Thirst and salt appetite elicited by hypovolemia in rats with chronic lesions of the nucleus of the solitary tract.

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The present study sought to determine the effects of reduced blood volume on water intake in rats with chronic lesions of the nucleus of the solitary tract (NTS), the brain stem site that receives afferent inputs from cardiac baroreceptors (14). Because hypovolemia normally stimulates salt appetite as well as thirst in rats (32), the present study also examined the NaCl intake of rats with chronic NTS lesions. If cardiac receptors are essential for stimulating water and NaCl intakes, then NTS lesions should impair both responses. However, the results indicated that in rats with chronic NTS lesions, PEG treatment continued to stimulate marked consumption of water and NaCl solution. These observations parallel recent findings that neurohypophysial vasopressin (VP) secretion induced by hypovolemia also remains intact in rats with chronic NTS lesions (20).

METHODS

Animals. Adult male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA), weighing ~300 g, were individually housed in a temperature-controlled room (22–23°C). Lights were on between 8:00 AM and 8:00 PM. Tap water and Purina Chow pellets were available ad libitum except where noted.

Electrolytic NTS lesions. NTS lesions were produced as described previously (20). Briefly, rats were anesthetized with halothane (2% in 100% O2 administered through a cone placed over the nose) and were injected with a ganglionic blocker (chlorisondamine, 2 mg/kg sc) and a VP1 receptor antagonist (1-[β-mercapto-β,β-cyclopentamethylene-propionyli],2-[O-methyl]-tyrosine)Arg8-VP; 10 µg/kg sc) to combat the acute hypertension that results from increased sympathicotrophic activity and VP secretion, respectively, after surgical lesions that destroy the baroceptive portion of the NTS. Rats not pretreated in this way develop fulminating hypertension after NTS lesions and die within several hours (4, 33). 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 brain stem was exposed via a limited craniotomy, and the area postrema was visualized with the aid of a surgical microscope. A Teflon-insulated tungsten electrode (200 µm OD with 375 µm of the tip exposed; A-M Systems, Everett, WA) was placed into the NTS (0.5 mm rostral to the caudal tip of the area postrema, 0.6 mm lateral to the midline, and 0.6 mm below the dorsal surface of the brain stem). Lesions were produced by passing anodal current (1 mA for 15 s) from a DC constant current source (Grass Instruments, Quincy, MA). After NTS lesions were made bilaterally, the wound was closed, halothane administration was terminated, and rats were given an antibiotic (Combicil; 0.1 ml im). Because water intake was initially reduced after surgery, rats were injected with isotonic saline (10 ml/day sc) until spontaneous drinking resumed 1–3 days later. Experiments began 7–14 days after surgery, when daily food and water intake had returned to normal levels.

METHODS

THIRST IS STIMULATED reliably in rats by extravascular administration of colloidal solution, which gradually leeches plasma isosmotically from the circulation into the injection site (5, 26). The water intake elicited by subcutaneous injection of polyethylene glycol (PEG) solution in rats has been found to be proportional to the induced plasma volume deficit (27) and to the associated activation of the renin-angiotensin system (13, 15). Although angiotensin II (ANG II) is a known dipsogen in rats (6, 13), it is not a necessary stimulus of thirst after PEG treatment in rats because water consumption is not altered when renin secretion is eliminated by bilateral nephrectomy (5, 29). Moreover, destruction of the subfornical organ, the site of ANG II receptors that mediate thirst elicited by circulating ANG II (24), raises the threshold of drinking after PEG treatment but does not otherwise affect water ingestion (11). Thus the predominant stimulus of thirst during hypovolemia has been attributed to a neural signal from cardiac volume-sensitive receptors (30).

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water intakes were normal and body weights had reached or surpassed preoperative levels.

Physiological assessment of NTS lesions. To determine whether the NTS lesions were effective, a series of reflex responses known to be processed through the NTS were measured. On the day that reflexes were examined, rats were anesthetized with halothane and a catheter was placed into the right femoral artery for recording arterial pressure (AP) and heart rate (HR) and for withdrawing blood samples. Another catheter was placed into the right femoral vein for administering drugs. The free ends of the two catheters were passed subcutaneously to exit the back between the scapulae. A teth and swivel system (Harvard Apparatus, Holliston, MA) allowed intravenous drug administration, blood sampling, and continuous recording of AP and HR (except during blood sampling) in conscious unrestrained animals.

