (Fig. 5B). At the end of the 8-h infusion period, both groups had a positive “water balance” (i.e., water ingested + 8 ml infused − urine excreted), which was not different between the groups (1.2 ± 2.3 ml in control rats and 1.2 ± 2.7 ml in rats with NTS-X). At the end of the 8-h period, Na\(^+\) excreted in urine was also comparable between the groups (6.7 ± 0.3 meq in control rats and 6.0 ± 0.4 meq in rats with NTS-X, representing 84 and 75% of the infused Na\(^+\) load, respectively).  

**Histological assessment of NTS-X.** Postmortem histological analysis of the lesion sites revealed that lesions were centered on the medial subnucleus of the NTS at the rostrocaudal level of the area postrema. All lesions involved some portion of the subadjacent dorsal motor nucleus of the vagus, although the commissural NTS, lateral NTS, and rostral portions of the NTS were typically spared. The histological appearance of the lesions was similar to those that we have reported previously (33).

### DISCUSSION

The present study was designed to determine whether elimination of cardiac and arterial baroreceptor inputs would potentiate drinking and VP release caused by intravenous infusion of ANG II in rats. The major findings of this study are that chronic NTS lesions do enhance ANG II-induced water intake, whereas chronic NTS lesions do not affect ANG II-induced release of VP. These results suggest that when stimulated by ANG II, release of VP and water intake are controlled by different central mechanisms. **NTS lesions as a model for baroreceptor denervation.** The present experiments used chronic NTS-X to eliminate cardiac and arterial baroreceptor inputs to the brain. As we have shown previously, rats with NTS-X have a normal mean AP with an increased lability of AP compared with baroreceptor-intact rats (33, 41), which is similar to what is seen after sinoaortic denervation (41). Elimination of arterial baroreceptor reflexes in the rats with NTS-X that were used in the present studies was demonstrated by a complete absence of change in HR to pharmacologically evoked increases and decreases in AP. The rats with NTS-X also lacked inputs from cardiac chemosensitive receptors, as demonstrated by an absence of cardiovascular responses to an intravenous injection of phenyl biguanide. Furthermore, we have previously shown that rats with NTS-X lack input from cardiac mechanosensitive receptors (32). Lesions of NTS would also be expected to interrupt other nonbaroreceptor vagal and glossopharyngeal afferent inputs from the viscera. **ANG II-induced water intake is potentiated in rats with NTS-X.** Intravenous infusion of ANG II elicited more water intake in rats with NTS-X than in weight-matched control rats. Similarly, previous studies have shown that chronic cardiac and arterial baroreceptor-denervated dogs drink more than control animals in

### Table 2. Urine volume and Na\(^+\) excretion during infusion of ANG II

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight, g</th>
<th>Urine Volume, ml</th>
<th>Water Balance, ml</th>
<th>Urine Na(^+), μeq</th>
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<tbody>
<tr>
<td>10 ng · kg(^{-1}) · min(^{-1})</td>
<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>336 ± 10</td>
<td>3.5 ± 0.8</td>
<td>−0.6 ± 0.8</td>
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<tr>
<td>NTS-X</td>
<td>9</td>
<td>334 ± 14</td>
<td>3.1 ± 0.9</td>
<td>0.4 ± 0.7</td>
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</tr>
<tr>
<td>100 ng · kg(^{-1}) · min(^{-1})</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>405 ± 16</td>
<td>4.8 ± 1.1</td>
<td>0.2 ± 1.2</td>
<td>436 ± 161</td>
</tr>
<tr>
<td>NTS-X</td>
<td>6</td>
<td>392 ± 17</td>
<td>3.5 ± 1.0</td>
<td>8.3 ± 1.7(†)</td>
<td>362 ± 109</td>
</tr>
<tr>
<td>250 ng · kg(^{-1}) · min(^{-1})</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>386 ± 10</td>
<td>6.3 ± 1.0</td>
<td>4.6 ± 2.1</td>
<td>862 ± 144</td>
</tr>
<tr>
<td>NTS-X</td>
<td>8</td>
<td>378 ± 5</td>
<td>4.1 ± 2.0</td>
<td>11.1 ± 1.1(†)</td>
<td>521 ± 168</td>
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Values are means ± SE. Water intakes of these rats are depicted in Fig. 3. The infusion of ANG II produced a urine volume and urine Na\(^+\) excretion that did not differ between control rats and rats with NTS-X. The 1-h infusion of 100 or 250 ng · kg\(^{-1}\) · min\(^{-1}\) ANG II produced a greater positive water balance in rats with NTS-X when compared with control rats at the same doses of ANG II (*P < 0.05) and to rats with NTS-X infused with 10 ng · kg\(^{-1}\) · min\(^{-1}\) ANG II (†P < 0.05).

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Fig. 4. Effect of infusion of 1 M NaCl on plasma VP levels in control rats (n = 6) and rats with NTS-X (n = 6). Plasma osmolalities and plasma Na\(^+\) from these rats are in Table 3. There were no significant differences between groups. *Significant difference from mean baseline value of control rats at this and all subsequent time points, P < 0.05. †Significant difference from mean baseline value of control rats at this and all subsequent time points, P < 0.05.
response to an intravenous infusion of ANG II (20). One interpretation of these results is that the increased AP associated with the ANG II infusion normally blunts water intake through inhibitory arterial baroreceptor inputs, and NTS-X potentiate drinking by eliminating this inhibitory effect. This interpretation is consistent with studies showing that ANG II-induced drinking is enhanced when the pressor effect of ANG II is counteracted pharmacologically in baroreceptor-intact rats (10, 31). However, two studies have reported that selective removal of arterial baroreceptor inputs in rats does not potentiate ANG II-induced water intake (18, 30). Importantly, neither study assessed baroreceptor reflex responses to decreased AP, and one study (30) reported that the denervated animals actually had residual HR responses to increased AP. Thus it seems possible that the rats in those studies were not totally denervated, and consequently the animals could still detect the inhibitory pressor signal produced by an intravenous infusion of ANG II. In this regard, we have previously argued that baroreceptor reflex testing is essential for determining whether sinoaortic denervation surgery effectively removes arterial baroreceptor inputs (35). For example, after such surgery, rats with small residual reflex HR responses to phenylephrine-evoked increases in AP have cardiovascular responses to acute inhibition of the NTS that are the same magnitude as in control rats, whereas these responses are absent in totally denervated rats (34).

Alternatively, the enhanced ANG II-induced drinking in rats with NTS-X may result from removal of cardiac inputs, either alone or in conjunction with the removal of arterial baroreceptor inputs. Similarly, the enhanced drinking to ANG II infusion in chronic cardiac and arterial baroreceptor-denervated dogs (20) may also result from removal of inhibitory cardiac signals. In the present study, infusion of ANG II likely stimulated cardiac receptors by two separate mechanisms. First, large increases in AP significantly elevate cardiac pressure (6) and can stimulate mechanosensitive cardiac afferents to elicit a reflexive inhibition of sympathetic nerve activity (5). Second, the infusion of subpressor doses of ANG II activates phenyl biguanide-sensitive cardiac afferents (1). Although the effect of stimulating chemosensitive cardiac afferents on water intake has not been reported, stimulation of cardiac mechanosensitive afferents does inhibit water intake produced by a subcutaneous injection of isoproterenol (19), a response that is largely dependent on circulating ANG II (29). Whether selective removal of cardiac inputs enhances ANG II-induced water intake in rats or dogs remains to be determined.