Arterial baroreceptor reflexes were examined by measuring changes in HR evoked either by increasing AP with phenylephrine (2 µg/kg iv) or by decreasing AP with sodium nitroprusside (5 µg/kg iv). As expected (21), phenylephrine elicited a decrease in HR and nitroprusside elicited an increase in HR in control rats. These arterial baroreceptor reflex responses were eliminated in ~60% of the rats prepared. Rats were included in the present studies only when NTS lesions eliminated these reflexes. Rats showing any residual HR response to either drug were dropped from further investigation.

Phenyl biguanide (50 µg/kg iv) was given to determine the effects of NTS lesions on the changes in AP and HR evoked by the direct stimulation of cardiac 5-HT 3 receptors with vagal afferents that project to the NTS. In control animals, phenyl biguanide produced marked hypotension and bradycardia (22), whereas those responses were absent in all rats with chronic NTS lesions that were used in these experiments. Although this test has the advantage of allowing cardiac reflex tests in conscious animals, phenyl biguanide stimulates cardiac afferents that are distinct from volume-sensitive afferents (9). To determine whether NTS lesions also effectively remove neural inputs from those cardiac receptors, and therefore whether the loss of AP and HR responses to phenyl biguanide is a legitimate test for predicting the elimination of volume-related baroreflexes mediated by those receptors, reflex renal sympathoinhibition in response to atrial stretch was determined in a separate subset of rats with NTS lesions and in control rats (n = 4 and 5).

Rats used to examine the effect of atrial stretch on renal sympathetic nerve activity (RSNA) were anesthetized with pentobarbital sodium (30 mg/kg ip, followed by 15 mg·kg⁻¹·h⁻¹ iv). Via a retroperitoneal approach, a branch of the left renal nerve was isolated and placed on a bipolar electrode constructed from Teflon-coated stainless steel wire (37). The electrode was anchored in place with a polynylsiloxane dental impression material (President Light Body, Coltene, Hudson, MA). RSNA was amplified (~10,000; CWE model BMA-831, Ardmore, PA) and filtered (20–20,000 Hz), and the signal was digitized and collected on a computer at a sampling rate of 10,000 Hz (Labview National Instruments). The digitized nerve signal was integrated over 1-s epochs. Background noise determined at the end of the experiment, after death caused by an overdose of pentobarbital sodium, was subtracted from integrated nerve activity values.

RSNA responses to a bolus injection of 500 µl of 0.15 M NaCl into the right atrium were measured using a protocol described previously (10). The cervical vagus nerves were then cut bilaterally and responses to the saline load were tested again. As shown in Fig. 1, RSNA in control rats was reduced to 30 ± 3% of baseline values (P < 0.01) during the 1-s period after the atrial saline load, and this effect was largely eliminated by bilateral vagotomy. In contrast, RSNA in the four rats with chronic NTS lesions was 83 ± 9% of baseline values after the atrial saline load (P > 0.05) and was not significantly affected by vagotomy; none of these rats had shown either AP or HR responses to phenyl biguanide. We also examined one rat whose NTS lesion appeared to be incomplete in that a small bradycardia was elicited by phenyl biguanide; this rat showed a marked suppression of RSNA in response to atrial injection of saline, similar to control rats. Collectively, these findings suggest that phenyl biguanide is an effective screen for the removal of inputs from volume-sensitive cardiac receptors in rats with NTS lesions.

Drinking studies. Baseline water balance was examined in rats 1–2 wk after NTS lesions had been produced and in weight-matched control animals (n = 8 and 7, respectively). Rats were placed in their home cages, below which a funnel was added to permit the collection of voided urine, and a calibrated tube containing water was placed on each cage. Food was withheld at 9 AM, and measurements were made of water intakes and urinary volumes (~0.5 ml) and Na⁺ concentrations (by sodium-selective electrode; Beckman Electrolyte Analyzer II) at 5 PM and then again at 9 AM the next morning.