Table 3. Plasma osmolality, plasma Na\(^+\), mean arterial pressure, and heart rate during infusion of 1 M NaCl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
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<tr>
<td>Plasma osmolality, mosmol/kgH(_2)O</td>
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<tr>
<td>Control</td>
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<td>295 ± 1</td>
<td>301 ± 2</td>
<td>303 ± 2</td>
<td>304 ± 2</td>
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<tr>
<td>NTS-X</td>
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<td>297 ± 2</td>
<td>302 ± 3</td>
<td>304 ± 1</td>
<td>305 ± 2</td>
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<tr>
<td>Plasma Na(^+), meq/l</td>
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<tr>
<td>Control</td>
<td>137.8 ± 0.8</td>
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<tr>
<td>NTS-X</td>
<td>138.6 ± 0.9</td>
<td>140.1 ± 0.7</td>
<td>142.2 ± 1.3</td>
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<td>Mean arterial pressure, mmHg</td>
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<tr>
<td>Control</td>
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<td>112 ± 5</td>
<td>107 ± 4</td>
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<td>NTS-X</td>
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<td>Heart rate, beats/min</td>
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<tr>
<td>Control</td>
<td>398 ± 27</td>
<td>407 ± 21</td>
<td>437 ± 26</td>
<td>435 ± 17</td>
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<tr>
<td>NTS-X</td>
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<td>384 ± 18</td>
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Values are means ± SE, \(n = 6\) in each group. Infusion of 1 M NaCl increased plasma Na\(^+\) and osmolality comparably in control rats and chronic NTS-X rats. The infusion did not alter mean arterial pressure or heart rate in control rats or rats with chronic NTS-X. Drinking water was not available during the infusion of hypertonic saline.

Fig. 5. Effect of infusion of 1 M NaCl on water intake (A) and urine excretion (B) in control rats (\(n = 7\)) and rats with NTS-X (\(n = 7\)). There were no significant differences between groups.
Increased water intake in response to intravenous ANG II is also seen in rats with lesions of the lateral parabrachial nucleus, which do not alter osmotically induced water intake (7, 25). These lesions also eliminate the inhibition of isoproterenol-induced water intake produced by stimulation of cardiac mechanoreceptors (27). However, it is unclear whether the removal of inhibitory cardiac signals is responsible for enhancing ANG II-induced water intake in rats with lesions of the lateral parabrachial nucleus, or whether NTS-X and lateral parabrachial lesions enhance ANG II-induced water intake by the destruction of a common central pathway.

Lesions of area postrema also enhance ANG II-induced water intake (8, 26). However, in contrast to the other models, rats with lesions of area postrema also increase intakes in response to a variety of other signals when they are intense enough to produce large intakes quickly (4), suggesting a loss of less specific postingestive inhibitory signals such as gastric distension (38). Although our NTS-X probably disrupted gastric distension signals (14), the putative loss of those signals is not likely to fully account for the enhanced ANG II-induced water intake seen in rats with NTS-X because the animals initiated drinking earlier than control rats. These latter findings suggest that the NTS-X allowed ANG II to have a bigger effect by producing a more powerful dipsogenic stimulus as opposed to eliminating an inhibitory signal produced by water consumption. In addition, the lack of overdinking in response to an infusion of hypertonic saline suggests that the enhanced drinking in response to ANG II in rats with NTS-X is selective for this stimulus.

ANG II-induced VP release is not potentiated in rats with NTS-X. In contrast to the effects of NTS-X on ANG II-evoked drinking, NTS-X did not enhance ANG II-evoked increases in plasma VP levels. These data suggest that ANG II-induced release of VP from the posterior pituitary in rats with NTS-X is comparable to that of control rats, assuming that the lesion does not significantly alter the clearance of the peptide from the blood. Therefore, the hypothesis that increased AP normally attenuates ANG II-induced VP release by a mechanism involving cardiac and/or arterial baroreceptors is not supported by the present study. Similarly, previous studies had shown that ANG II-induced VP release is not enhanced in chronic cardiac and arterial baroreceptor-denervated dogs (3).