Drinking in response to hypovolemia produced by subcutaneous injection of PEG was examined in four ways. In each experiment, rats with chronic NTS lesions and weight-matched control animals were briefly anesthetized with halothane and injected subcutaneously with 5 ml of 30% PEG solution (wt/wt; Carbowax, compound 20-M, Union Carbide). Then they were placed in their home cages in which food was not accessible. Rats regained consciousness within 5–10 min. A total of 20 rats with NTS lesions and 24 weight-matched control animals were used in these four studies of fluid intake in response to colloidal-induced plasma volume deficits. The first two experiments examined the effects of chronic NTS lesions on thirst during hypovolemia. In one experiment, rats
with NTS lesions and weight-matched control animals (n = 10 and 15, respectively) were given access to water immediately after PEG treatment, and their intakes were measured hourly for 7 h. Urine volumes and Na⁺ concentrations also were measured hourly. Because renal retention of ingested water produces an osmotic dilution of body fluids that limits water consumption (28), thereby making it more difficult to discern a possible reduction in thirst elicited by PEG treatment in rats with NTS lesions, the experiment was repeated 1 wk later in most of the same animals, but this time rats with chronic NTS lesions and weight-matched control animals were given 0.15 M NaCl to drink instead of water (n = 8 and 8).

The other two experiments examined the effects of chronic NTS lesions on thirst and salt appetite during hypovolemia. In these studies, rats were allowed to drink from two bottles, one containing water and the other 0.5 M NaCl; rats do not normally drink this hypertonic saline solution in large quantities because it is apparently unpalatable, whereas they do so when they have a strong salt appetite. In one experiment, another group of rats with chronic NTS lesions and weight-matched control animals were given PEG treatment (n = 10 and 9, respectively), 1 h after which water and 0.5 M NaCl became available as drinking fluids. Measurements of intake and urinary volume and Na⁺ concentration were made hourly for 7 h subsequently; additional measurements were made 12, 16, and 24 h after the PEG treatment. In the other experiment, conducted 1 wk later using some of the same animals (n = 5 and 5), access to the two drinking fluids was delayed for 24 h after PEG treatment, which allowed substantial hypovolemia to develop before the drinking test began; intakes of water and 0.5 M NaCl by rats with chronic NTS lesions and weight-matched control animals were measured every 15 min for 1 h and then hourly for 4 h. Frequent measurements of fluid intake were made initially because PEG-treated rats are known to drink rapidly under these testing conditions (31).

Blood volume analysis. In other rats with chronic NTS lesions and control animals (n = 6 and 6), blood volume was determined by dye dilution (36). Rats were anesthetized with halothane for implantation of catheters (dead space 0.5 ml) in the femoral artery and femoral vein. Evans blue dye (final concentration of 0.5% solution, chosen to fall in the midrange of the standard curve based on expected blood volumes) was injected into the femoral venous catheter and flushed with 100 µl of isotonic saline. Ten minutes later, a 1-ml blood sample was obtained through the femoral arterial catheter, after which rats were instrumented as described above for testing reflexes in the conscious state.

A small portion of the blood sample was used to measure hematocrit, and the remainder was centrifuged (10,000 g × 2 min). A 200-µl aliquot of plasma was diluted in 1.8 ml of saline, and absorbance of light at 605 nm was measured by spectrophotometry (Beckman, model DU 640). Values were compared with a standard curve generated by plotting 0–100 mg of dye into 1-ml aliquots of pooled rat blood and measuring the absorbance of light in plasma. Because basal hematocrits were equivalent among donors and the two groups of rats, values obtained from their plasma samples provided estimates of relative blood volume.

PEG-induced renin secretion. In two additional groups of rats with chronic NTS lesions and control animals (n = 6 and 6), a 0.5-ml blood sample was withdrawn for measurement of plasma renin activity (PRA; by radioimmunoassay) and protein concentration (P₉₀; by refractometry) on the morning after baroreceptor reflexes were tested. Samples were centrifuged (10,000 g × 2 min), plasma was removed, and red blood cells were resuspended in an equal volume of isotonic saline and returned to the animals. Then rats were anesthetized with halothane, injected subcutaneously with 5 ml of 30% PEG, and returned to their cages, where food and drinking water were not available. Seven hours later, a 0.5-ml blood sample was withdrawn from these conscious rats through the femoral artery for a second measurement of PRA and P₉₀.

Aliquots of plasma (0.1 ml) were stored at −70°C until assayed for PRA. Samples were adjusted to a pH of 6.5 by adding 0.1 ml of 20 mM maleate buffer and incubated at 37°C for 1 h in the presence of 1 mM phenylmethylsulfonyl fluoride to generate angiotensin I (ANG I). Aliquots of the incubate were analyzed by radioimmunoassay for ANG I content (33), and values were expressed as nanograms of ANG I generated per hour per milliliter of plasma.

Histological analysis. As mentioned, the effectiveness of the NTS lesions was judged by determining whether baroreceptor reflexes were completely eliminated by the lesions; that was the case for all animals used in this study. Nevertheless, after completion of testing, histological analysis of the brains was performed in many of the rats to confirm that the brain damage included destruction of the medial NTS. Rats with NTS lesions were anesthetized with an overdose of Equithesin and perfused intracardially with 0.15 M NaCl followed by 10% Formalin solution. Brain stems were removed and cut in 33-µm sections along the rostral-caudal axis. Sections were mounted and stained for Nissl substance with cresyl violet or neutral red, and brain stem lesion sites were examined by light microscopy.

Sources of chemicals and drugs. Chemicals and drugs were purchased from commercial sources as follows: nitroprusside, phenylephrine, PEG, and sodium chloride from Sigma Chemical (St. Louis, MO); halothane from Halocarbon Laboratories (River Edge, NJ); phenyl biguaine from Aldrich Chemical (Milwaukee, WI); and 1-[β-(m-mercapto-β-p-cyclopentamethylenepropiony1)]2-(O-methyl)-tyrosine[Arg8-VP (VP, antagonist, Manning compound) from Bachem (Torrance, CA). Chlorsidomine (Ecolid) was generously donated by Ciba-Geigy (Summit, NJ), and the ANG I antibody was generously donated by Dr. J. van Sealey (New York, NY). Other chemicals and reagents were obtained from standard commercial suppliers.

Statistical analysis. Data are expressed as means ± SE. Blood volumes and hematocrits of rats with NTS lesions and control animals were compared by Student’s t-test. PRA and P₉₀ before and after PEG treatment were compared by ANOVA with repeated measures followed by post hoc Fisher’s tests when significant F values were obtained. Baseline fluid intakes and urine volumes were compared by t-tests, and data from the drinking tests were examined by ANOVA with repeated measures followed by post hoc Fisher’s tests when significant F values were obtained.

RESULTS

Baseline water balance and blood volume. In rats with chronic NTS lesions drinking ad libitum, daily water intakes and urine volumes were similar to those observed in control animals. As shown in Table 1, ~90% of the 24-h water intakes of both groups of rats occurred during the last 16 h of the test period, which included the dark phase of the light-dark cycle, and significantly more urine was excreted then as well. Urinary Na⁺ excretion was comparable in the two groups.

The baseline blood volumes of the two groups were comparable (7.0 ± 0.4 ml/100 g body wt in six control animals, 6.5 ± 0.4 ml/100 g body wt in six rats with
chronic NTS lesions; \( P > 0.05 \), as were their hematocrit values (45 ± 2 and 43 ± 1, respectively; \( P > 0.05 \)).

PEG-induced fluid intake. Injection of 30% PEG elicited a steady increase in water intake of control rats (7.6 ± 1.5 ml in 7 h; Fig. 2), as expected (27). Rats with chronic NTS lesions had a similar drinking response (10.1 ± 1.2 ml in 7 h; Fig. 2). Although rats with NTS lesions drank more water during the 7-h test period than control rats did (2.4 ± 0.5 and 1.3 ± 0.2 ml, respectively; \( P < 0.05 \)), cumulative water intake at the end of the 7-h period did not differ significantly between the two groups (7.8 ± 1.1 and 6.3 ± 1.4 ml, respectively; \( P > 0.05 \)).

Fluid intakes were much greater when PEG-treated rats drank 0.15 M NaCl instead of water (both B values <0.001), but they remained comparable in rats with chronic NTS lesions and control animals (31.8 ± 3.8 and 31.8 ± 5.2 ml, respectively, in 7 h; \( P > 0.05 \); Fig. 3). Excreted urine volumes also were comparable in the 7-h test period (7.8 ± 1.1 and 6.3 ± 1.4 ml, respectively; \( P > 0.05 \)).

When given both water and 0.5 M NaCl to drink soon after PEG treatment, during hours 1–8, control rats drank water in volumes comparable to those seen during the first 7 h of the one-bottle test (9.4 ± 1.0 ml), but no saline. However, by 24 h substantial volumes of both fluids had been consumed (52.3 ± 2.0 ml of water, 16.2 ± 1.7 ml of 0.5 M NaCl; Fig. 4), as expected (32). Cumulative 24-h urine volumes (16.3 ± 2.4 ml) and Na⁺ losses (1.3 ± 0.5 meq) were much smaller than intakes (both B values <0.001), indicating a substantial renal retention of ingested fluid.