The most straightforward interpretation of these data is that the increased AP produced by an intravenous infusion of ANG II does not inhibit the release of VP. However, it has been well established that acute increases in AP inhibit the firing of VP neurons in anesthetized rats (e.g., Refs. 15, 28). Indeed, doses of ANG II that markedly increase AP above normal resting levels inhibit the firing of putative VP neurons (24). Conversely, high doses of ANG II increase the firing rate of VP neurons when baseline AP is low or when arterial baroreceptor inputs are removed by sinoaortic denervation (11, 23). The apparent dichotomy between these studies and the present findings may be explained by several factors. For example, the duration of the hypertensive stimuli may be important; rapid increases in AP inhibit the firing rate of VP neurons, but this effect appears to be transient (28) and therefore not relevant to increases in AP lasting more than a few minutes. Alternatively, changes in firing rates of VP neurons may not always reflect changes in circulating VP levels. Furthermore, the firing properties of magnocellular VP neurons in conscious rats may be markedly different from those in anesthetized rats prepared for recording from magnocellular neurons. The effects of increases in AP on firing rates of VP neurons have been studied only in anesthetized preparations, which typically have very high circulating levels of VP. Indeed, the bursting pattern that discriminates VP neurons from oxytocin neurons in electrophysiological studies reflects a very stimulated state of VP release in contrast to the slow, irregular firing rate that is characteristic of a resting state (16). Thus, although the inhibition of VP neuronal firing by increased AP has been an effective tool for identifying these cells, the physiological significance of this phenomenon is not known and may not predict the response to sustained hypertension in conscious animals.

Finally, it is possible that increased AP blunts VP release under physiological conditions, but this effect is not apparent in these experiments because it is not mediated via arterial baroreceptors or because animals with chronic baroreceptor denervation adapt to the loss of baroreceptor signals. Both of these explanations are consistent with the observations that in dogs ANG II-induced VP release is not enhanced by arterial plus cardiac baroreceptor denervation (3), whereas it is enhanced by prevention of ANG II hypertension with the vasodilators nitroprusside or hydralazine (2). On the basis of the present data, any adaptation would not involve a general decrease in sensitivity to ANG II because ANG II-induced drinking is enhanced in chronically denervated animals. In addition, VP neurons in chronically denervated animals are not less sensitive to stimulation because VP release to osmotic challenges is normal.

Cardiovascular responses to ANG II are altered in rats with NTS-X. In the present study, intravenous infusion of ANG II increased AP in a dose-related manner, a response produced primarily by direct constriction of arterioles. In rats with NTS-X, infusion of ANG II produced a larger increase in AP, which likely results from a loss of baroreflex-mediated inhibition of sympathetic vasomotor tone and a larger cardiac output (13). Moreover, in these animals ANG II did not produce a decrease in HR in association with the increase in AP; instead, an increase in HR was observed. Infusion of ANG II has been shown previously to increase HR in arterial baroreceptor-denervated dogs (13, 17), and this response likely results from a direct positive chronotropic effect of ANG II mediated by AT_1 receptors (22) and an increase in sympathetic outflow (13, 22, 42).
The release of VP and water intake are known to be stimulated by decreased blood volume, hypotension, and increased plasma osmolality (39). These responses, which aid in body fluid homeostasis, are triggered by a combination of neural and circulating signals and are often thought to be regulated parallel. Neural signals from cardiac and arterial baroreceptors are coordinated with circulating signals such as ANG II to stimulate the release of VP and ingestion of water. Circulating ANG II acts at the subfornical organ to stimulate central pathways regulating VP release and drinking (36, 37). It has been argued that the ability of ANG II to stimulate these responses is underestimated when exogenous ANG II is given systemically, because in a normotensive, water-replete animal ANG II also increases AP. The notion that increased AP, acting through baroreceptor inputs, counteracts the stimulatory effects of ANG II has become an accepted theory despite some seemingly conflicting results. In the case of water intake, the present study supports the idea that an inhibitory signal, possibly increased AP, produced by systemic ANG II blunts the ingestion of water and that this response is mediated by some input through the NTS. This increased sensitivity to ANG II may also contribute to the increased water intake in response to hypovolemia seen in rats with NTS-X (32), which lack neural volume-related signals thought to be involved in hypovolemia-stimulated thirst. In contrast, the present study does not support the idea that sustained hypertension limits the release of VP produced by ANG II and therefore resembles results obtained in studies of chronic cardiac plus arterial baroreceptor-denervated dogs (3, 20). These findings allow the conclusion that although VP release and water intake are stimulated by the same signals under several of the same conditions, the central mechanisms regulating these responses are distinct.

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