PEG-treated rats with chronic NTS lesions drank both fluids in amounts that were not significantly different from the 24-h intakes of PEG-treated control animals (59.4 ± 3.3 ml of water, 19.7 ± 1.6 ml of 0.5 M NaCl). Furthermore, the pattern of drinking during the test was similar between rats with chronic NTS lesions and control animals (Fig. 4), as were the relatively low cumulative 24-h urine volumes (24.2 ± 3.7 ml) and Na⁺ losses (1.9 ± 0.4 meq). When a 24-h delay was imposed between the PEG treatment and the drinking test, both control rats and rats with chronic NTS lesions excreted little urinary volume (6.0 ± 1.1 and 6.5 ± 0.7 ml, respectively) or Na⁺ (0.2 ± 0.1 and 0.1 ± 0.1 meq, respectively) during this period. During the subsequent 5-h test, control rats

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### Table 1. Baseline water balance and urinary Na⁺ excretion in rats with chronic lesions of the NTS and in control animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Intake, ml</th>
<th>Urine Volume, ml</th>
<th>Urine Na⁺, meq</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–8 h</td>
<td>2.1 ± 0.6</td>
<td>6.7 ± 0.3</td>
<td>768 ± 41</td>
</tr>
<tr>
<td>Control</td>
<td>2.5 ± 0.8</td>
<td>8.0 ± 0.4</td>
<td>518 ± 58</td>
</tr>
<tr>
<td>NTS lesions</td>
<td>23.6 ± 4.1</td>
<td>24.4 ± 4.8</td>
<td>700 ± 74</td>
</tr>
<tr>
<td>8–24 h</td>
<td>20.7 ± 2.9</td>
<td>20.8 ± 3.4</td>
<td>907 ± 218</td>
</tr>
<tr>
<td>Control</td>
<td>25.7 ± 4.5</td>
<td>31.1 ± 4.8</td>
<td>1,468 ± 99</td>
</tr>
<tr>
<td>NTS lesions</td>
<td>23.2 ± 2.6</td>
<td>28.9 ± 3.2</td>
<td>1,424 ± 255</td>
</tr>
</tbody>
</table>

Values are means ± SE. NTS, nucleus of the solitary tract. Rats were given drinking water but not food during the 24-h test period. Body weights of rats with chronic NTS lesions (381 ± 5 g, n = 8) were comparable to those of control animals (377 ± 7 g, n = 7).

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Fig. 2. Cumulative intakes of water by rats with chronic NTSx (●) and control rats (○). Values are means ± SE; \( n = 10 \) and 15, respectively. The 7-h drinking tests began immediately after subcutaneous injection of 30% polyethylene glycol (PEG) solution.

Fig. 3. Cumulative intakes of 0.15 M NaCl by rats with chronic NTSx (●) and control rats (○). Values are means ± SE; \( n = 8 \) and 8, respectively. The 7-h drinking tests began immediately after subcutaneous injection of 30% PEG solution.

Fig. 4. Cumulative intakes of water (squares) and 0.5 M NaCl (circles) by rats with chronic NTSx (filled symbols) and control rats (open symbols). Values are means ± SE; \( n = 10 \) and 9, respectively. The drinking tests began 1 h after subcutaneous injection of 30% PEG solution and continued for 24 h postinjection.
drank both fluids immediately and in large volumes 
(29.5 ± 2.5 ml of water, 10.3 ± 1.7 ml of 0.5 M NaCl, in 5 h; Fig. 5), as noted previously (31). In contrast, rats 
with chronic NTS lesions drank significantly more fluid in 5 h (46.2 ± 6.7 ml of water, 14.9 ± 2.4 ml of 0.5 M 
NaCl; both P values <0.001; Fig. 5). Both groups 
excreted relatively little urinary volume (7.7 ± 0.9 and 
12.9 ± 3.3 ml, respectively) or Na⁺ (0.6 ± 0.2 and 0.8 ± 
0.1 meq, respectively) during the drinking test, again 
indicating a substantial renal retention of ingested 
fluid.

PEG-induced renin secretion. In control rats, a signifi-
cant increase in P Prep of 2.4 g/dl was observed in blood 
samples taken 7 h after PEG treatment (Table 2), which corresponds to a decrease in plasma volume of 
29% (27). This hypovolemia was associated with a 
10-fold increase in PRA (Table 2), as expected (15).

Table 2. Plasma protein concentrations and renin activity in rats with chronic lesions of the NTS 
and in control animals

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Plasma Protein, g/dl</th>
<th>Renin Activity, ng ANG I·ml⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>5.9±0.1</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>NTS lesions</td>
<td>6</td>
<td>5.6±0.2</td>
<td>1.7±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood samples were obtained 7 h after 
subcutaneous injection of 30% polyethylene glycol (PEG) solution, to 
induce hypovolemia. *Significant differences from values before PEG 
treatment.

Fig. 6. Photomicrograph of a representative lesion of NTS. Arrows 
point to glial sheath marking the edges of the lesion. In this rat, as 
was typical of many of the rats with chronic NTS lesions in this study, 
the damaged area was infiltrated by vascular tissue and therefore 
does not appear as a hole. The box in the schematic drawing of a brain 
stem section (from Ref. 34) shows the location of the photomicro-
graph. The bar in the bottom right corner of the photomicrograph 
represents 100 µm. AP, area postrema; C, central canal.

Additionally, rats with chronic NTS lesions, PEG treatment pro-
duced a comparable degree of hypovolemia (32% reduction in plasma volume) and increase in PRA (Table 2).

Histological analysis. The NTS lesions were found to be 
similar to those we have described previously (20). They were centered on the medial NTS at the rostral-
caudal level of the area postrema, and usually involved 
portions of the adjacent dorsal motor nucleus of the 
vagus and the area postrema but not the hypoglossal 
nucleus. Figure 6 shows a representative photomicro-
graph of the damaged area.

DISCUSSION

Reduced blood volume elicits a constellation of compen-
satory behavioral and physiological responses, in-
cluding ingestion of fluids and secretion of VP from the 
posterior pituitary (7, 29). Mechanosensitive receptors 
in the heart with vagal afferent inputs to the brain 
stem are generally thought to play an important role in 
initiating these adaptive responses to hypovolemia.

The principal finding of the present study was that 
removal of these neural inputs, by destruction of their 
projection sites in the NTS, did not diminish water or 
saline drinking stimulated by PEG-induced hypovole-
mia in rats. This result complements our previous 
report that chronic NTS lesions did not prevent secre-
tion of VP stimulated either by PEG treatment or 
 hemorrhage (20). Collectively, these findings suggest
that volume-sensitive cardiac vagal afferents are not crucial for initiating counterregulatory responses to hypovolemia in rats.

A critical assumption of the present investigation is that the NTS lesions destroyed the brain stem site that receives vagal afferents projecting from cardiac mechanoceptors, as intended. In support of this assumption, NTS lesions were found to abolish the AP and HR responses to phenyl biguanide, which are known to be triggered by stimulation of cardiac vagal afferents projecting to NTS (22), in all rats used in this study. Additionally, in the subset of animals that were tested, RSNA responses to volume stimulation of the right atrium also was eliminated. Furthermore, bilateral cervical vagotomy is known to have no effect on hypovolemia-induced VP secretion in rats with chronic NTS lesions (20), thus ruling out the possibility that remaining vagal afferents projecting to the NTS were responsible for mediating the induced VP secretion and, by implication, the induced drinking.

The abrupt cessation of arterial baroreceptor input produced by electrolytic lesions of the medial subnucleus of the NTS seems to mimic severe hypotension; that is, secretion of VP and activation of the sympathoadrenal system increase so markedly that, unless prevented by appropriate drug treatments, blood pressure rises rapidly to lethal levels (33). However, fulminating hypertension does not develop when the protective drug treatments are discontinued 1 day after surgery, and rats with NTS lesions then eat sufficient food to maintain their body weights while drinking water and excreting urine in normal quantities. The present results further indicate that rats with chronic NTS lesions drink fluids steadily and in normal amounts when given a PEG treatment that gradually reduced plasma volume by 30% or more. Indeed, they did not show impairments in drinking even when rats were tested soon after PEG treatment because rats can no longer detect some consequence of ingested fluid acting in the upper gastrointestinal tract, perhaps gastric distension, that normally inhibits ongoing fluid intake (2). Consistent with this hypothesis are observations that rats with chronic NTS lesions, similar to those used in the present experiments, also do not respond to cholecystokinin-induced stimulation of gastric vagal afferents (8). A similarly enhanced fluid consumption would not be expected when rats were tested soon after PEG treatment because their smaller intakes then should be much less influenced by the putative loss of a rapid feedback mechanism of inhibition (see also Ref. 3).

The present studies also indicate that chronic NTS lesions do not abolish the secretion of renin in response to PEG-induced hypovolemia. The same result was obtained previously when renin secretion was stimulated by hemorrhage in rats with chronic NTS lesions (19). These observations demonstrate that renin secretion during hypovolemia need not be activated by neural input from cardiovascular baroreceptors to the brain stem. Instead, the induced renin secretion may have resulted from a reflexive increase in sympathetic outflow to the kidneys that is initiated by unknown afferents not processed through the NTS (see below). Such an explanation would be consistent with the report by Hubbard et al. (12) that renin release evoked by severe hypotension in rats with NTS lesions could be markedly attenuated either by pretreatment with a β-adrenergic receptor blocker or by prior renal denervation. Hypovolemia-induced renin release in rats with chronic NTS lesions additionally may result from alterations in renal perfusion pressure caused by hypovolemia (1). Further experiments are needed to elucidate the mechanisms that mediate renin secretion during hypovolemia in rats with chronic NTS lesions.

Secretion of renin after PEG treatment allows the possibility that ANG II, a known dipsogen, mediates the drinking response of rats with chronic NTS lesions in the absence of neural input from cardiac volume-sensitive receptors. Although previous studies have demonstrated that ANG II is not necessary for mediat-
ing hypovolemic thirst in neurologically intact rats (5, 11, 29), it remains possible that afferent neural signals from the heart and ANG II together stimulate hypovolemic thirst in rats, and that water intake is not impaired either by chronic NTS lesions or by bilateral nephrectomy because in the absence of one stimulus the other becomes more effective. According to this hypothesis, rats with chronic NTS lesions should continue to drink in response to the peripheral administration of ANG II; indeed, they should be more sensitive to exogenous ANG II than control rats. This hypothesis remains to be tested.

The signals of thirst during hypovolemia have been studied in species other than rats, and the results obtained are not similar to those now reported. For example, a specific loss of the drinking response to hypovolemia has been produced by cardiac denervation in dogs subjected to hemorrhage (18, 25) and by crushing the right atrial appendage in sheep subjected to ultrafiltration (38). It remains to be determined whether the differences between those results and the present observations reflect differences in the relative roles of volume-sensitive cardiac receptors and of ANG II or other signals in the stimulation of thirst during hypovolemia among the species being studied, differences in the treatments that were used to produce hypovolemia, differences in how the cardiac neural input was eliminated, or differences in some other variable altogether.

Recent observations suggest that the neural input from cardiac volume-sensitive receptors and the bloodborne input from ANG II are not the only stimuli of VP secretion in rats during hypovolemia (19). Thus a third signal for VP secretion may exist, perhaps mediated by input from renal afferent nerves, that ascends through the spinal cord to the brain but bypasses the NTS. That same signal might similarly play a role in stimulating thirst and salt appetite during hypovolemia, as suggested previously (23, 35). Further investigations are needed to evaluate the role of ANG II and of renal afferent nerves in the stimulation of drinking by plasma volume deficits in rats with chronic NTS lesions.

In summary, the removal of cardiac and arterial baroreceptor inputs by chronic lesions of NTS does not prevent the stimulation of thirst and salt appetite during plasma volume deficits induced by PEG treatment in rats. These data are in agreement with our earlier report that hypovolemia continues to stimulate VP secretion in rats with chronic NTS lesions (20). Although cardiac receptors with vagal afferents may normally contribute to the ingestion of fluids during hypovolemia, they clearly are not essential for the behavioral response to decreased blood volume in rats. Hypovolemia also stimulates the release of renin in rats with chronic NTS lesions, and it remains possible that ANG II plays an important role in mediating the observed fluid ingestion. Neuronal input to the brain from renal afferent nerves may also contribute to thirst and salt appetite. In general, it seems plausible that the responses of rats to deficits in blood volume likely involve multiple neural and humoral signals, and consequently removal of one such signal should not prevent the coordinated compensatory responses that contribute to volume regulation.

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